EFFECTS OF MONOVALENT CATION IONOPHORES ON CALCIUM UPTAKE BY RABBIT SKELETAL MUSCLE SARCOPLASMIC RETICULUM VESICLES

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1. Introduction

The ability of ionophores such as A23187 and X537A to increase the calcium permeability of sarcoplasmic reticulum vesicles by facilitating calcium movement across lipid membranes is now well characterized [1,2]. Addition of low concentrations of X537A, which caused only slight calcium release from calcium-filled vesicles, was accompanied by a marked slowing of the spontaneous calcium release that normally occurs with these vesicles [3]. Although both X537A and A23187 have broad cation specificities, allowing monovalent as well as divalent cations to cross lipid membranes [4], previous reports have indicated that the monovalent cation specific ionophores [1] and uncouplers of oxidative phosphorylation [5,6] were without effect on sarcoplasmic reticulum vesicles. In an effort to understand the mechanism by which X537A inhibits calcium release from sarcoplasmic reticulum vesicles, we reinvestigated the effects of monovalent cation ionophores and uncouplers of oxidative phosphorylation (proton ionophores) on calcium uptake and calcium release by this preparation.

2. Methods and materials

Sarcoplasmic reticulum vesicles were prepared from rabbit hind-leg white muscle as in [7]. This preparation was further purified into 'light' and 'heavy' fractions on a 20–60% (w/v) linear sucrose gradient [8,9]. The light fraction, which contained < 5 μM K^+, was used in all the experiments reported here; it was stored at 0°C and used within 3 days. Calcium uptake reactions were carried out at 25°C in 40 mM histidine (pH 6.8), 50 mM Tris phosphate, 5 mM MgATP, 120 mM KCl, 72 μM $^{45}CaCl_2$, 0.15 mg/ml pyruvate kinase, 5 mM phosphoenolpyruvate and 6 μg/ml sarcoplasmic reticulum protein. ^{45}Ca uptake by the vesicles was determined as in [3]. Ionophores were added in ethanol such that the final concentration of ethanol in the reaction mixture did not exceed 0.5% (v/v). These low ethanol concentrations were without effect on calcium content [10]. Protein was determined by the Lowry method [11] using bovine serum albumin as standard. Phosphoenolpyruvate, potassium salt, was obtained from Boehringer Mannheim. Pyruvate kinase from rabbit muscle (type III), gramicidin, valinomycin and carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) were obtained from the Sigma Chemical Company. Nigericin was a generous gift of Robert Hosley (Lilly Research Laboratories). Water used in all solutions was deionized and glass distilled.

3. Results

We have previously demonstrated that, with ATP as substrate, calcium uptake by sarcoplasmic reticulum vesicles exhibits time-dependent changes [12]. Similar results were obtained with the 'light' fraction of sarcoplasmic reticulum vesicle (fig.1a), which can accumulate more calcium than the unfractionated sarcoplasmic reticulum vesicles (data not shown) [6]. These time-dependent changes in calcium content were also observed in vesicles preincubated 2 h in the

