Mechanisms of Ageing and Development, 49 (1989) 105—117
Elsevier Scientific Publishers Ireland Ltd.

A COMPARATIVE STUDY OF THE Ca^{2+}-Mg^{2+} DEPENDENT ATPASE FROM SKELETAL MUSCLES OF YOUNG, ADULT AND OLD RATS

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(Received September 5th, 1988)

SUMMARY

Sarcoplasmic reticulum (SR) vesicles isolated from skeletal muscle of Sprague—Dawley rats ranging in age from 4 months to 28 months were studied and compared. A marked decline, with age, was observed in the amount of (total) SR proteins isolated per gram of muscle tissue used. This decline is in line with the known loss of muscle fiber mass and size with advancing age; however, whether the magnitudes of these two effects are indeed identical, remains to be studied. In contrast, no analogous age-related change was detected in the amount of SR protein per unit mass of rat cardiac muscle. The calcium contents, per mg protein, in SR vesicles isolated from rats of all age groups studied did not differ significantly, and represented only a small fraction of the total capacity of the vesicles for this cation. This capacity was found to decline at old age and this effect, combined with the age-related decrease in the concentration of SR proteins in the tissue, indicate a significant decline in calcium sequestration ability in old muscle. Both basal (Ca^{2+} independent) and calcium stimulated ATPase activities were found not to be affected by age. In contrast, the efficiency of Ca^{2+} transport across the SR membrane, as reflected by the number of calcium ions pumped into the vesicles per ATP cleaved, declined from a value of 0.37 at 3—4 months to 0.15 at 24 months. This change may represent an age-related reduction in the fraction of coupled SR vesicles, possibly due to alterations in the membrane. SR vesicle preparations from both young and old rats displayed strongly biphasic inactivation kinetics when incubated at 37°C. This may reflect the heterogeneity of muscles in the tissue used, or be due to the presence of a mixture of coupled and uncoupled vesicles in the SR preparations. The rate of the first step in the ATPase inactivation, in which about 75% of the activity is lost, was found to be affected by age, the old SR vesicles being markedly more labile than their young counterparts. In contrast, no difference was detected between

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0047-6374/89/$03.50
Printed and Published in Ireland

the inactivation kinetics of young and old ATPase proteins dissolved in Triton X-100 and the inactivation was monophasic down to less than 6% of the original activity. These results indicate that the age-related modifications in the stability of the SR calcium pump system involve the membrane but not the ATPase protein. The inactivation of the SR ATPase is believed to proceed via dissociation of the dimeric enzyme to (unstable) subunits. It is therefore likely that changes in the SR membrane in old muscle render the ATPase more dissociable.

Key words: Rat muscle; Calcium pump; Sarcoplasmic reticulum; Enzyme aging

INTRODUCTION

The Ca^{2+} and Mg^{2+} dependent sarcoplasmic reticulum (SR) ATPase plays a major role in promoting muscle relaxation by sequestering Ca^{2+} from the cytoplasm [1—5]. This enzyme has been shown to be a dimer consisting of two identical subunits of about 115 000 Mol. wt, and is the major protein constituent of the SR membrane [6,7]. Vesicular SR membrane fragments, isolated by differential centrifugation of muscle homogenates, maintain the ability to pump calcium, at the expense of ATP, from the medium into the intravesicular lumen. Such vesicles are therefore a convenient model system and have, indeed found extensive use in studies of the SR calcium pump [1—7]. Several studies have revealed decreased rates of Ca^{2+} uptake by SR preparations derived from cardiac tissue of old rats as compared with the rates displayed by preparations from young hearts [8—12]. In a recent study by Narayanan [10] it was found that while cardiac SR showed an age-related decline in Ca^{2+} pumping rate (but not in Ca^{2+} stimulated ATPase activity) the Ca^{2+} pumping activity of the sarcolemma was significantly increased at old age. The reduction in Ca^{2+} sequestration ability by the SR in old cardiac tissue was suggested to be a major factor in the observed prolongation of cardiac relaxation duration with aging [8,11].

