Sarco/endoplasmic reticulum Ca^{2+}-ATPase isoforms: diverse responses to acidosis

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The effects of acidic pH on the kinetics of Ca^{2+}-ATPase isoforms from intracellular membranes of skeletal muscle, cardiac muscle, cerebellum and blood platelets were studied. At neutral pH, all four Ca^{2+}-ATPase isoforms exhibited similar Ca^{2+}-concentration requirements for half-maximal rates of Ca^{2+} uptake and ATP hydrolysis. A decrease in the pH from 7.0 to 6.0 promoted a decrease in both the apparent affinity for Ca^{2+} [increasing half-maximal activation ($K_{0.5}$)] and the maximal velocity (V_{max}) of Ca^{2+} uptake. With skeletal muscle vesicles these effects were 5 to 10 times smaller than those observed with all the other isoforms. Acidification of the medium from pH 7.0 to 6.5 caused the release of Ca^{2+} from loaded vesicles and a decrease in the amount of Ca^{2+} retained by the vesicles at the steady state. With the vesicles derived from skeletal muscle these effects were smaller than for vesicles derived from other tissues. The rate of passive Ca^{2+} efflux from skeletal and cardiac muscle vesicles, loaded with Ca^{2+} and diluted in a medium containing none of the ligands of Ca^{2+}-ATPase, was the same at pH 7.0 and 6.0. In contrast, the rate of Ca^{2+} efflux from cerebellar and platelet vesicles increased 2-fold after acidification of the medium. The effects of DMSO, Mg^{2+} with P_i and arsenate on the rate of Ca^{2+} efflux varied among the different preparations tested. The differences became more pronounced when the pH of the medium was decreased from 7.0 to 6.0. It is proposed that the kinetic differences among the Ca^{2+}-ATPase isoforms may reflect different adaptations to cellular acidosis, such as that which occurs during ischaemia.

INTRODUCTION

Calcium signalling is involved in the control of numerous physiological events, such as muscle contraction, secretory processes and platelet aggregation [1,2]. The Ca^{2+}-ATPase from sarco/endoplasmic reticulum (SERCA) is essential for the maintenance of a low cytosolic Ca^{2+} concentration. This enzyme pumps Ca^{2+} from the cytosol into the lumen of the reticulum, using the energy derived from ATP hydrolysis [3,4]. Three distinct genes encode SERCA isoforms, but the physiological meaning of isoform diversity is not clear. The SERCA 1 gene is expressed in fast skeletal muscle [5], whereas the SERCA 2 gene gives rise to SERCA 2a and SERCA 2b isoforms by alternative splicing [6,7]. The SERCA 2a isoform is expressed in cardiac and slow skeletal muscle, while SERCA 2b is ubiquitously expressed and is the dominant isoform found in the cerebellum [7–9]. SERCA 3 is expressed in non-muscular tissues, such as in blood platelets and lymphoid tissues [10–12]. In addition to SERCA 3, blood platelets express the SERCA 2b isoform [13], and it has been proposed that the two isoforms found in blood platelets are located in different Ca^{2+} pools of the cell [14].

Several studies demonstrate that a decrease in intracellular pH is accompanied by an increase in cytosolic Ca^{2+} concentration. This has been observed in different cell types, including muscles, neurons and blood platelets [15–19]. During cellular ischaemia and energy failure, the production of lactic acid promotes a decrease in the intracellular pH to values as low as 6.0 [20–23]. Intracellular Ca^{2+} overload often occurs during severe ischaemia and intracellular acidosis, and is a key event leading to cellular injury [16,24,25]. The occurrence of reversible cellular acidosis is very common in skeletal muscle. During muscle fatigue, the internal pH falls to 6.5–6.3, and the cells usually recover [16,19]. On the other hand, lactic acidosis has been proposed as one of the main factors involved in cellular death following cerebral ischaemia [26] and platelet storage [27,28]. Some neuronal subtypes, such as hippocampal CA1 neurons, are particularly susceptible to ischaemia [25]. Because intracellular Ca^{2+} often increases during acidosis, we postulated that the Ca^{2+}-ATPase isoforms from different tissues might exhibit distinct adaptations to acidosis, with the skeletal muscle isoform being more resistant to acidic pH than the other isoforms. Therefore, in this work, we compared the effects of pH on the kinetics of the Ca^{2+}-ATPase found in the intracellular membranes of skeletal muscle, cardiac muscle, cerebellum and human blood platelets.

