Biochimica et Biophysica Acta, 1064 (1991) 329–334

ADONIS 000527369100206G

BBAMEM 75222

Effects of Mg^{2+} and ATP on the phosphate transporter of sarcoplasmic reticulum

H.I. Stefanova, S.D. Jane, J.M. East and A.G. Lee

Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton (U.K.)

(Received 16 October 1990)

Key words: Sarcoplasmic reticulum; Phosphate transporter; Chloride channel

The extra uptake of Ca^{2+} by vesicles of sarcoplasmic reticulum (SR) observed in the presence of P_i, attributable to transport of P_i by the P_i-transporter, has been studied. It has been shown that the P_i transporter is stimulated by ATP. Single channel conductance measurements have shown that the Cl^- channel in the SR membrane is impermeable to P_i. It is suggested that the transporter could be an an ion antiporter system. Studies of uptake as a function of pH and Mg^{2+} concentration suggest that transport of $MgHPO_4$ and $H_2PO_4^-$ are faster than transport of HPO_4^{2-}. For oxalate and pyrophosphate, Mg^{2+} binding inhibits transport. It is suggested that protonation of lysine residue(s) at the anion binding site increase the rate of transport.

Introduction

The sarcoplasmic reticulum (SR) of skeletal muscle contains a transporter for P_i ions which also carries oxalate and pyrophosphate [1–5]. Movement of these ions into the lumen of the SR results in the precipitation of the corresponding Ca^{2+} salts with an increase in the level of Ca^{2+} accumulated by the SR [6,7]. It was suggested in the preceding paper that phosphonoformic acid was also transported by the P_i transporter, whereas other phosphonocarboxylic acids were transported poorly, if at all, but were inhibitors of P_i transport [5].

The mechanism of the transporter is unknown. Carley and Racker [4] showed that, as well as P_i, external Ca^{2+}, Mg^{2+} and ATP were required for uptake of P_i. Since these same ions are required for Ca^{2+} uptake mediated by the (Ca^{2+}-Mg^{2+})-ATPase in the SR, this could suggest that P_i transport was linked to the formation of a Ca^{2+} gradient. However, although uptake of P_i was markedly reduced in the presence of the Ca^{2+} ionophore A23187, it was reported still to occur, leading Carley and Racker [4] to suggest that a Ca^{2+} gradient was not necessary [4]. Carley and Racker [4] also showed that uptake was not driven by an inside positive membrane potential generated by the (Ca^{2+}-Mg^{2+})-ATPase, since uptake of P_i was unaffected by the addition of valinomycin. In the previous paper, it was shown that P_i transport was protected by ATP against modification by acetic anhydride or phenylglyoxal, suggesting a binding site for ATP on the transporter [5]. The role of such a binding site remains to be determined. Also unclear is the species of P_i actually transported, since, in a medium containing both Mg^{2+} and P_i, a mixture of protonated and Mg^{2+}-bound species of P_i will be present. In this paper we explore the effects of ATP and Mg^{2+} on the uptake of P_i and oxalate.

Materials and Methods

AnalaR reagents and murexide were obtained from BDH Chemicals and Hepes (Ultrol) was from Calbiochem. SR was prepared as described in Stefanova et al. [5]. For some experiments SR was fractionated into light, intermediate and heavy fractions following the protocol of Meissner [24]. Ca^{2+} fluxes were monitored photometrically by using the dye murexide. Spectra were run on a Shimadzu UV3000 dual-wavelength spectrophotometer with a wavelength pair of 507 nm-542 nm, at 25°C, as described [5]. Ca^{2+} uptake was initiated by addition of ATP; at the Mg^{2+} concentration usually employed (5 mM) the change in free Ca^{2+} concentration due to binding of Ca^{2+} to ATP was negligible. At lower concentrations of Mg^{2+}, a decrease

Abbreviations: SR, sarcoplasmic reticulum; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone.

Correspondence: A.G. Lee, Department of Biochemistry, University of Southampton, Southampton, SO9 3TU, U.K.

in free Ca^{2+} concentration was observed on addition of ATP to buffer in the absence of SR due to the formation of CaATP; this change was subtracted from the traces obtained in the presence of SR.