An important functional property of the SR ATPase is the tight coupling of ATP hydrolysis to calcium transport, the efficiency of pumping being reflected by the number of calcium ions transported per ATP cleaved [13—15]. Under optimal conditions SR vesicles prepared from rabbit skeletal muscle display a Ca/ATP ratio of 2. However, we have recently shown that this ratio may be modulated by external conditions and significantly reduced under conditions of "energy stress" that exist when Ca^{2+} concentrations in the medium become very low [16].

It is well established that the fidelity of the SR membrane is of utmost importance to the pump's performance and that changes in the membrane composition may have dramatic effects on the ATPase activity and calcium pumping rate as well as on the pumping efficiency [17,18]. Changes, with age, in the membrane component, as well as the ATPase protein may thus potentially affect the performance of the system

In the present study several functional aspects of the SR ATPase from skeletal

muscle of young and old rats were studied and compared. A large decrease, with age, in the quantity of SR protein per gram of wet muscle was found, a decrease not detected in SR preparations from cardiac muscle. This, combined with our finding that a significant reduction occurs in the capacity of old SR vesicles to accumulate calcium ions, may markedly impair Ca^{2+} sequestration in old muscle. We also report here that the origin of these aging effects appears to be mostly in the SR membrane as some of the differences between young and old SR vesicles disappear upon solvation of the ATPase in a non-ionic detergent.

MATERIALS AND METHODS

Materials

ATP, Hepes, 3-(*N*-Morpholino) propane sulfonic acid (Mops), oxalic acid, calcium acetate and polyvinylpyrrolidone were purchased from Sigma Chemical Co. Ammonium molybdate was obtained from MCB Manufacturing Chemists, Inc. Hydroxylamine hydrochloride was supplied by Fisher Scientific Co.

Animals

Sprague—Dawley rats of the following age groups were used in the preparation of skeletal muscle SR: 4 months (young); 10—12 months (adult); 17 months (aging) and 28 months (old). Cardiac muscle SR were prepared from rats of 2 months, 4 months, 12—13 months, 22 months and 28—29 months. The animals were killed by decapitation, and the heart and skeletal muscles from the hind legs were removed for the preparation of SR vesicles.

Isolation of SR membrane fragments

SR vesicles were isolated from rat cardiac and skeletal muscle by differential centrifugation as described by Champeil et al. [19]. The vesicles were suspended by homogenization in a medium containing 0.3 M sucrose, 100 mM KCl, 10 mM Hepes (pH 7.4) and stored at $-70°C$.

Protein and ATPase activity assays

The total protein concentration of the SR vesicles was determined according to the method of Lowry et al. [20] following solvation of the membrane proteins by the addition of sodium dodecyl sulfate (from a 10% stock solution) to a final concentration of 1%. Bovine serum albumin in the presence of 1% sodium dodecyl sulfate served as the standard.

ATPase activity was determined at 25 °C in a medium containing 100 mM KCl, 5mM $MgCl_2$, 5 mM potassium oxalate, 2 mM ATP, varying concentrations of calcium chloride (in the range of 0—100 μM) in 20 mM Mops buffer, pH 7.0. Aliquots (500 μl) were taken out at various times and the reaction quenched with 200μl of cold 10% trichloroacetic acid. The amounts of ATP hydrolyzed were

determined spectrophotometrically from the inorganic phosphate content in the aliquot by the method of Ohnishi and Gall [21] using calibrated Na_2HPO_4 solutions as the standard.

The calcium content of the vesicles was measured by atomic absorption spectrophotometry (Instrumentation Laboratory Inc.) using a hollow cathode calcium lamp (model 62510) and a wavelength of 422.7 nm. Calibration curves were obtained by adding 1-mM aliquots of calcium acetate solution in distilled water.

ATPase inactivation experiments

Aliquots (100 μl) of SR vesicles prepared from young or old rats were freed from sucrose by centrifugation through a column packed with 3 ml of Sephadex G-50 [22] and diluted to the desired concentration in a medium containing 20 mM Mops, 5 mM $MgCl_2$, 0.4 mM $CaCl_2$, pH 7.0. The intact vesicles, as well as samples of ATPase solubilized by adding Triton X-100 (from a 10% stock solution) to a final concentration of 1%, were incubated at the desired temperature. Aliquots were taken out at various times and their ATPase activity assayed.