MATERIALS AND METHODS

Vesicle preparation

The light fraction of skeletal muscle sarcoplasmic reticulum was prepared from rabbit fast skeletal muscle, as described by Eletr and Inesi [29]. Canine cardiac sarcoplasmic vesicles were prepared according to the method of Harigaya and Schwartz [30]. Cerebella of adult rats were dissected, and vesicles from the endoplasmic reticulum were prepared as described by Supattapone et al. [31]. Vesicles derived from the dense tubules of human blood platelets were prepared as described by Le Peuch et al. [32]. The vesicles were stored in liquid nitrogen until use. Protein concentration was estimated by the procedure of Lowry et al. [33]. The Ca^{2+}

Abbreviations used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

uptake was not due to contamination by membranes other than sarco/endoplasmic reticulum. In agreement with previous reports, thapsigargin (2 μM) inhibited a large fraction (70–85 %) of the Ca^{2+} uptake in cerebellum and abolished Ca^{2+} uptake in platelet, cardiac and skeletal muscle vesicles [34–36].

Ca^{2+} uptake

Ca^{2+} uptake was assayed at 35 °C in a medium containing 50 mM Mops, 5 mM $MgCl_2$, 3 mM ATP, 100 mM KCl, 0.2 mM EGTA and different concentrations of radioactive $^{45}CaCl_2$, to yield various free Ca^{2+} concentrations. Other additions are described in the legends of the relevant Figures and Tables. The concentrations of vesicles varied from 5 μg of protein/ml, in the case of skeletal muscle, to a maximum of 50 μg of protein/ml, in the case of vesicles derived from either cerebellum or platelets. The reaction was started by the addition of the vesicles and after 5 min (sarcoplasmic reticulum) or 20 min (cerebellum or platelets) it was arrested by filtration of 0.4 ml samples through Millipore filters (0.45 μm) [37]. The filters were flushed six times with 5 ml of 3 mM $La(NO_3)_3$, and the remaining radioactivity was counted in a scintillation counter. The free Ca^{2+} concentration was calculated using the association constants of Schwarzenbach et al. [38] in a computer program described by Fabiato and Fabiato [39] and modified by Sorenson et al. [40].

ATPase activity

Ca^{2+}-dependent ATPase activity from leaky vesicles (in the presence of 5 μM A23187) was determined with the use of [γ-^{32}P]ATP and measured both in the absence and in the presence of 1 mM EGTA [41].

The Ca^{2+}-stimulated ATPase activity was inhibited by thapsigargin (2 μM) in all vesicle preparations tested. Other transport ATPases, such as Na^+/K^+-ATPase, F_1-ATPase and calmodulin-dependent plasma membrane Ca^{2+}-ATPase, are not inhibited by thapsigargin [35,36].

Ca^{2+} efflux

For the Ca^{2+} efflux experiments, the vesicles were preloaded with Ca^{2+} in a medium containing 50 mM Mops/Tris (pH 7.0), 5 mM $MgCl_2$, 20 mM P_i, 0.02–0.3 mM $^{45}CaCl_2$, 3 mM ATP, 5 μM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and 0.05–0.1 mg of protein/ml. After 30–60 min incubation at 35 °C, the vesicles were centrifuged at 40000 ***g*** for 40 min, the supernatant was discarded and the walls of the tubes were blotted to minimize the volume of residual loading medium. The pellets were kept on ice and resuspended in ice-cold 0.2 M sucrose. The Ca^{2+}-loaded vesicles were quickly diluted into efflux media to a final concentration of 0.02–0.05 mg of protein/ml. The loaded vesicles were never left resuspended for more than 2 min before dilution into the efflux medium. The efflux was arrested as described above for Ca^{2+} uptake.

Phosphorylation of phospholamban

Assay medium and experimental procedures for phospholamban phosphorylation were as described by Tada et al. [42].