Concentrations of species of P_i and oxalate in the presence of Mg^{2+} were calculated using log proton binding constants of 6.71, and 3.81, respectively, and log Mg^{2+} binding constants of 1.88 and 2.39, respectively [8].

Planar bilayers were formed from a 1 : 1 molar ratio of egg yolk phosphatidylethanolamine and bovine brain phosphatidylserine (Lipid Products) dissolved in decane at a lipid concentration of 4 mg/ml. The lipid solution was painted across a 300 μm hole drilled in a polycarbonate cup. Aliquots of the intermediate SR fraction [24] (50 μg protein/ml final concentration) were added to the cis chamber and fusion was induced by stirring and the application of a negative holding potential across the bilayer of -40 mV. The buffer was 10 mM Hepes-Tris (pH 7.4) containing 5 mM $CaCl_2$ and 100 μM EGTA, with choline chloride and P_i added as required. Experiments were performed at room temperature.

Current flow through the bilayer was monitored using a patch and cell clamp amplifier (Bioiogic RK300) as a current-to-voltage converter via glass salt bridges in series with Ag/AgCl electrodes. The output of the amplifier was displayed on an oscilloscope, digitized using a pulse code modulator (Biologic PCM) and stored on videotape for analysis. Data stored on videotape was reconverted to an analogue signal using the output channel of the pulse code modulator and low-pass filtered at a corner frequency of 300 Hz (Frequency Devices 902LP Filter) and digitized at 10 kHz prior to analysis using Satori software (Intracel).

Results

Effect of ATP on P_i and oxalate transport

The uptake of Ca^{2+} that follows addition of ATP to a suspension of SR vesicles can be monitored spectrophotometrically using murexide to measure the concentration of Ca^{2+} in the external medium [5]. As shown previously [1,2,5], in the presence of P_i the initial fast phase of uptake of Ca^{2+} is followed by a second, slower phase of uptake that can be attributed to the transport of P_i followed by precipitation of $CaHPO_4$ within the vesicles. It has been shown that uptake of Ca^{2+} can also be driven by pseudo substrates such as acetyl phosphate [9]. Fig. 1 shows that in the presence of P_i rapid Ca^{2+} uptake driven by acetyl phosphate is also followed by a second slower phase of uptake, but that the rate of the second phase is slower than observed for uptake driven by ATP (compare Fig. 1, Ref. 5). A slow phase of uptake is also observed in the presence of oxalate, but again this is considerably slower

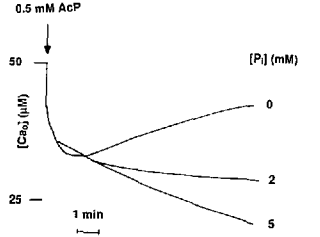

Fig. 1. Uptake and release of Ca^{2+} are shown following the addition of acetyl phosphate (0.5 mM) to SR vesicles (0.13 mg protein/ml) in buffer (40 mM Hepes-KOH, 100 mM KCl, 5 mM $MgSO_4$ (pH 6.3)) at an initial Ca^{2+} concentration of 50 μM, in the presence of the given concentrations (mM) of P_i.

in the presence of acetyl phosphate than ATP (data not shown).

The slower rate of the slow phase of accumulation of Ca^{2+} observed with acetyl phosphate than with ATP suggests that uptake of P_i (and oxalate) might be stimulated by ATP. This possibility was explored further by studying the effect of addition of ATP during the uptake process. As shown in Fig. 2, in the absence of P_i, fast uptake of Ca^{2+} is followed by a slow phase of Ca^{2+} release. It was shown previously that this slow phase of release occurs with ATP in the system; a second, faster phase of Ca^{2+} release is observed after all the added ATP has been hydrolysed by the (Ca^{2+}-Mg^{2+})-ATPase [9]. That spontaneous release occurs in the presence of ATP is confirmed by Fig. 2, which shows that addition of 1 mM ATP during the release phase does not prevent the release of Ca^{2+}. The effect of addition of P_i depends markedly on the time of addition following initiation of uptake (Fig. 3A). Ad-