Calcium capacity determination

This was based on the well established stimulation of the ATPase activity of SR in the presence of calcium, and on our previous observation [16] that when the amount of calcium added to SR suspensions does not exceed the capacity of the vesicles to accumulate this ion, then the fast, Ca^{2+} dependent, rate of ATP hydrolysis subsides back to the slower basal (Ca^{2+} independent) level once all the medium calcium has been transported into the vesicles. The plot of ATP hydrolyzed *vs.* time then assumes a typical S shape as shown in Fig. 1 for the lower concentrations of added calcium. However, when the amount of calcium in the medium does exceed the capacity of the vesicles then the concentration of free Ca^{2+} in the medium remains high enough to drive the ATPase activity and the rate of ATP hydrolysis is not reduced. By adding increasing concentrations of calcium to a fixed amount of SR vesicles and determining the level above which the high rate of ATPase activity is maintained indefinitely, one can evaluate the vesicles capacity for calcium. This method was used here to compare these capacities in SR preparations from young and old rats.

RESULTS

Table 1 depicts the protein and calcium concentrations in SR vesicles isolated from skeletal muscles of Sprague—Dawley rats ranging in age from 2 to 28 months, showing a striking decline, with age, in the quantity of SR protein per gram of wet tissue. In contrast, no change with age was found in the amount of protein in SR vesicles isolated from cardiac tissue, the level of total protein being 3.0 ± 0.5 mg/g wet tissue for all age groups. The calcium contents of SR vesicles as isolated from

TABLE I

PROTEIN AND CALCIUM CONCENTRATIONS IN RAT MUSCLE SR PREPARATIONS

Age of rats (Months)	*Protein concentration[a] (mg/g Tissue)*	*Calcium concentration[b] (nmol/mg Protein)*
3—4	0.8	35 ± 5[c]
10—12	0.5	36 ± 6[d]
17	0.2	36 ± 9[d]
24		44 ± 3[d]
28	0.1	36 ± 4[d]

[a]Total protein concentration in SR preparations determined by the Lowry assay method.
[b]Determined by atomic absorption spectrometry as described in the text.
[c]Mean ± S.E.M. (n = 4).
[d]Mean ± S.E.M. (n = 2).

skeletal muscles of animals of the various age groups tested were found to be similar and are also presented in Table I. It should be noted that these concentrations of calcium represent only a small fraction of the capacity of the SR vesicles for this ion.

The kinetics of ATP hydrolysis by SR vesicles from young and old rats are depicted in Fig. 1. The stimulation of ATPase activity by calcium is revealed in the two preparations by the marked increase in the rate of ATP hydrolysis upon the addition of this cation. Thus, the rates change from the basal values of 500 nmol/min per mg (young) and 140 nmol/mg per min (old) to 1430 nmol/min per mg and 1100 nmol/mg per min, respectively, in the presence of Ca^{2+}. When the rates in the presence of Ca^{2+} are corrected for the contribution from basal ATPase activities by subtracting the respective values of the latter, the Ca stimulated ATPase activities of the young and old SR vesicles are seen to be practically identical (930 nmol/min per mg and 960 nmol/min per mg for young and old vesicles, respectively). An analogous similarity between the ATPase activities of SR vesicles from young and old rat heart was reported by Narayanan [9]; however the degree of stimulation of the activity by Ca^{2+} in the cardiac muscle was several fold lower than that found by us for the skeletal muscle SR. Also, the basal activity in the cardiac system was significantly higher.