Materials

ATP, FCCP, arsenate, cAMP-dependent protein kinase and cAMP were obtained from Sigma (St. Louis, MO, U.S.A.). $^{45}CaCl_2$ was purchased from Du Pont–New England Nuclear (Wilmington, DE, U.S.A.). All other reagents were of analytical grade.

RESULTS AND DISCUSSION

Effect of acidic pH on Ca^{2+} transport

For the ATPase from skeletal muscle, previous reports have shown that the optimal pH for Ca^{2+} uptake is 7.0, and acidification of the medium promotes both a change in the equilibrium constant of different partial reactions of the catalytic cycle and a decrease in Ca^{2+} affinity [43–48]. At pH 7.0, the Ca^{2+} concentration required for half-maximal activation ($K_{0.5}$) of both Ca^{2+} uptake and ATPase activity was found to vary between 0.14 and 0.36 μM depending on the vesicle preparation used (Figures 1 and 2 and Table 1). These values are in the same range as those reported by other authors [49–51] for vesicles derived from skeletal muscle and for the different isoforms expressed in COS cells. As far as we know there are no data in the literature comparing the effect of acid pH values on the affinity for Ca^{2+} of the different SERCA isoforms. At pH 6.0 the Ca^{2+} affinity of the skeletal muscle isoform was higher than in the other isoforms by factors ranging from 3 to more than 11 (Table 1), while at pH 7.0 this difference varied between 1.1- and 2.6-fold. Notice in Figures 1 and 2 that at pH 6.0 the values of both Ca^{2+} transport and ATPase activity measured with platelets preparation were low, and saturation was not clearly achieved in the presence of 100 μM Ca^{2+}. Thus, for this particular condition, the values of both $K_{0.5}$ and V_{max} are higher than the values shown in Table 1. Except for platelet vesicles, the apparent cooperativity of Ca^{2+}-ATPase activity (n_H) was little affected by acidification of the medium (Table 1).

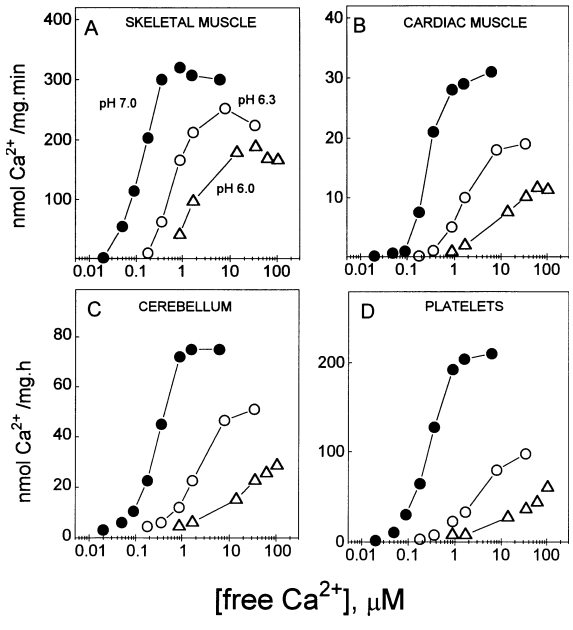

Figure 1 Ca^{2+}-dependence of Ca^{2+} uptake

The initial velocity of Ca^{2+} uptake in skeletal muscle (**A**), cardiac muscle (**B**), cerebellar (**C**) and platelet (**D**) vesicles was measured in media containing 50 mM Mops/Tris, 5 mM $MgCl_2$, 5 mM P_i, 3 mM ATP, 100 mM KCl, 5 μM FCCP and a mixture of $^{45}CaCl_2$ and EGTA, to yield various free Ca^{2+} concentrations at pH 7.0 (●), pH 6.3 (○) and pH 6.0 (△). The reaction was started by the addition of vesicles to a final concentration of 5 μg (**A**), 20 μg (**B**) or 50 μg (**C** and **D**) and stopped after (**A**) 2 min, (**B**) 5 min and (**C** and **D**) 15 min. The values are representative of three experiments with two different preparations.

