CHARGE TRANSFER DURING Ca^{2+} UPTAKE BY RABBIT SKELETAL MUSCLE SARCOPLASMIC RETICULUM VESICLES AS MEASURED WITH OXANOL VI

Karl E. O. ÅKERMAN and C. Henrik J. WOLFF
Department of Medical Chemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

Received 5 February 1979

1. Introduction

Optical probes for measurements of potential charges in subcellular membrane fractions are currently of wide interest [1]. The most specific probes in this context are the cyanines [1,2], safranine [3–5] and oxanols [1,6–10]. The cyanines and safranine are positively charged and hence accumulate towards the negative pole of the membrane. Thus they are suitable for measurements of potentials with negative polarity inside membrane vesicles. The oxanols are negatively charged and hence more suitable for detecting potentials with the opposite polarity (especially oxanol VI). Oxanol VI has thus been used in studies on the generation of potentials with positive internal polarity in submitochondrial particles [7], thylakoids [8] and chromatophores [9]. Oxanol V is less sensitive with submitochondrial particles and also senses potentials with negative internal polarity in mitochondria [10]. Changes in the fluorescence of the nonspecific probe [11] ANS^- [12] and a cyanine [13] indicating the buildup of a potential with positive internal polarity during Ca^{2+} uptake by sarcoplasmic reticulum vesicles have been reported [12,13]. Furthermore as measured with ANS^- a 50 mV potential change could be detected [14] during Ca^{2+} uptake into reconstituted Ca^{2+}-ATPase vesicles. The extent of Ca^{2+} uptake into the vesicles was found [14] to decrease with an increased positive polarity inside the vesicles.

Abbreviations: ANS, 8-anilino-1-naphtalenesulfonic acid; EGTA, ethyleneglycol bis (α-aminoethyl (ether)-*N,N*-tetra-acetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid

We show here that a very fast shift in the spectrum of oxanol VI occurs during Ca^{2+} uptake by sarcoplasmic reticulum vesicles indicating a buildup of a membrane potential with positive polarity inside the vesicles. The spectral change is completely reversed by addition of the 2 H^+/Ca^{2+} exchanger A23187. Smaller but reproducible changes in the probe response are, on the other hand, observed upon efforts to change the electric conductivity of the membrane by the addition of a cation conducter, valinomycin, a freely permeable anion, SCN^- or a H^+/cation exchanger, nigericin.

2. Materials and methods

Sarcoplasmic reticulum (SR) vesicles were prepared from the hind-leg muscles of female rabbits essentially as in [15]. The vesicles were suspended in a medium containing 100 mM KCl, 10 mM Hepes(pH 7.3)–KOH. The preparation was used within 2–3 days. The spectral changes of safranine [3–5], oxanol V and oxanol VI [6–10] were measured in an Aminco DW_2 spectrophotometer. Protein was measured by the Lowry method [16]. For further experimental details see figure legends.

Valinomycin was purchased from Sigma Chemicals Co., St Louis, MO. Nigericin was obtained from Dr M. Gorman (Eli Lilly Co.) A23187 from Dr Hamill (Eli Lilly Co.) and the oxanols were donated by Professor B. Chance (Johnson Res. Found. PA). All the other reagents were commercial products of highest grade.