In contrast to the age-invariant ATPase activity, the capacity of the vesicles for pumping calcium ion was found to be modified with age. When increasing concentrations of calcium were added to a fixed amount of vesicle preparations the old vesicles became saturated with calcium at the range of 400—600 nmol/mg SR protein while young vesicles were able to accumulate this cation to levels in the range of 600—1000 nmol/mg SR protein. As noted earlier, this is not due to different contents of calcium inside the vesicles as isolated from young and old animals since

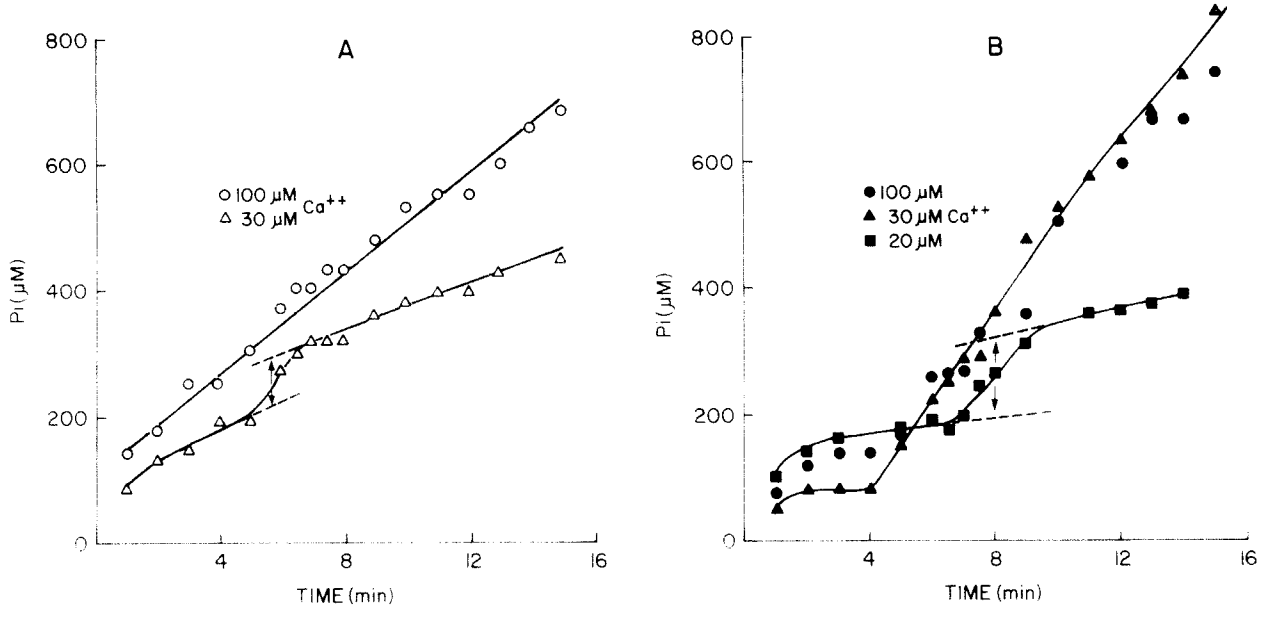

Fig. 1. Time course of ATP hydrolysis by the calcium dependent SR ATPase from rat skeletal muscle. SR vesicles suspension (10 ml, 50 μg of protein per ml) were incubated at 25 °C in a medium containing 100 mM KCl, 5 mM $MgCl_2$, 5 mM potassium oxalate, 2 mM ATP and 20 mM Mops (pH 7.0). Aliquots (500 μl) of the sample were taken at various times, the reaction quenched by acid, and their inorganic phosphate contents were determined as described in the experimental section. Calcium chloride was added into the SR suspension from 10 mM or 100 mM stock solutions to give a final concentration of 20 μM (■) 30 μM (△, ▲) and 100 μM (○, ●). Hollow symbols represent the data derived from SR preparations isolated from 3- to 4-month-old rats (A), while solid symbols are used to describe the data obtained with SR vesicles from 24-month-old rats (B). The broken lines are extrapolations of the ATP hydrolysis before Ca^{++} addition (basal activity) and following its full sequestration. The arrows indicate the net increase in inorganic phosphate (from ATP hydrolysis) during the burst of Ca^{++} stimulated ATPase activity.

these contents were found to be identical and not significant compared to the amounts of calcium used in the experiments described here.