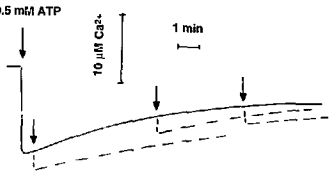

Fig. 2. Uptake and release of Ca^{2+} are shown following the addition of ATP (0.5 mM) to SR vesicles (solid line). Also shown is the effect of addition of 1 mM ATP at the given times following initiation of uptake (dotted lines). The decrease in free Ca^{2+} concentration following the second addition of ATP can be attributed to the formation of a small amount of CaATP at the relatively high ATP/Mg^{2+} ratio. The protein concentration was 0.08 mg/ml and the buffer 40 mM Hepes-KOH, 100 mM KCl, 5 mM $MgSO_4$ (pH 6.3) with an initial Ca^{2+} concentration of 50 μM.

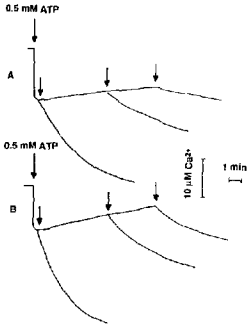

Fig. 3. Uptake and release of Ca^{2+} are shown following the addition of ATP (0.5 mM) to SR vesicles (0.08 mg/ml) followed by the addition of 5 mM P_i (A) or 5 mM P_i plus 0.5 mM ATP (B) at the given times. Other conditions as in Fig. 2.

dition of P_i 0.5 min following initiation of uptake leads to a very marked second phase of uptake, comparable to that seen following the addition of ATP to SR vesicles incubated in the presence of P_i (see Fig. 1 of Ref. 5). However, addition of P_i 10 min after the initiation of uptake results in little further uptake of Ca^{2+}. As shown in Fig. 3B, simultaneous addition of P_i and ATP leads to a more marked second phase of uptake than addition of P_i alone, the difference being particularly marked at long times. These results serve to confirm that uptake of P_i is not simply driven by a Ca^{2+} gradient across the SR membrane, but is stimulated by ATP. Very similar results were obtained following the addition of oxalate and oxalate plus ATP (data not shown).

Effect of pH and Mg^{2+} on P_i and oxalate transport

In the Mg^{2+}-containing medium used to study uptake of Ca^{2+}, P_i will be present as a mixture of HPO_4^{2-}, $H_2PO_4^-$ and $MgHPO_4$, the relative proportions depending on pH and Mg^{2+} concentration (Table I). As shown in Fig. 4, the second slow phase of Ca^{2+} accumulation observed in the presence of P_i increases markedly in rate with increasing concentration of Mg^{2+} and is also more marked at acid pH. The effect of oxalate (Fig. 5) is also more marked at acid pH, but the slow phase of accumulation in this case is inhibited by Mg^{2+}. As for oxalate, the slow phase of uptake observed in the presence of pyrophosphate is also reduced by Mg^{2+} (Fig. 6).

Single-channel recordings of the Cl^- channel

Fig. 7A shows typical conductance fluctuations caused by fusion of SR vesicles with a planar bilayer in

TABLE I

Relative proportions of species of P_i and oxalate as a function of pH and Mg^{2+}

The relative proportions were calculated using the binding constants given in Materials and Methods.

pH	Mg^{2+} (mM)	Relative proportions		
		HPO_4^{2-}	$H_2PO_4^-$	$MgHPO_4$
5.3	1	0.04	0.96	0
	5	0.04	0.95	0.01
	10	0.04	0.93	0.03
8.1	1	0.90	0.03	0.07
	5	0.70	0.03	0.27
	10	0.56	0.02	0.42
		$C_2O_4^{2-}$	$HC_2O_4^-$	MgC_2O_4
5.3	1	0.79	0.02	0.19
	5	0.44	0.01	0.54
	10	0.29	0.01	0.70
7.2	1	0.80	0.02	0.20
	5	0.45	0	0.55
	10	0.29	0	0.71

the presence of 250 mM and 50 mM choline chloride in the *cis* and *trans* chambers, respectively, at a holding potential of 0 mV. Since the K^+ channels of SR do not carry choline$^+$ [23], the carrier of current under these conditions will be Cl^- [14,15]. In these traces, open events are represented as downward deflections, so that under these conditions the channel spends much of its time in an open state. Channel currents were measured over the range of holding potentials -40 mV to $+60$