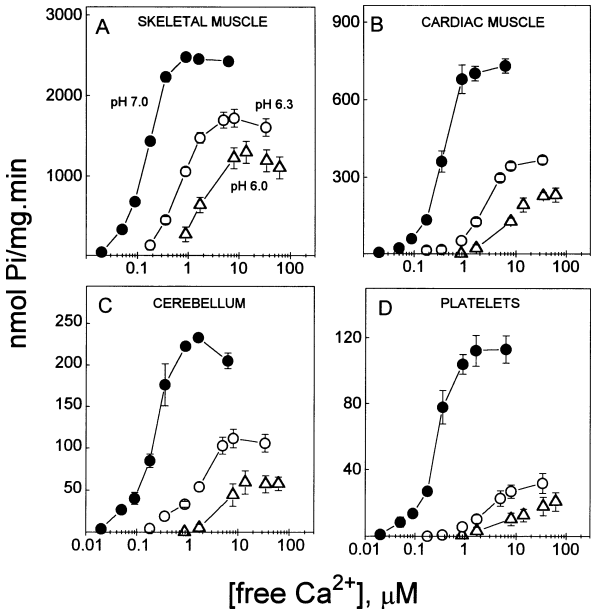

Figure 2 Ca^{2+}-dependence of Ca^{2+}-ATPase activity

The initial velocity of the Ca^{2+}-dependent ATPase activity was measured in a medium containing 50 mM Mops/Tris, 5 mM $MgCl_2$, 5 mM P_i, 3 mM [γ-^{32}P]ATP, 100 mM KCl, 100 μM ouabain, 10 mM NaN_3, 5 μM A23187 and a mixture of $CaCl_2$ and EGTA, to yield various free Ca^{2+} concentrations at pH 7.0 (●), pH 6.3 (○) and pH 6.0 (△). Other conditions were as in Figure 1. The values are means ± S.E.M. of three to five experiments with three different preparations. The lack of error bars indicates that the S.E.M. is smaller than the symbols.

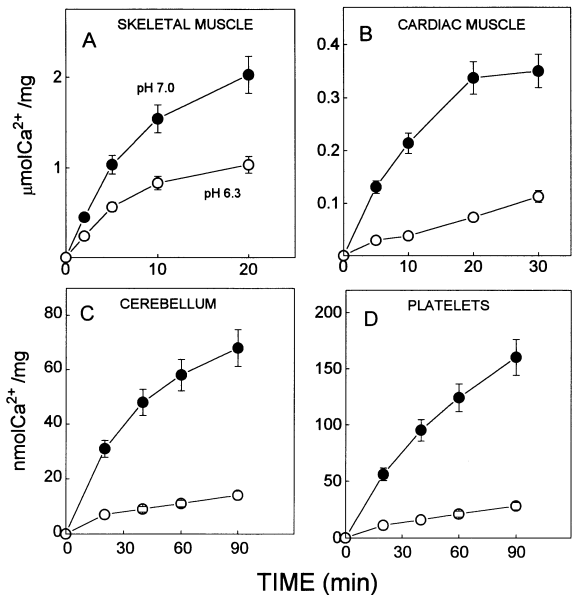

Figure 3 Time-course of Ca^{2+} uptake at 1 μM free Ca^{2+} concentration

The Ca^{2+} uptake of skeletal muscle (**A**), cardiac muscle (**B**), cerebellar (**C**) and platelet (**D**) vesicles was measured using a medium containing 50 mM Mops/Tris, 5 mM $MgCl_2$, 5 mM P_i, 3 mM ATP, 100 mM KCl, 5 μM FCCP and a mixture of [^{45}Ca]$CaCl_2$ and EGTA, to yield 1 μM free Ca^{2+} concentration at pH 7.0 (●) or at pH 6.3 (○), with 5 μg (**A**), 20 μg (**B**) or 50 μg (**C** and **D**) of vesicle protein/ml. The values are means ± S.E.M. of three experiments with three different preparations. The lack of error bars indicates that the S.E.M. is smaller than the symbols.

