Biochimica et Biophysica Acta, 332 (1974) 313–315

BBA 76547

CALCIUM EFFLUX FROM SARCOPLASMIC RETICULUM VESICLES

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(Received October 11th, 1973)

SUMMARY

Ca^{2+} efflux was studied in sarcoplasmic reticulum vesicles isolated from rabbit skeletal muscle. In experimental conditions in which the Ca^{2+} pump is reversed, the rate of Ca^{2+} efflux varies with the ADP, orthophosphate and Mg^{2+} concentrations of the assay medium and is inhibited by Na^+.

A highly efficient ATP-dependent system for Ca^{2+} transport has been described in sarcoplasmic reticulum vesicles isolated from skeletal muscle. This system plays a key role in the process of excitation–contraction coupling in muscle cells [1,2]. Though the Ca^{2+} uptake proceeds at a very fast rate, the Ca^{2+} accumulated by the sarcoplasmic reticulum vesicles is released slowly when the pump is arrested by different experimental procedures which do not damage the sarcoplasmic reticulum vesicle membrane [3].

Recently it has been shown that the rate of Ca^{2+} efflux is greatly enhanced when pre-loaded vesicles are incubated in a medium containing ADP and P_i. Coupled with the release of Ca^{2+} there is ATP formation, thus characterizing the reversal of the Ca^{2+} pump [3–7]. Different results have been reported for the role of Mg^{2+}, ADP and P_i on the reversal of the Ca^{2+} pump. Panet and Selinger [7] observed that Mg^{2+} inhibits the Ca^{2+} efflux, that the rate of Ca^{2+} outflow is activated by either P_i or ADP alone, and that the effect of ADP and P_i is not additive. On the other hand, Barlogie et al. [3] reported that Mg^{2+} does not inhibit the Ca^{2+} efflux and that the increment of Ca^{2+} efflux was only observed in the presence of Mg^{2+}, ADP and P_i.

In this communication, the role of Mg^{2+}, ADP and P_i on the activation of Ca^{2+} efflux of pre-loaded sarcoplasmic reticulum vesicles was further studied.

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle as previously described [8].

The sarcoplasmic reticulum vesicles were loaded with calcium oxalate by incubating at room temperature 3.3 mg of sarcoplasmic reticulum vesicle protein in 1 ml of a medium containing 20 mM Tris–maleate buffer, pH 7.0; 20 mM KCl, 2 mM ITP, 5 mM $MgCl_2$, 1 mM $^{45}CaCl_2$, 1 mM ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) and 5 mM potassium oxalate. After 5 min incubation at room temperature, 95–98% of the ^{45}Ca of the assay medium was removed by the sarcoplasmic reticulum vesicles. Efflux experiments were initiated by adding 0.17 ml

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid.

of medium containing the loaded sarcoplasmic reticulum vesicles in 5 ml of a solution containing 10 mM Tris–maleate buffer (pH 7.0), 1 mM EGTA and variable concentrations of Mg^{2+}, ADP and P_i. Ca^{2+} incorporation or release was determined by measuring the ^{45}Ca in the solution after removing the sarcoplasmic reticulum vesicles by means of Millipore filters [9].

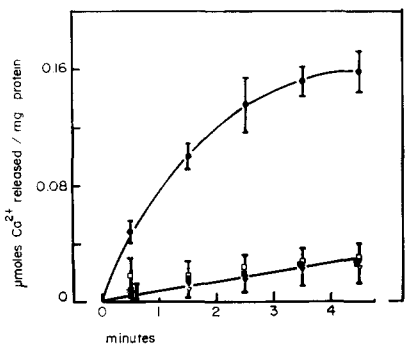

Fig. 1. Activation of Ca^{2+} efflux by ADP, P_i and Mg^{2+}. Sarcoplasmic reticulum vesicles were previously loaded with calcium oxalate as described in the text, and then incubated in media containing 10 mM Tris–maleate buffer (pH 7.0), 1 mM EGTA and (●) 2 mM ADP, 20 mM P_i and 5 mM $MgCl_2$; (□) 2 mM ADP and 20 mM P_i; (■) 20 mM P_i and 5 mM $MgCl_2$; (▽) 2 mM ADP and 5 mM $MgCl_2$; (▼) no additions. The experimental conditions were as described in the text. The values represent the average ± S.E. of 6 experiments.

Fig. 1 shows that the rate of Ca^{2+} efflux was only activated in the presence of ADP, P_i and Mg^{2+}. If any of these reagents was omitted, the rate of Ca^{2+} efflux is drastically decreased. Fig. 2 shows a Lineweaver–Burk plot of the initial rate of Ca^{2+} efflux as a function of the P_i concentration, in the presence of 0.5 mM ADP. In four experiments the K_m for P_i found was 4.7 mM ± S.E. 0.9. Fig. 2 also shows that raising

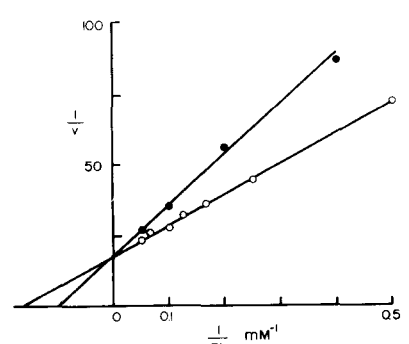

Fig. 2. Competition of ADP and P_i for the sarcoplasmic reticulum vesicle membrane. The initial rate (1 min) of Ca^{2+} efflux of sarcoplasmic reticulum vesicles loaded with calcium oxalate was measured in media containing 10 mM Tris–maleate buffer (pH 7.0), 1 mM EGTA, 5 mM $MgCl_2$, variable concentrations of P_i ranging from 1 to 20 mM, and 0.5 mM ADP (○—○) or 2.0 mM ADP (●—●). The experimental conditions were as described in the text. The figure shows a typical experiment. Essentially the same results were observed in four different sarcoplasmic reticulum vesicle preparations tested.

the ADP concentration to 2 mM results in a decrease of the Ca^{2+} efflux rate due to a competition of ADP with P_i for the binding site on the sarcoplasmic reticulum vesicle membrane.

In previous papers it has been shown that Na^+ and K^+ might inhibit the Ca^{2+} transport depending on the substrate used [9–12]. In eight different sarcoplasmic reticulum vesicle preparations the Ca^{2+} efflux was measured in media containing 10 mM Tris–maleate buffer pH 7.0, 1 mM EGTA, 2 mM ADP, 4 mM P_i, 2 mM $MgCl_2$ and either 5 mM KCl, 120 mM KCl or 120 mM NaCl. The rates of Ca^{2+} efflux found were, respectively, 11.2±S.E. 2.6; 9.5±S.E. 1.9 and 8.0±S.E. 1.3 μmoles Ca^{2+} per mg protein per min. The inhibition caused by Na^+ was statistically significant ($P<0.05$).

In conclusion, this communication shows that in conditions in which the Ca^{2+} pump of the sarcoplasmic reticulum vesicles is reversed, the rate of Ca^{2+} efflux is Mg^{2+} dependent, is inhibited by 120 mM Na^+ but not by 120 mM K^+, and that the maximal rate of Ca^{2+} efflux varies with the ADP: P_i ratio used, excess of ADP being inhibitory.

ACKNOWLEDGEMENTS

This work was supported by the Conselho Nacional de Pesquisas (T.C. No. 16.293), by the Conselho de Ensino e Pesquisas para Graduados da U.F.R.J. and by the Banco Nacional de Desenvolvimento Econômico (FUNTEC 143).

H. M. is Recipient of a fellowship from the Coordenação do Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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