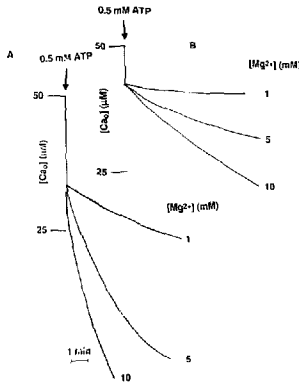

Fig. 4. Uptake of Ca^{2+} is shown following the addition of ATP (0.5 mM) to SR vesicles (0.08 mg protein/ml) in the presence of P_i (5 mM) at pH 5.3 (A) and pH 8.1 (B) at the given concentrations of Mg^{2+}. Other conditions as in Fig. 2.

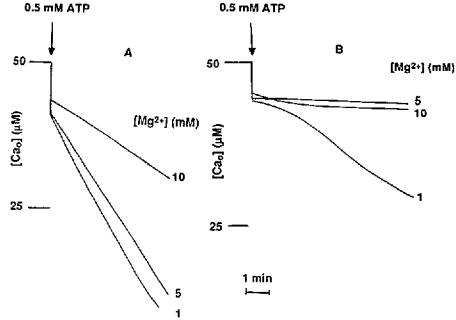

Fig. 5. Uptake of Ca^{2+} is shown following the addition of ATP (0.5 mM) to SR vesicles (0.03 mg protein/ml) in the presence of oxalate (1 mM) at pH 5.3 (A) and pH 7.2 (B) at the given concentrations of Mg^{2+}. Other conditions as in Fig. 2.

mV and gave a conductance of 87 pS comparable to the value of 95 pS reported by Rousseau et al. [15] under similar conditions. As shown in Fig. 7B, with 50 mM choline chloride in each chamber and 50 mM P_i in the *cis* chamber with a holding potential of 0 mV, no conductance steps were observed, showing that the Cl^- channel is impermeable to P_i.

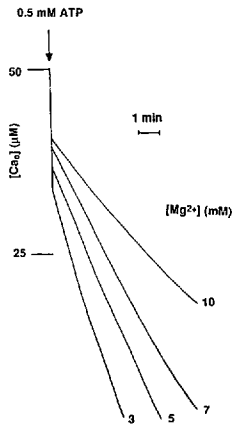

Fig. 6. Uptake of Ca^{2+} is shown following the addition of ATP (0.5 mM) to SR vesicles (0.03 mg protein/ml) in the presence of pyrophosphate (2 mM) at pH 7.2 at the given concentrations of Mg^{2+}. Other conditions as in Fig. 2.

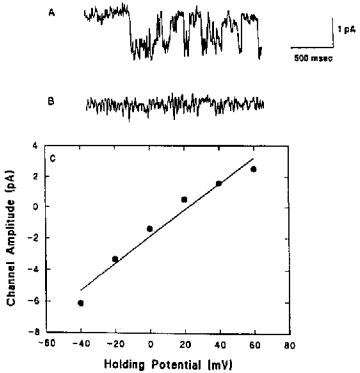

Fig. 7. Single Cl^- channel recordings (A) in the presence of 25 mM choline chloride *cis* and 50 mM choline chloride *trans* and (B) 50 mM choline chloride in both chambers and 50 mM P_i *cis*, at a holding potential of 0 mV. Open events are represented by downward deflections. (C) Current voltage relationship recorded in the presence of 250 mM choline chloride *cis* and 50 mM choline chloride *trans*. Data points represent the average of at least six determinations at each holding potential.