Table 1 Kinetic parameters of the Ca^{2+}-ATPase at different pH values

The values of $K_{0.5}$ (μM), V_{max} (nmol $P_i \cdot mg^{-1} \cdot min^{-1}$) and n_H were obtained from experiments like those shown in Figure 2. $K_{0.5}$ and n_H were calculated from Hill plots (log $V/[V_{max} - V]$ versus log [free Ca^{2+}]). The values are means ± S.E.M. of three to five experiments with three different preparations.

	Skeletal muscle	Cardiac muscle	Cerebellum	Platelets
pH 7.0				
$K_{0.5}$	0.14 ± 0.02	0.36 ± 0.01	0.15 ± 0.02	0.25 ± 0.05
V_{max}	2650 ± 150	734 ± 200	234 ± 3	114 ± 9
n_H	1.86 ± 0.11	1.80 ± 0.16	1.99 ± 0.24	1.7 ± 0.13
pH 6.3				
$K_{0.5}$	0.72 ± 0.2	2.70 ± 0.37	1.70 ± 0.22	4.70 ± 1.20
V_{max}	1700 ± 100	341 ± 74	112 ± 14	39 ± 4
n_H	1.72 ± 0.13	1.75 ± 0.18	1.4 ± 0.15	1.08 ± 0.05
pH 6.0				
$K_{0.5}$	2.20 ± 0.37	7.30 ± 1.11	5.71 ± 1.62	> 20
V_{max}	1300 ± 140	221 ± 67	63 ± 10	> 20
n_H	1.60 ± 0.04	1.75 ± 0.20	1.87 ± 0.07	–

In addition to its effect on Ca^{2+} affinity, lowering the pH also caused a significant decrease in the V_{max} for Ca^{2+} uptake and ATP hydrolysis. In skeletal muscle there was a 1.3-fold decrease in V_{max} when the pH was lowered from 7.0 to 6.3; in blood platelets, this decrease was 2.9-fold (Table 1 and Figures 1 and 2).

The effect of acidification in cardiac muscle vesicles does not seem to be related to interaction of the Ca^{2+}-ATPase with the regulatory protein phospholamban. Inactivation of this regulatory protein by phosphorylation did not modify the change in V_{max} observed in Figure 2 after acidification (results not shown).

During muscle activity, the free Ca^{2+} concentration in the cytosol increases from 0.1 μM (resting cells) to 1.0 μM (contracting cells) [19]. In the presence of 1 μM free Ca^{2+} concentration, both the initial velocity and the steady-state level of Ca^{2+} uptake were inhibited at acidic pH, but this inhibition was less pronounced in skeletal muscle vesicles (Figure 3). Acidification of the medium from pH 7.0 to 6.5 promoted release of Ca^{2+} and a decrease in the amount of Ca^{2+} retained by the vesicles at steady state (Figure 4). The relative amount of Ca^{2+} released by the vesicles derived from skeletal muscle was smaller than that released by vesicles derived from other tissues (compare Figures 4A and 4B). These results are compatible with those published by Fabiato and Fabiato [52] who observed a decrease in the rate of Ca^{2+} loading in skinned muscle fibres at acid pH values, which was more apparent in cardiac (SERCA 2a) than in skeletal (SERCA 1) muscle. Mandel et al. [53] reported that the rate of phosphorylation of the Ca^{2+}-ATPase decreases at acid pH, and that this effect was more pronounced in cardiac than in skeletal muscle vesicles.

Role of P_i concentration

The values reported for P_i concentration in skeletal muscle under resting conditions are quite variable, ranging from 0.6 to 8 mM. This variability is related to the technical limitations of the methods available for these measurements (for review, see [16]). The intracellular P_i concentration increases to values as high as

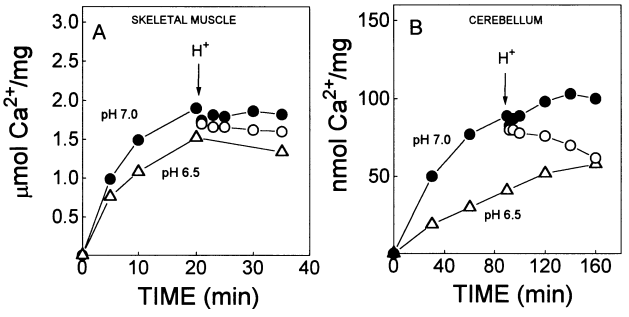

Figure 4 Acidification-induced Ca^{2+} efflux from skeletal muscle (A) and cerebellar (B) vesicles