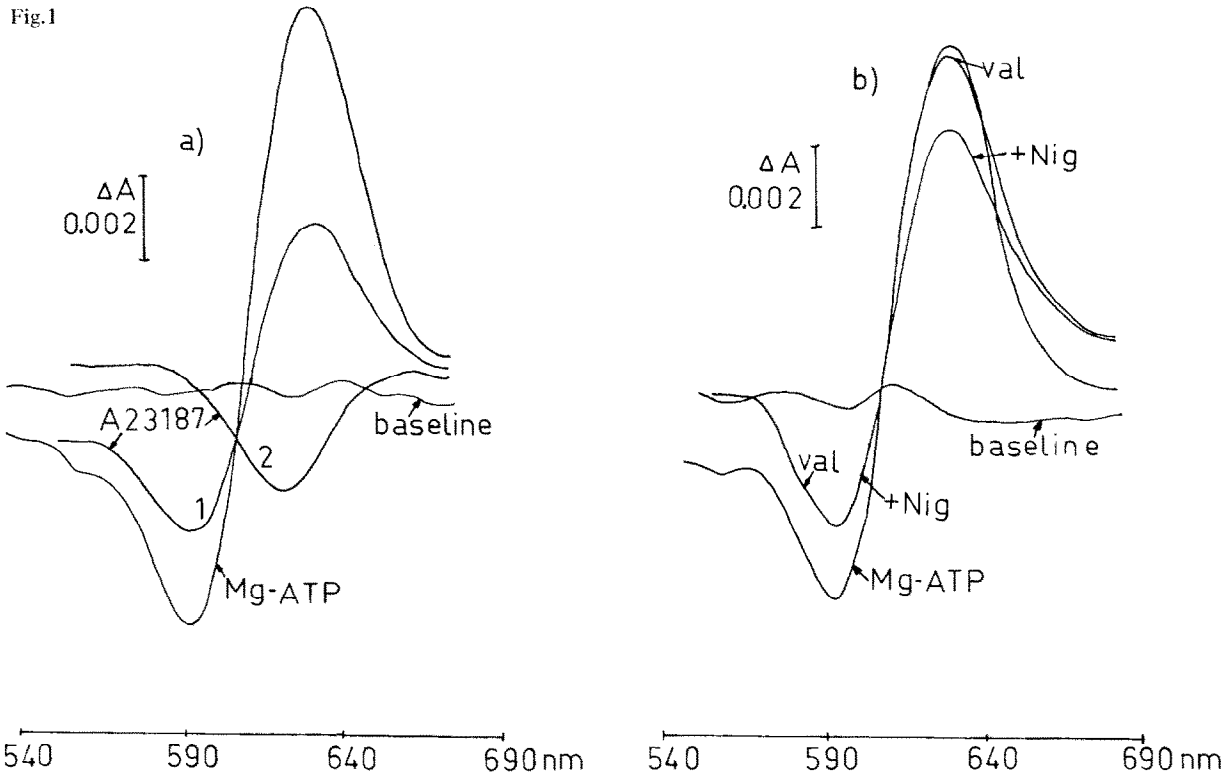
3. Results

An addition of Mg-ATP to a sarcoplasmic reticulum (SR) vesicle suspension causes a fast shift in the spectrum of oxanol VI in the presence of Ca^{2+}. The shift is composed of an increase in A_{625} and a decrease in A_{590}. A similar spectral change occurs upon energization of submitochondrial particles [7] or chromatophores [9] and indicates a buildup of a positive polarity inside the membrane vesicles [9]. In similar conditions no or only nonspecific changes in the spectrum of safranine or oxanol V can be detected (not shown). Figure 1a also shows that the signal is completely reversed by the addition of an electroneutral 2 H^+/Ca^{2+} exchanger A23187 [17]. In this case a small mirror decrease in A_{625} is seen indicating that the membrane potential of the vesicles is more negative internally in these conditions than before the Mg-ATP addition. No significant change in the spectrum of oxanol VI alone is observed upon Ca^{2+} addition up to 5 mM or ethanol up to 5% (not shown). Oxanol VI furthermore does not react to changes in pH in the range between pH 4–8 [10]. Furthermore, no changes in the probe response can be observed in the absence of SR vesicles in the experimental conditions. With the safranine method [3–5] only nonspecific changes can be detected upon A23187 addition (not shown) suggesting that the membrane potential is still positive internally or near zero.

Figures 1b,c show the effects of valinomycin and KSCN on the spectral change induced by Mg-ATP. Valinomycin causes a small decrease of the response ($\Delta A_{625-590}$ decreased ~15%) and possibly a nonspecific increase in the absorbance at all wavelengths (contraction?). The response of valinomycin is enhanced somewhat by nigericin ($\Delta A_{625-590}$ decreased ~20%). A small change ($\Delta A_{625-590}$ decreased ~25%) in the Mg-ATP response is also seen upon addition of KSCN.

In fig.2 the time dependence of the spectral changes are seen. Addition of Mg-ATP to the suspension causes a fast response followed by a slower one and subsequently a rather steady level. A further addition of A23187 causes a very fast reversal, of the change, which is not abolished by Mg-ATP or EGTA (fig.2a).