Discussion

The mechanism of the P_i transporter is unknown. Uptake of P_i and oxalate in the absence of Ca^{2+} is low [2,4,10] but, in the absence of Ca^{2+} to precipitate the anion in the SR lumen, it is not clear that equilibration of P_i or oxalate between the internal and external mediums would have been detectable, because of the small internal volume of the SR vesicle (approx. 4 μl/mg protein; Ref. 11). Light scatter experiments suggest half-permeation times of 11 and 40 s for P_i and oxalate, respectively, at 23°C, compared to 0.4 s for Cl^- [12]. Direct measurements of the rate of efflux of P_i from passively loaded SR vesicles give a half-time for efflux of approx. 30 s [13]. The high permeability of the SR membrane to Cl^- can be attributed to the presence of Cl^- channels which have been characterized electrophysiologically [14,15]. Fig. 7 shows single-channel recordings for the Cl^- channel of SR. The mean single-channel conductance recorded with 250 and 50 mM Cl^-, respectively, in the *cis* and *trans* chambers was 87 ps, comparable to that reported by Rousseau et a. [15]. This value is also comparable to that reported for surface membrane Cl^-channels such as that in cultured neurones [25]. It has been reported that the permeability of the Cl^-channel to multivalent anions is low [14]. Fig. 7B shows that no current is carried by P_i through the Cl^-channel, suggesting that the Cl^- channel has no role in transport of P_i. Further, it has been reported that ATP has no effect on the Cl^- channel [15] whereas, as described below, the P_i transporter appears to be stimulated by ATP.

A non-specific anion channel has been reported in the inner mitochondrial membrane that transports Cl^-, P_i, HCO_3^-, malate and succinate, and is inhibited by Mg^{2+} [21]. It has also been reported that the anion transporter (Band 3) of red blood cells will transport P_i instead of HCO_3^-, although at a slower rate [22].

In other systems, uptake of P_i is an active process. In mitochondria, transport of P_i is linked to the movement of H^+ [16] and the transporter is related to the ADP/ATP transporter [17,18]. Na^+-linked P_i transport is common in mammalian cells and, for example, in brush-border membranes, two such transport systems have been identified; one a low-affinity (K_m 1.0 mM) high-capacity system and the other a high-affinity (K_m 0.1 mM) low-capacity system [19,20]. Carley and Racker [4] have reported that uptake of P_i by SR requires ATP, Ca^{2+} and Mg^{2+}. They also suggested that the formation of a Ca^{2+} gradient was not necessary for uptake, since they observed that addition of the Ca^{2+}-ionophore A23187 did not prevent uptake of P_i, although uptake was reduced under these conditions. Uptake of P_i was also shown not to be driven by a membrane potential, inside positive, since addition of valinomycin did not prevent uptake [5].

As shown in Fig. 1, Ca^{2+} uptake by SR mediated by the (Ca^{2+}-Mg^{2+})-ATPase can be driven by acetyl phosphate rather than the usual substrate, ATP. A second slow phase of uptake is seen following the initial fast phase of uptake in the presence of P_i, although the rate of this second phase is less than is observed in the presence of ATP as substrate (compare Fig. 1, Ref. 5). Similarly, a second phase of uptake is seen in the presence of oxalate with acetyl phosphate as substrate, but again the rate of uptake is markedly less than with ATP as substrate (data not shown). These differences cannot be attributed to an effect of ADP generated from ATP by the (Ca^{2+}-Mg^{2+})-ATPase, since addition of ADP to SR vesicles in the presence of acetyl phosphate and P_i leads to a reduction in the magnitude of the initial fast phase of uptake with no effect on the rate of the second phase (data not shown). These results suggest that the P_i transporter is stimulated by ATP. In the previous paper [5] it was shown that the presence of ATP could reduce the inhibitory effects of labelling SR with acetic anhydride or phenylglyoxal, suggesting the presence of a binding site for ATP on the transporter.