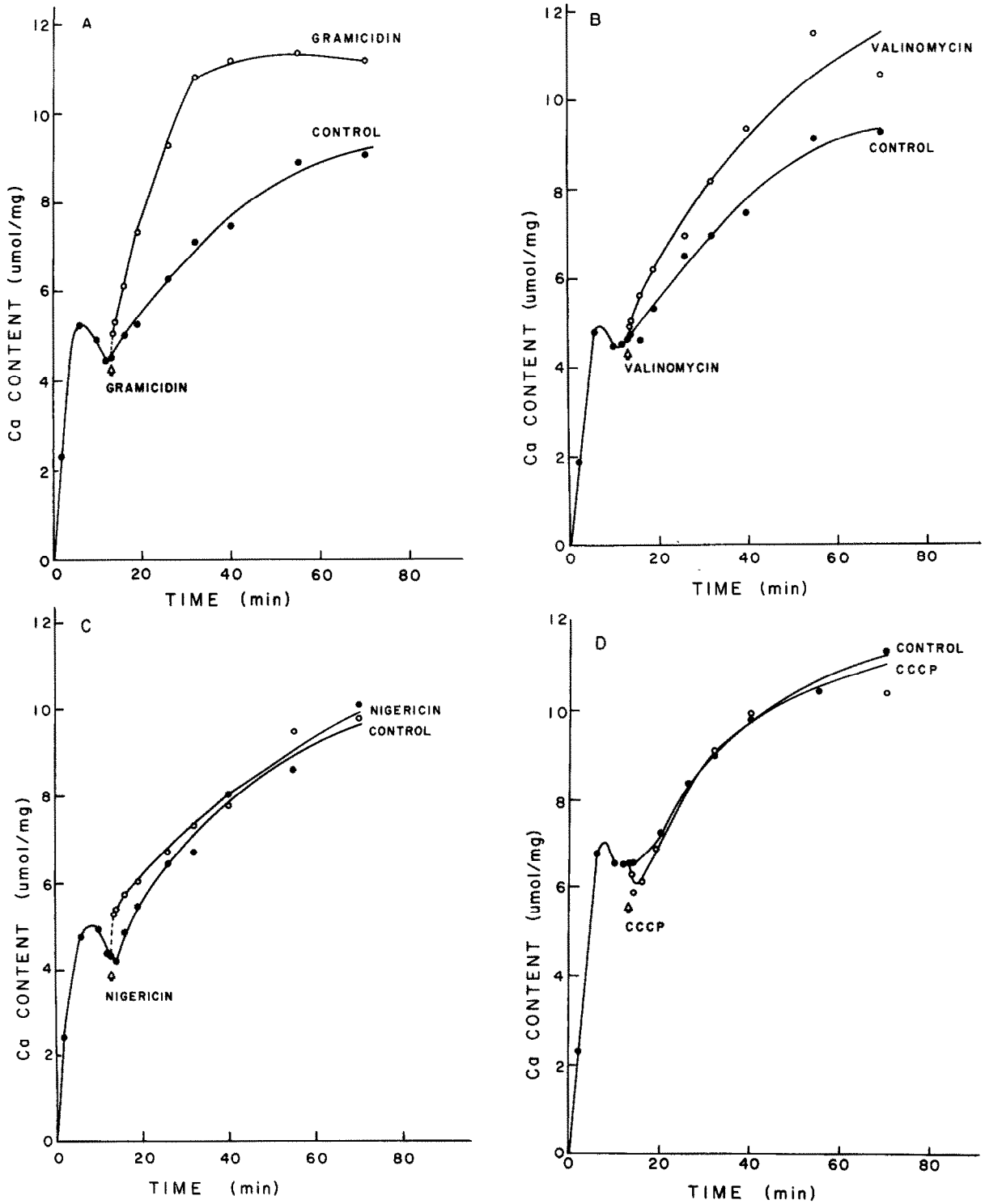

Fig.1. Effect of ionophores on calcium uptake by sarcoplasmic reticulum vesicles. Calcium uptake was carried out as described in section 2 and at 12 min ionophore was added (final conc. 5 μg/ml. (•) Control; (○) ionophore-containing media.

absence of MgATP, indicating that these changes were not due to denaturation of the vesicles during the 1 h reaction period.

Addition of the channel-forming quasi-ionophore gramicidin to a reaction mixture containing partially calcium-filled sarcoplasmic reticulum vesicles, 12 min after the start of the calcium uptake reaction, led to an immediate stimulation of the calcium uptake rate (fig.1a). This caused the 'release' phase of the calcium uptake process to revert to an 'uptake' phase. Although the extent to which gramicidin stimulated the rate of calcium uptake tended to decrease ~10 min after addition of the ionophore, the calcium content of the vesicles in the ionophore-containing medium always remained higher than that of control vesicles. Similar ionophore effects were observed with unfractionated sarcoplasmic reticulum vesicles except that initial uptake rates and total calcium contents were considerably lower. Preincubation of the vesicles in KCl at < 800 mM for 24 h prior to initiation of calcium uptake had no effect on the gramicidin stimulation of calcium uptake such as seen in fig.1a.