As discussed in the experimental section the rate of ATP hydrolysis by SR vesicles declines back to the basal level once all the medium calcium has been sequestered. This pattern is indeed followed by both young and old SR preparations at low concentrations of Ca^{2+} (Fig. 1). The amount of ATP expended to drive the pumping of a given amount of Ca^{2+} can be evaluated from the amplitude of the increase in inorganic phosphate concentration in the medium following the addition of Ca^{2+} (these amplitudes are indicated by the arrows in Fig. 1 A, B). The Ca^{2+} transport stoichiometry (i.e. the number of calcium ions transported per ATP cleaved) was calculated from these amplitudes to be 0.37 and 0.15 for SR preparations from young and old rats, respectively. While these Ca/ATP ratios are very close to the values reported by Narayanan [10] for rat cardiac SR (0.34 and 0.18, respectively for vesicles from 6- and 24-month-old rats) they are considerably smaller than the values of 1.5—1.8 found for rabbit muscle SR under similar conditions [16,23]. The 58% decline in pumping stoichiometry of skeletal muscle SR between the ages of 4 and 28 months reported here, is remarkable, and deviates from the results of Bertrand et al. [24] who reported a slight increase, with age, in the Ca/ATP ratio in Fischer rat SR. Possible explanations for this discrepancy in the values of transport efficiency of the pump will be given in the Discussion.

The upper panel in Fig. 2 presents the results of inactivation of the SR ATPase upon incubation of intact vesicles at 37 °C. Under these conditions the inactivation was found to be biphasic — both young and old vesicles lost 75% of their ATPase activity by a first order mechanism whereas the remaining activity was resistent to prolonged (i.e. 22 h) incubation. This behavior may reflect an age-independent heterogeneity of vesicles, or of the ATPase protein itself. The rate of the initial inactivation step is strongly affected by age, the young vesicles showing markedly higher heat stability than their old counterparts. Thus, the inactivation rate constants calculated from our data are $(2.4 \pm 0.9) \times 10^{-3}$ min^{-1} for young and $(7.5 \pm 2.7) \times 10^{-3}$ min^{-1} for old SR preparations.

The kinetics of heat inactivation of the SR ATPase dissolved in Triton X-100 are presented in the lower panel of Fig. 2. In contrast to the results described above the dissolved ATPase shows no age-related change in its inactivation rate. Thus, the rate constants obtained from our data at 37 °C are $(14.8 \pm 2.4) \times 10^{-3}$ min^{-1} for the young enzyme and $(14 \pm 1.6) \times 10^{-3}$ min^{-1} for the old ATPase, while at 30 °C the respective rates are $(5.6 \pm 0.6) \times 10^{-3}$ min^{-1} and $(4.9 \pm 0.5) \times 10^{-3}$ min^{-1}. The inactivation at both temperatures follows first order kinetics (at 37 °C the reaction was followed to below 6% of the original activity) and shows no heterogeneity. It is therefore indicated by these data that the biphasic kinetics as observed in heat inactivation of the ATPase in intact vesicles reflects heterogeneity in vesicles due to differences in the membranes (or in membrane enzyme interactions) but not changes, with aging, in the enzyme itself.