Fig.1



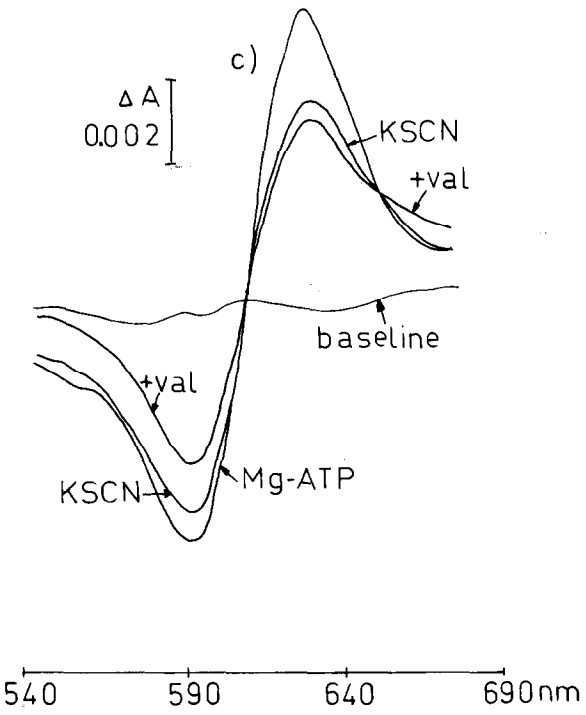


Fig.1. Effect of A23187, valinomycin, KSCN and nigericin on Mg-ATP-induced change in the spectrum of oxanol VI in the presence of SR vesicles. SR vesicles (0.26 mg/ml) were suspended in a medium containing 50 mM KCl, 20 mM Tris, 10 mM Hepes(pH 6.9)–KOH, 10 μM $CaCl_2$ and 1 μM oxanol VI (room temperature) and the baseline was drawn as indicated. In (a) additions: 200 μM Mg-ATP and 2.5 μM A23187. The spectrum was drawn immediately after the A23187 addition (1) and within 2 min (2). In (b) additions: 200 μM Mg-ATP, 40 ng/ml valinomycin (Val) and 4 ng/ml nigericin (Nig) as indicated. The spectra were drawn within 1–2 min after the additions. In (c) additions: 200 μM Mg-ATP, 10 mM KSCN and 40 ng/ml valinomycin (Val) as indicated. The spectra were drawn within 1–2 min after the additions.

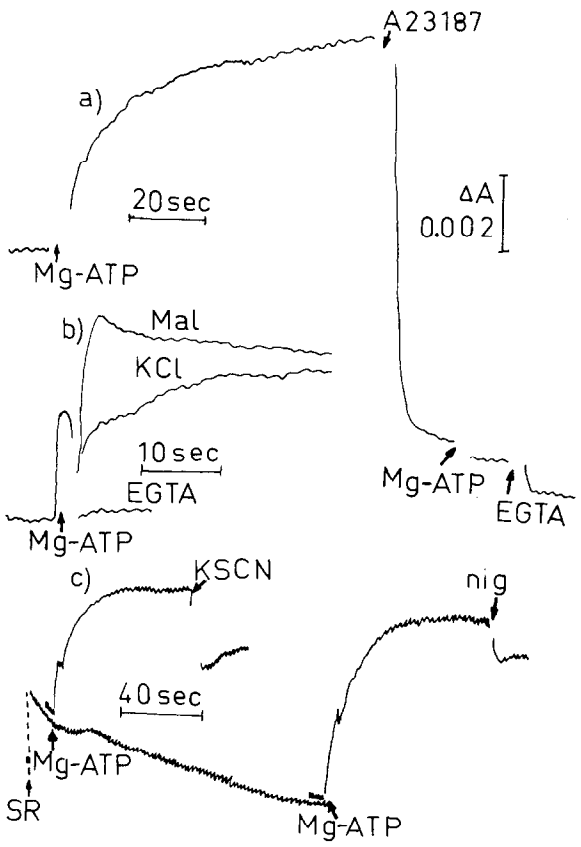


Fig.2. Time dependence of Mg-ATP-induced oxanol VI response. Conditions as in fig.1. Dual wavelength technique, wavelength pair 620–650. In (a) additions: 200 μM Mg-ATP, 7 μM A23187, 200 μM Mg-ATP and 2 mM EGTA as indicated. In (b) same as in (a) except that the SR vesicles were suspended into a 60 mM maleate medium adjusted with KOH to pH 6.9 (Mal) or the same medium as fig.1 in the absence (KCl) or presence of 2 mM EGTA as indicated. In (c) vesicle suspension (SR), 200 μM Mg-ATP, 10 mM KSCN or 4 ng/ml nigericin (Nig) as indicated. Protein concentration: (a) 0.4 mg/ml, (b,c) 0.26 mg/ml. Upward deflections indicate an increased positive polarity inside the vesicles.

