ACTIVATION OF CALCIUM EFFLUX BY ADP AND INORGANIC PHOSPHATE

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1. Introduction

The release of calcium from sarcoplasmic vesicles preloaded with calcium oxalate or calcium phosphate has been studied under various conditions such as the addition of high concentration of EGTA to ATP containing calcium uptake media or the depletion of EGTA containing uptake media from ATP [1–3]. Under both conditions, the observed initial release rates are unidirectional flux rates because the calcium activity in the solution surrounding the vesicles is very low. When the vesicles were loaded with 300–500 nmoles calcium oxalate/mg of vesicular protein, the rates of release were found to be about 100 times slower than the initial rate of calcium uptake [1]. It has been shown that the very slow calcium efflux must be attributed to a low calcium permeability of the membranes, since the hydrolysis of membranal lipids or the binding of surface active agents induces a very fast calcium release. The effect of these membrane modifying treatments cannot be reversed. In this report it is shown that in contrast to these unspecific and irreversible permeability changes the calcium efflux is most specifically and reversibly activated by inorganic phosphate in combination with ADP. The experimental conditions are given in the legends to the figures.

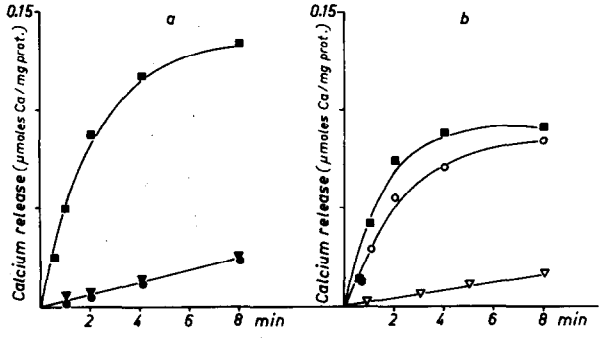

Fig. 1(a). Activation of calcium release from sarcoplasmic vesicles by ADP plus inorganic phosphate. 10 mg of sarcoplasmic vesicles prepared according to Makinose and Hasselbach [1] were loaded with ^{45}Ca oxalate in 3 ml of a solution containing (mM) 1 ITP, 5 $MgCl_2$, 5 oxalate, 1 EGTA, 1 $^{45}CaCl_2$, 50 KCl and 20 histidine (pH 7.0). After an incubation period of 10 min the suspension was diluted 30 fold by solutions containing (mM) 40 KCl, 20 histidine (pH 7.0), 5 $MgCl_2$, 1 EGTA (T = 20°) and ■ 2 ADP plus 20 phosphate, ▼ 2 ADP, ● 20 phosphate. Aliquots were filtered through Sartorius filters (450 n) and the ^{45}Ca was determined by liquid scintillation counting. (b) Inhibition of the activated efflux by reduction of the ADP or the magnesium concentration. The vesicles were loaded as described in (a). The release media contained (mM) 40 KCl, 20 histidine, 1.0 EGTA (T = 20°) and ■ 2 ADP, 20 phosphate, 5 $MgCl_2$, ○ 0.01 ADP, 20 phosphate, 5 $MgCl_2$, ▽ 2 ADP, 20 phosphate, 5 EDTA.

2. Results and remarks

The efflux activating effect of ADP plus inorganic phosphate is clearly demonstrated by the result of release experiments depicted in fig. 1. In these experiments the vesicles have been loaded with calcium oxalate using ITP as energy donator instead of ATP to prevent ADP accumulation in the solution. When the ITP present in the assay is used up the suspension of calcium loaded vesicles is diluted by different release solutions containing in addition to EGTA inorganic phosphate, ADP and inorganic phosphate plus ADP. Fig. 1a shows that the calcium efflux rate is enhanced more than 10 fold in the medium containing both phosphate and ADP in comparison to the other solutions. The calcium ef-

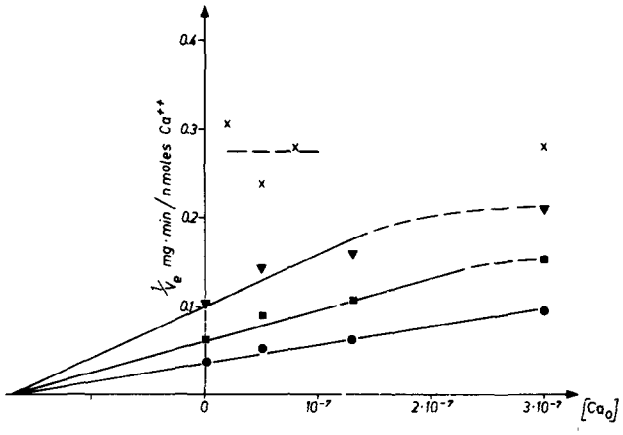

Fig. 2. Inhibition of calcium efflux by ionized calcium. The vesicle were loaded as described in fig. 1. The medium contained instead of 1 mM ITP 1 mM ATP as energy source. The release media contained (mM) 40 KCl, 20 histidine pH 7.0, 5 $MgCl_2$, 1 EGTA and x no phosphate, ▼0.3 pnosphate, ■ 1 phosphate, ● 5 phosphate. ADP was introduced with the uptake medium; its final concentration was 0.03 mM. The free calcium concentration indicated at the abscissa was adjusted by the addition of $CaCl_2$ to the release media. As stability constant for CaEGTA at pH 7.0 a value of 6.7 was used. *Ordinate:* reciprocal plot of the release rate measured during the first minute.

flux is just optimally activated when the assay medium contains 0.05 mM ADP and about 3 mM phosphate. This calcium efflux mechanism has been found to be inhibited by ionized calcium and the lack of magnesium. The assay medium contains no free magnesium (5 mM EGTA present); the calcium efflux is not activated by phosphate and ADP (fig. 1b). Inhibiting concentrations of free calcium in the medium have been found to be identical with those that activate the calcium uptake. For a number of activating phosphate concentrations the identical inhibition constant of 200 nM has been obtained (fig. 2). Since the value of the inhibition constant coincides with the calcium concentration that must be present in the assay medium outside the vesicles when half maximal activation of the calcium uptake is achieved, one has to assume that the inhibition occurs on the outer surface of the vesicles at the same site where calcium transport is activated. This finding excludes a simple diffusion mechanism for calcium exit; it rather implies that membrane components involved in the active uptake of calcium also take part in calcium efflux. Furthermore, since the calcium efflux is activated by the reaction products which are liberated during calcium influx it is suggestive to assume that the osmotic energy which becomes available during calcium efflux is used for ATP synthesis.

Acknowledgement

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