Beil et al. [26] and Carley and Racker [4] reported no active accumulation of P_i or oxalate in the absence of Ca^{2+}, making it unlikely that transport of P_i is due to a P_i-ATPase. Further, the 1 : 1 stoichiometry of uptake of Ca^{2+} and oxalate or P_i [2,5] makes any independent pumping of oxalate or P_i unlikely. Carley and Racker [4] reported uptake of P_i in the presence of A23187 which prevented the formation of a Ca^{2+} gradient across the SR membrane, and Tate et al. [10] observed that incubation of SR vesicles at alkaline pH led to a reduction in accumulation of Ca^{2+} with no effect on the uptake of oxalate. These experiments argue against a simple uniport mechanism for P_i movement across the membrane, driven by the precipitation of the corresponding Ca^{2+} salt within the SR. Carley and Racker [4] observed that addition of valinomycin to SR vesicles had no effect on uptake of P_i arguing against uptake being driven by a membrane potential. We have found that addition of the proton uncoupler FCCP (130 μM) to SR vesicles results in a slight increase in the rate of the slow phase of Ca^{2+} accumulation in the presence of P_i (data not shown) arguing against uptake of P_i being coupled to a H^+ gradient.

It is possible that uptake of P_i is by an antiport mechanism, coupling P_i uptake to efflux of some other anion. It has been established that the (Ca^{2+}-Mg^{2+})-ATPase is electrogenic [27], so that uptake of Ca^{2+} in SR vesicles must be accompanied by counterion movement, but the nature of the counterion has yet to be established. In this context, the observation of a high concentration of carbonic anhydrase within the SR lumen [28] is of interest. Fast diffusion of CO_2 across lipid bilayers combined with an anion HCO_3^- antiporter could give an efficient buffering system [28,29].

Transport of P_i by such an antiporter could then be possible, by analogy with the transport of P_i by the anion antiporter (Band 3) of red blood cells [22]. The importance of buffering of H^+ within the SR and a possible role for H^+ as the counterion in Ca^{2+} transport has been discussed in a recent paper [30].

It is also necessary to establish the ionic species of P_i and oxalate carried by the transporter. A potential complication here is that the rate of the slow phase of Ca^{2+} uptake seen in the presence of P_i could follow either from a slow rate of P_i transport across the membrane or from a slow rate of precipitation of $CaHPO_4$ within the SR. If transport were fast and precipitation slow, a two-phase uptake of P_i would be expected, the first phase, with a rate determined by the rate of transport, being fast and the second phase, determined by the rate of precipitation, being slow. As shown in the previous paper [5] this is not observed, and we conclude that the slow phase of Ca^{2+} uptake seen in the presence of P_i reflects a slow rate of transport. This is also consistent with the inhibitory effects seen with the phosphonocarboxylic acids, since it is hard to see how they could reduce the rate of P_i uptake if the rate of P_i uptake were determined by the rate of precipitation. We conclude that any observed effects of pH and Mg^{2+} on the rate of the slow phase of Ca^{2+} uptake will reflect changes in the rate of the P_i transporter.

In solutions containing P_i or oxalate and Mg^{2+}, a variety of species will be present, depending on pH (Table I). The increase in the rate of the P_i transporter with increasing Mg^{2+} concentration at pH 8.1 (Fig. 4) suggests that transport of HPO_4^{2-} is slower than of $MgHPO_4$ (see Table I). The faster rate of the P_i transporter at pH 5.3 than at pH 8.1 (Fig. 4) suggests that the rate of transport of $H_2PO_4^-$ is faster than HPO_4^{2-}. The effect of Mg^{2+} on the rate of transport at pH 5.3 (Table I) could follow from changes in the relative concentrations of MgATP and ATP (which varies significantly with Mg^{2+} concentration at pH 5.3 but not at pH 8.1); the concentration of $MgHPO_4$ is low at all Mg^{2+} concentrations at pH 5.3 (Table I).

The effect of Mg^{2+} on the uptake of oxalate is the opposite of that seen with P_i (Fig. 5), with increasing Mg^{2+} leading to a decrease in the rate of transport. These results suggest (see Table I) that $C_2O_4^{2-}$ is the predominant species carried, and that the Mg^{2+}-bound species (in which presumably the Mg^{2+} bridges the two O atoms) is a poor substrate. The slower rates seen at all Mg^{2+} concentrations at pH 7.2 than at pH 5.3 presumably reflects effects of protonation of residues on the transporter itself; as shown in Table I, there are no significant changes in the relative concentrations of oxalate species over this pH range. The chemical labelling studies reported in Ref. 5 suggest the presence of amine residues at the P_i binding site of the transporter, and it is likely that protonation of such residues at low pH will lead to increased anion binding. As shown in Fig. 6, Mg^{2+} also inhibits transport of pyrophosphate, consistent with poor uptake of a Mg^{2+}-bridged species.