Ca^{2+} uptake was measured in a medium with the same composition as described in the legend to Figure 1, either at pH 7.0 (●) or 6.5 (△). After 20 min (**A**) or 90 min (**B**) (arrow), the vesicles loaded at pH 7.0 were diluted in a medium of the same composition, but buffered at a lower pH to yield a final pH of 6.5, and several aliquots were filtered at different incubation intervals (○). The controls were also diluted in a medium at pH 7.0. The free Ca^{2+} concentration was maintained fixed at 1 μM by means of a Ca^{2+}/EGTA buffer. The values are representative of three different experiments performed with two different vesicle preparations.

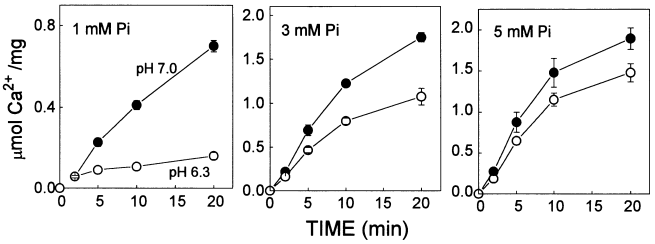

Figure 5 P_i attenuates the inhibition of Ca^{2+} uptake in acidic pH by skeletal muscle vesicles

Ca^{2+} uptake was measured at 35 °C in a medium containing 50 mM Mops/Tris, 5 mM $MgCl_2$, 3 mM ATP, 100 mM KCl, 5 μM FCCP and a mixture of $^{45}CaCl_2$ and EGTA, to yield 30 μM free Ca^{2+} concentration and 10 μg/ml skeletal muscle vesicle protein at various P_i concentrations, either at pH 7.0 (●) or pH 6.3 (○). The values are means ± S.E.M. of four experiments with two different preparations. The lack of error bars indicates that the S.E.M. is smaller than the symbols.

20 mM during the acidosis that occurs in fatigued muscle [16,54]. P_i stimulates Ca^{2+} uptake in sarcoplasmic reticulum vesicles by different mechanisms. One is related to the complexation of P_i with Ca^{2+} inside the vesicles, leading to a decrease in the free Ca^{2+} concentration in the vesicles lumen, which reduces back-inhibition of the Ca^{2+}-ATPase [3,4]. A second mechanism involves phosphorylation of the enzyme by P_i, which in turn inhibits passive Ca^{2+} efflux through the Ca^{2+}-ATPase [55]. The inhibition of Ca^{2+} transport by acid pH varied depending on the P_i concentration in the medium (Figure 5). An increase in P_i from 1 to 5 mM attenuated the inhibition of Ca^{2+} uptake by acid pH in the skeletal muscle vesicles (Figure 5) and in all other vesicle preparations tested (results not shown). At present we cannot rule out an indirect effect of pH acting upon the proportions of the various ionized forms of P_i and on their difusion across the membrane vesicles. However, regardless of the mechanism, the data in Figure 5 suggest that an increase in P_i concentration in the cells may prevent the inhibition of Ca^{2+} transport observed during acidosis.