When the experimental medium contains K-maleate only a very fast response of Mg-ATP is seen followed by a slow decay (fig.2b). The difference in the signal between the K-maleate and KCl media varies somewhat from preparation to preparation but is always qualitatively equal. If EGTA is added prior to Mg-ATP only a nonsignificant change is seen (fig.2b). In preliminary experiments using a Ca^{2+}–EGTA buffer system we have found that the signal appears at $Ca^{2+} > 10^{-7}$ M and that a saturation occurs at $> 10^{-6}$ M (not shown) in accordance with data on Ca^{2+}-stimulated ATPase activity [18] or kinetics of Ca^{2+} uptake [19,20]. A more thorough analysis on the Ca^{2+} dependence in different conditions will be reported in the future. Figure 2c shows that upon dilution of the vesicle suspension into the experimental medium a small drift towards a more negative potential is seen and that the magnitude of the change caused by

Mg-ATP depends on at which stage of this drift Mg-ATP is added. It is also shown that both nigericin alone and KSCN cause a slight reversal of the signal.

4. Discussion

The results indicate that a membrane potential with positive internal polarity is formed during Ca^{2+} uptake by SR vesicles. The membrane potential is probably generated as a result of an active electrogenic transport of Ca^{2+} across the vesicle membrane. This is in agreement with the results in [14] on reconstituted Ca^{2+}-ATPase liposomes. The workers also showed that when a more negative internal potential was induced with valinomycin in the presence of different K^+ gradients more Ca^{2+} was taken up. We have as yet been unable to quantitate the oxanol VI response with K^+ diffusion potentials because of the high endogenous permeability of SR vesicles towards K^+ [21].

The fact that A23187, known to induce Ca^{2+} release from SR vesicles [22], reverses the potential change induced by Ca^{2+} (see fig.1,2), would suggest that maintainance of the membrane potential is dependent on an ability of the vesicles to keep up an outwards-directed Ca^{2+} gradient across the membrane. On the other hand, 2 H^+/Ca^{2+} exchange might lead to the creation of an outwards-directed proton electrochemical potential across the vesicle membrane. In fact a proton gradient (alkaline inside) is created during Ca^{2+} uptake across SR vesicle membranes [13]. If this proton translocation is electronic it could lead to a more negative potential inside the SR vesicles.

Addition of KSCN after Ca^{2+} uptake causes a slight reversal of the membrane potential induced by Mg-ATP probably because of SCN^- influx. Also valinomycin has a similar effect, suggesting that also this cation conducter is able to cause a charge compensation for electrogenic Ca^{2+} influx by increasing K^+ efflux. Indeed valinomycin has been shown to decrease the half-time of K^+ equilibration across the SR vesicle membrane to the same as that of Cl^- (0.5 s, [21]). The effect of valinomycin is increased by addition of a H^+/K^+ exchanger nigericin. The effect of nigericin would be due to an equilibration of the K^+ gradient by mediating a distribution of K^+ across the membrane according to the proton or K^+ gradients. On the other hand, nigericin also causes electrogenic K^+ transport in some conditions [23]. In this context it is of interest that both valinomycin and nigericin increase time-dependent Ca^{2+} uptake by SR vesicles as shown [24]. This could be due to a better charge compensation for electrogenic Ca^{2+} uptake in light of the present results and those in [14] even if suggested in [24] that the effect of these ionophores are due to structural changes in the membrane.

The response caused by Mg-ATP in a K-maleate medium as compared to the KCl medium is also of interest especially because of the very high permeability of SR vesicles towards Cl^- [21]. The slower response in the KCl medium would thus be due to a partial charge compensation for Ca^{2+} influx by uptake of Cl^-. A further point of possible importance in this context is the finding [25] that SR vesicles transiently take up more Ca^{2+} from a K-maleate medium but release the Ca^{2+} taken up again much faster than in a KCl medium. Furthermore, if Ca^{2+} uptake is carried out in a KMS medium (MS^- less permeable than Cl^-, [21]) and the medium is switched for a KCl medium a fast Ca^{2+} release from SR vesicles can be noted [25–28]. A similar result has been obtained with skinned muscle fibers [29]. The efflux is probably not simply a result of depolarisation due to Cl^- influx since no effect is observed upon a change from KMS to choline-Cl [26] and a depolarisation is expected to enhance Ca^{2+} uptake [14].

It is concluded that influx of Ca^{2+} across the membrane of SR vesicles appears to cause a potential change in the membrane with positive polarity inside the vesicles. The potential change appears to be only partially compensated for by either K^+ efflux or Cl^- influx.

Acknowledgements

This study was aided by a grant by the Sigrid Juse'lius Foundation. The authors wish to thank Dr M. K. F. Wikström for discussion and criticism and Ms Annikki Saulamo for typing the manuscript.

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