Acknowledgment

We thank the SERC for financial support.

References

1 Hasselbach, W. and Makinose, M. (1961) Biochem. Z. 333, 518–528.
2 Martonosi, A. and Feretos, R. (1964) J. Biol. Chem. 239, 648–6,58.
3 Hasselbach, W. and Weber, H.H. (1974) in Membrane Proteins in Transport and Phosphorylation (Azzone, G.F., Klingenberg, M.E. and Siliprandi, N., eds.), pp. 103–111, North-Holland Publishing Company, Amsterdam.
4 Carley, W.W. and Racker, E. (1982) Biochim. Biophys. Acta 680, 187–193.
5 Stefanova, H.I., East, J.M. and Lee, A.G. (1990) Biochim. Biophys. Acta, 1064, 321–328.
6 Hasselbach, W. (1964) Prog. Biophys. Mol. Biol. 14, 169–222.
7 De Meis, L., Hasselbach, W. and Machado, R.D. (1974) J. Cell. Biol. 62, 50[illegible].
8 Sillen, L.G. a[illegible] Martell, A.E. (1971) Stability Constants of Metal-ion Complexes, Chemical Society, London.
9 McWhirter, J.M., Gould, G.W., East, J.M. and Lee, A.G. (1987) Biochem. J. 245, 731–738.
10 Tate, C.A., Chu, A., McMillin-Wood, J., Van Winkle, W.B. and Entman, M.L. (1981) J. Biol. Chem. 256, 2934–2939.
11 Martonosi, A.N. (1984) Physiol. Rev. 64, 1240–1320.
12 Komentani, T. and Kasai, M. (1978) J. Membr. Biol. 41, 295–308.
13 Campbell, K.P. and MacLennan, D.H. (1980) Ann. N.Y. Acad. Sci. 358, 328–331.
14 Tanifuji, M., Sokabe, M. and Kasai, M. (1987) J. Membr. Biol. 99, 103–111.
15 Rousseau, E., Roberson, M. and Meissner, G. (1988) Eur. Biophys. J. 16, 143–151.
16 LaNoue, K.F. and Schoolweeth, A.C. (1979) Annu. Rev. Biochem. 48, 871–922.
17 Aquila, H., Link, T.A. and Klingenberg, M. (1987) FEBS Lett. 212, 1–9.
18 Ferreira, G.C., Pratt, R.D. and Pedersen, P.C. (1989) J. Biol. Chem. 264, 15628–15633.
19 Bonjour, J.P. and Caverzasio, J. (1984) Rev. Physiol. Biochem. Pharm. 100, 161–214.
20 Tenenhouse, H.S., Klugerman, A.H. and Neal, J.L. (1989) Biochim. Biophys. Acta 984, 207–213.
21 Garlid, K.D. and Beavis, A.D. (1986) Biochim. Biophys. Acta 853, 187–204.
22 Labotka, R.J. and Omachi, A. (1987) J. Biol. Chem. 262, 305–311.
23 Coronado, R. and Miller, C. (1982) J. Gen. Physiol. 79, 529–547.
24 Meissner, G. (1975) Biochim. Biophys. Acta 389, 51–68.
25 Franciolini, F. and Petris, A. (1988) Arch. Biochem. Biophys. 261, 97–102.
26 Beil, F.U., Von Chak, D., Hasselbach, W. and Weber, H.H. (1977) Z. Naturforsch. (C) 32, 281–287.
27 Zimniak, P. and Racker, E. (1978) J. Biol. Chem. 253, 4631–4637.
28 Bruns, W., Dermietzel, R. and Gros, G. (1986) J. Physiol 371, 351–354.
29 Lea, T.J. and Ashley, C.C. (1981) J. Membr. Biol. 61, 115–125.
30 Levy, D., Seigneurel, M., Bluzat, A. and Rigaud, J.L. (1990) J. Biol. Chem. 265, 19524–19534.