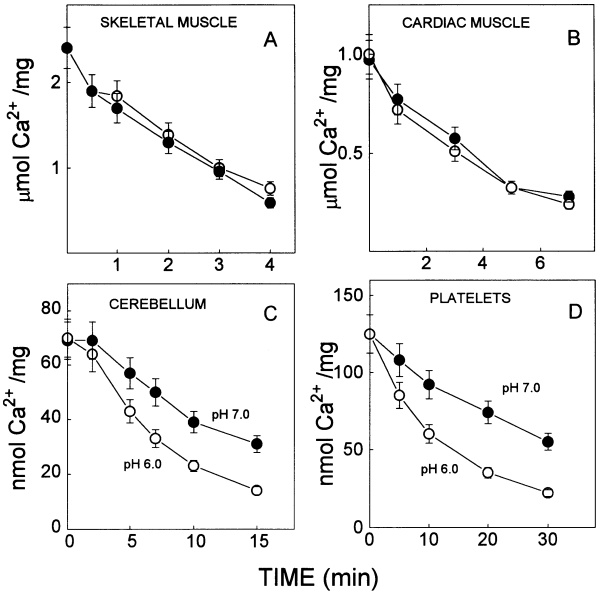

Figure 6 Time course of Ca^{2+} efflux from skeletal muscle (A), cardiac muscle (B), cerebellar (C) and platelet (D) vesicles

Microsomal vesicles were actively loaded with $^{45}CaCl_2$ using 20 mM P_i as a Ca^{2+} precipitating agent as described in the Materials and methods section. The loaded vesicles were diluted to a final concentration of 50 μg/ml in medium containing 50 mM Mops/Tris/5 mM EGTA either at pH 7.0 (●) or pH 6.0 (○). The Figure shows the Ca^{2+} remaining in the vesicles after different incubation intervals. The values are means ± S.E.M. of three to five experiments with three different vesicle preparations.

pH-dependence of Ca^{2+} efflux

It has been shown in previous reports that Ca^{2+} leaks through the skeletal muscle Ca^{2+}-ATPase when vesicles previously loaded with Ca^{2+} are incubated in media containing none of the ligands of the ATPase [55,56]. This efflux is not coupled to the synthesis of ATP and is referred to as passive Ca^{2+} efflux. The rate of passive Ca^{2+} efflux is decreased when thapsigargin or one of the ligands of the pump, such as Ca^{2+}, Mg^{2+} or K^+, is added to the efflux medium [55–57]. The effectiveness of cations in blocking the passive efflux of Ca^{2+} from skeletal muscle vesicles is decreased at acidic pH [48]. Agents that increase the phosphorylation of the Ca^{2+}-ATPase by P_i, such as high P_i concentration and DMSO, also decrease passive Ca^{2+} efflux [55].

The rate of passive Ca^{2+} efflux at pH 7.0 varied in the different vesicle preparations tested, being highest in the preparation from skeletal muscle (Figure 6). This difference is probably related to differences in the number of Ca^{2+}-ATPase units present in each vesicle preparation, and not to different amounts of Ca^{2+} load in the lumen of the vesicle. During Ca^{2+} loading, P_i was used as a Ca^{2+} precipitating agent. Thus, the concentration of free Ca^{2+} inside the different vesicle preparations is determined by the solubility product of calcium phosphate, and was the same in all vesicle preparations regardless of the total amount of Ca^{2+} accumulated [58].

The rate of Ca^{2+} efflux from skeletal and cardiac muscle vesicles did not vary when the pH was decreased from 7.0 to 6.0 (Figure 6A, B; Table 2). In contrast, a significant increase in Ca^{2+} efflux rate was measured at acid pH in cerebellar and platelet vesicles (Figures 6C and 6D). Thus, an increase in the rate of Ca^{2+} efflux may contribute to a decrease in the amount of Ca^{2+}

Table 2 Effects of DMSO, P_i and arsenate on Ca^{2+} efflux from different vesicle preparations

The vesicles were loaded with Ca^{2+} as described in the Materials and methods section. The efflux media contained 50 mM Mops/Tris (pH 7.0 or 6.0) and 5 mM EGTA (control), plus either 20% (v/v) DMSO, or 4 mM $MgCl_2$ and 4 mM P_i, or 4 mM $MgCl_2$ and 4 mM sodium arsenate. Ca^{2+} efflux rate was calculated from experiments like those shown in Figure 6. The percentage relative to the control efflux rate is shown in parentheses beside each value. The values are means ± S.E.M. of three or four experiments with two different vesicle preparations. N.D., not determined.