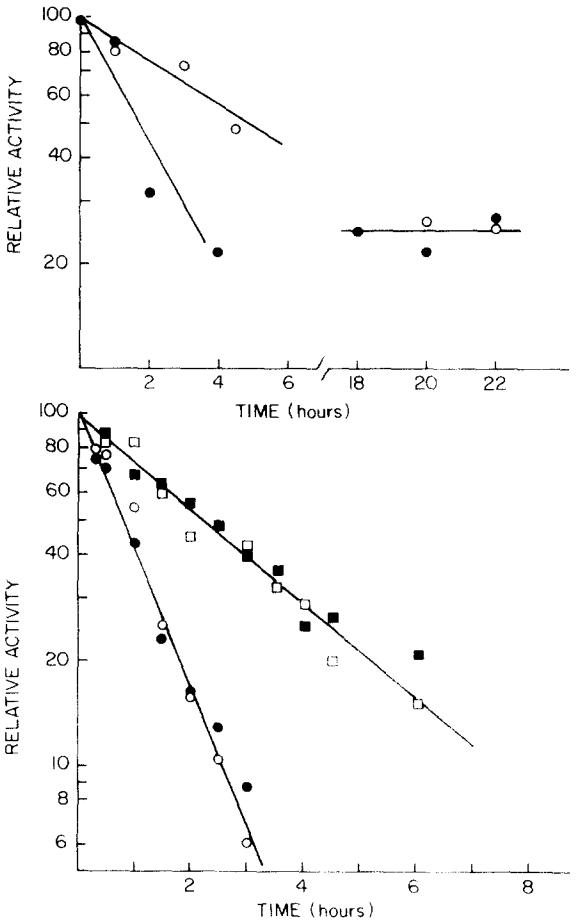

Fig. 2. Inactivation of the calcium dependent SR ATPase during incubation at 37°C (circles) or at 30°C (squares) in 20 mM Mops, 5 mM $MgCl_2$, 0.4 mM $CaCl_2$, pH 7.0. Hollow symbols depict the data obtained using SR preparations derived from skeletal muscles of 4-month-old rats while solid symbols represent the data obtained for SR vesicles from 28-month-old rats. The upper panel presents the inactivation pattern of the ATPase in intact vesicles while the lower panel depicts the inactivation of the same preparations dissolved in 1% Triton X-100 to give a protein concentration of 1 mg/ml. Aliquots of the samples were removed at various time intervals and their enzyme activities determined.

DISCUSSION

Alterations in functional as well as structural properties of a number of enzymes isolated from various tissues of old rats, compared with the enzyme forms isolated from young animals, have been well documented [25—30]. The details of these structural modifications and their mechanisms of development were the focus of numerous studies but are still not understood satisfactorily. While most of the

studies cited above addressed soluble enzymes, the present investigation focuses on the membrane-bound calcium ATPase from rat muscle sarcoplasmic reticulum. This system is considerably more complex, since here the aging effects may involve the ATPase protein, the phospholipid membrane, one (or more) of the other SR membrane proteins or any combination of these three components. The fidelity of the composition of the SR membrane is indeed essential for the effective performance of the calcium pump [17,18] and a modification with age of this membrane may be expected to have profound effects on the system.

In the present study we found that some properties of the SR calcium pump are unmodified by age. These include the calcium content in the vesicles as isolated as well as both their basal and calcium stimulated specific ATPase activities. The latter observation is in full agreement with the recent report by Narayanan [10] that the ATPase activity in rat cardiac SR does not depend on the animals age. An obvious conclusion from these results is that the concentration of the ATPase enzyme relative to the total protein content of skeletal muscle SR, as well as its specific ATPase activity are unaffected by age. This lack of inactivation in the old tissue is somewhat atypical since in many other enzymes reduced activity is a symptom of aging [25—28].

Several properties of the calcium pump were found to be affected by age. Thus, a significant reduction in the capacity of the SR vesicles for calcium (maximal number of nmoles Ca^{2+} accumulated per mg SR protein) was detected in vesicles from old rats relative to their young counterparts. This, combined with the marked age-related decrease in the amount of SR protein per unit mass of skeletal muscle (Table I) indicates a very substantial loss in the ability of the old muscle to sequester calcium. A large decrease in mass is one of the most evident effects of aging in skeletal muscle leading to the decreased muscular strength which is commonly observed in the aged. This type of muscle atrophy is known to involve decreases in both number and size of muscle fibers [31,32]. Indeed Fujisawa [33,34] reported uniformly severe muscular atrophy in rats above 600 days of age, resulting in a great reduction in the mass of active fibers, while Campbell et al. [35] demonstrated the decreased muscular performance in old humans to be primarily associated with a smaller number of functional motor units. Our finding that the amount of SR protein per gram of wet tissue declines with aging correlates well with this evidence. Whether this agreement is quantitative (i.e. the same relative decline in SR protein as in fiber mass) remains to be studied.