The effect of gramicidin to cause renewed calcium uptake is shared by the ionophores valinomycin (fig.1b) and nigericin (fig.1c). However, at 5 μg/ml, the latter ionophores did not stimulate calcium uptake to the same extent as did gramicidin.

The effects of varying the concentration of gramicidin and valinomycin added to sarcoplasmic reticulum vesicles 12 min after the start of the reaction are given in table 1. In this table the differences between the calcium contents of the control and the ionophore-treated vesicles 8 min later (t = 20 min) are tabulated. It is apparent that both of these ionophores stimulated calcium uptake over a wide range of ionophore concentrations.

Table 1
Effect of ionophore concentration on the stimulation of the calcium content of sarcoplasmic reticulum vesicles

[Ionophore] (μg/ml)	% Increase in Ca content at 20 min due to presence of ionophore		
	Gramicidin D	Valinomycin	CCCP
0.1	7%	5%	−1%
1	13%	13%	−1%
10	30%	21%	0%
30	23%	17%	−5%

Calcium uptake was as described in section 2. At 12 min ionophore was added and the calcium content of control and ionophore-containing vesicles were compared at 20 min

When CCCP, an uncoupler of oxidative phosphorylation [11], was added to vesicles at 12 min, no significan stimulation of calcium uptake was seen; rather, CCCP reduced calcium content slightly (fig.1d). Over a range of concentrations CCCP had no stimulatory effect on calcium uptake by the vesicles (table 1). When different combinations of the ionophores were added to vesicles after 12 min of calcium filling, calcium uptake was stimulated in all cases; the degree of stimulation being greater than when just one ionophore was added alone.

4. Discussion

Time-dependent changes in calcium uptake and release have been reported in a number of studies [13–15]. The more complex time-dependent changes in calcium content described in fig.1, which were highly reproducible, cannot be attributed to substrate depletion because they were not attentuated when an ATP-regenerating system was included in the reaction mixtures. Furthermore, they were not due to nonspecific changes in vesicle membranes such as might occur during the prolonged reaction time since preincubation of the vesicles for < 2 h had no significant effect on the characteristics of these time-dependent changes in calcium content.

The present findings demonstrate that the monovalent cation ionophores gramicidin, valinomycin and nigericin stimulate calcium uptake by partially calcium-filled sarcoplasmic reticulum vesicles, without a significant time lag. Gramicidin and valinomycin activate calcium uptake over a range of concentrations, both being most effective at ~ 10 μg/ml (table 1). Because vesicles preincubated in either 800 mM KCl or < 5 μM KCl exhibited properties similar to those shown in fig.1, it appears that the ionophore-induced stimulation of calcium uptake does not require a K^+ gradient across these membranes. Thus, it is unlikely that these effects arise from a changing transmembrane potential due to K^+ fluxes.

It is unlikely that the stimulation of calcium

uptake we have observed is due to ionophore-mediated proton movement across the sarcoplasmic reticulum membrane because addition of CCCP did not reproduce this effect. Although, mitochondrial uncouplers may not act as proton ionophores in sarcoplasmic reticulum [6], valinomycin, which has little capacity to act as an ionophore for protons [16], stimulated calcium uptake in a manner similar to gramicidin and nigericin (fig.1). This stimulation by valinomycin was observed when either nigericin or CCCP was added with the valinomycin at 12 min; these being conditions which should have inhibited the stimulation of calcium uptake if it was due to dissipation of a proton gradient [16].

The present findings suggest that the effect of low concentrations of X537A to decrease spontaneous calcium release from calcium-filled sarcoplasmic reticulum vesicles [3] may arise from an effect similar to that seen with the monovalent cation ionophores (fig.1). In addition, our results indicate that the monovalent cation ionophores stimulate calcium uptake of sarcoplasmic reticulum vesicles by a mechanism not involving either dissipation of a proton gradient or a transmembrane potential due to K^+. Instead, we suggest that these ionophores exert their effect by modifying lipid–protein interactions in the membrane rather than by equilibrating monovalent cations across the sarcoplasmic reticulum.

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