	Skeletal muscle		Cardiac muscle		Cerebellum		Platelets	
	pH 7.0	pH 6.0	pH 7.0	pH 6.0	pH 7.0	pH 6.0	pH 7.0	pH 6.0
Control	450 ± 40 (100%)	440 ± 36 (100%)	140 ± 9 (100%)	135 ± 12 (100%)	3.0 ± 0.2 (100%)	4.5 ± 0.3 (100%)	2.5 ± 0.2 (100%)	4.8 ± 0.3 (100%)
DMSO 20% (v/v)	185 ± 19 (41%)	283 ± 19 (64%)	67 ± 6 (48%)	148 ± 9 (110%)	2.1 ± 0.1 (70%)	4.2 ± 0.1 (94%)	1.9 ± 0.1 (77%)	4.9 ± 0.1 (102%)
4 mM Mg^{2+} plus 4 mM P_i	225 ± 19 (50%)	271 ± 33 (62%)	N.D.	58 ± 10 (43%)	N.D.	2.2 ± 0.6 (49%)	N.D.	3.6 ± 0.2 (75%)
4 mM Mg^{2+} plus 4 mM arsenate	662 ± 40 (150%)	620 ± 32 (140%)	184 ± 11 (131%)	144 ± 10 (107%)	3.7 ± 0.3 (123%)	3.8 ± 0.3 (84%)	2.8 ± 0.1 (110%)	4.3 ± 0.5 (90%)

accumulated by cerebellar and platelet vesicles at acid pH values. The ryanodine Ca^{2+} channel is completely closed at pH $\leqslant$ 6.5 [59–62]. Similarly, the opening of the inositol trisphosphate Ca^{2+} channel is also inhibited at acid pH values [63]. Therefore, the possibility remains that the Ca^{2+} pump may be a pathway for Ca^{2+} release during cellular acidosis such as that which occurs following cellular ischaemia.

In agreement with a previous report [55], the Ca^{2+} efflux rate, measured at pH 7.0, from skeletal muscle vesicles decreased after the addition of either DMSO or Mg^{2+} plus P_i (Table 2). The decrease in Ca^{2+} efflux was not a common feature of all the SERCA isoforms (Table 2). The Ca^{2+} efflux from cardiac muscle and cerebellar vesicles was less sensitive to DMSO, particularly at pH 6.0, but remained sensitive to Mg^{2+} plus P_i (Table 2). On the other hand, the rate of Ca^{2+} efflux from platelet vesicles was practically unaffected by either DMSO or Mg^{2+} plus P_i at all pH values tested (Table 2).

It has previously been reported that a large increase in the passive efflux of Ca^{2+} is promoted by several drugs that inhibit the phosphorylation of Ca^{2+}-ATPase by P_i, such as arsenate [64–66], phenothiazines [67], local anaesthetics [68], fatty acids [69], heparin [70] and ethanol [71]. In these studies the effect of arsenate was measured only at pH 7.0, and it was found to vary depending on the Ca^{2+}-ATPase isoform studied, being less pronounced in platelet vesicles than in skeletal muscle [14]. We have now shown that 4 mM arsenate stimulated Ca^{2+} efflux from skeletal muscle vesicles at both pH 6.0 and pH 7.0 (Table 2). However, the small increase in Ca^{2+} efflux (10–30%) promoted by arsenate in cardiac muscle, cerebellar and platelet vesicles was abolished when the pH of the medium was lowered from 7.0 to 6.0 (Table 2).

During catalysis, Ca^{2+}-ATPase cycles between two different conformations, defined in the literature as E_1 and E_2 [3]. Agents such as P_i, arsenate, vanadate and trifluoperazine, which interact with the E_2 conformation of the skeletal muscle Ca^{2+}-ATPase, have different effects, depending on the isoform studied [14,50,72]. Because pH modifies the equilibrium between the E_1 and E_2 conformations of SERCA 1 [46], we raise the possibility that the differences observed among the isoforms after acidification are due either to differences in E_2 conformation or to a change in the E_1–E_2 equilibrium. Lytton et al. [50] proposed a different E_1–E_2 equilibrium for SERCA 3 when compared with SERCA 1 and SERCA 2, and suggested that these isoforms differ in their sensitivity to Ca^{2+} and vanadate for this reason. The same reasoning may apply to the differences observed in this report.

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