The reduced capacity for calcium (per mg SR protein) found in vesicles from old muscle is an intrinsic property of the preparation and does not depend on the quantity of ATPase enzyme present in the tissue. It may reflect alterations in the SR membrane leading to an increase in the rate of calcium efflux. Such leaky membrane would also result in a reduced efficiency of pumping (i.e. more ATP having to be spent for the net pumping of a given amount of calcium) in line with our observation of a reduced Ca/ATP stoichiometry. This mechanism would, however, require that

the ATPase activity subside, after Ca^{2+} sequestration, to a level significantly above that of the basal (Ca^{2+} independent) rate since, due to Ca^{2+} efflux, this ion is continuously being readmitted into the medium. Inspection of Fig. 1B clearly shows that this is not the case. A leaky membrane is therefore not likely to be the cause of the age-related reduction in capacity for calcium.

It is possible to explain this decline in capacity by assuming that the SR vesicle population itself is heterogeneous and contains a fraction in which ATP cleavage is not coupled to Ca^{2+} pumping. If this latter fraction is increased at old age (at the expense of coupled vesicles) then the pumping potential (rate and capacity) per mg SR protein would decline. Moreover, one would expect the Ca/ATP ratio to decline with aging as is indeed observed. It may be possible to test this hypothesis experimentally by utilizing the increased density of vesicles loaded with calcium phosphate which can be separated from uncoupled 'vacant' vesicles by centrifugation [36]. Such experimentation will be attempted with young and old SR in our future work.

Several other potential mechanisms may be proposed to explain the age-related reduction in SR capacity for calcium. Thus, the level of Ca^{2+} binding proteins in the SR vesicles may decline with age, or alternatively the maximal calcium gradient that the SR pump can support across the membrane may be reduced. These seem unlikely in view of the fact that our experiments were conducted in presence of 5 mM oxalate which effectively binds the bulk of the calcium inside the vesicles making any change in the (relatively small) amount of Ca^{2+} bound to membrane proteins of little consequence. Also by buffering calcium concentrations both in the medium and inside the vesicles the oxalate ensures pumping across a constant gradient (the gradient rises only when the medium calcium is almost completely depleted and its concentration begins to fall) hence the ability of the pump to operate against a large gradient is not tested in these experiments.

The remarkable age-related decrease in the Ca/ATP ratio of the SR calcium pump is a finding of great interest. A similar decrease in transport stoichiometry has been reported before for rat cardiac SR by Narayanan [9,10]. In contrast, Bertrand et al. [24] found no reduction, and even a slight increase, with aging in both the Ca/ATP stoichiometry and the calcium pumping rate in SR membrane preparations from skeletal muscle of Fischer rats. While the differences between these authors results and the ones reported in the present study may have their origin in the different strains of rats used, it is pertinent to note that several other important experimental conditions differed. Thus, in the previous study only the basal (Ca^{2+} independent) ATPase activity was determined and the values reported were unusually high (greater than the rates usually found for Ca^{2+} stimulated ATPase activities [9,10,16]), indicating that the SR samples were highly uncoupled. The results could thus represent pumping by only a very small fraction of the SR preparation, which also is unaltered with age. Alternatively the uncoupling could be due to the presence of 20 mM NaCl in the medium, sodium being an efficient inducer of Ca^{2+} release from SR vesicles.

The Ca/ATP ratio observed for young SR vesicles in our study, while in good agreement with the value obtained for the cardiac ATPase [10], is considerably smaller than the ratio obtained for rabbit muscle SR [16]. It is possible, though unlikely, that the intrinsic transport stoichiometry is lower in rat, relative to rabbit SR. A more plausible explanation is that the lower stoichiometry reflects a heterogeneity of vesicles in the SR preparations due either to the fact that no attempt to resolve the rat tissue into individual muscles was made in the present study, or to the presence of a fraction of uncoupled vesicles in the preparation. The age-related reduction in the Ca/ATP ratio may thus represent an increased degree of uncoupling in old SR ATPase units. An explanation for this increase may be found in the observation that the SR ion pump rapidly becomes uncoupled when exposed to mild non-reducing conditions [38]. A significant increase in the oxidation potential has indeed been found to take place in muscle upon aging [39] making this a viable mechanism that may lead to the observed effects. Incubation of young SR under redox potential as exists in old muscle may test this hypothesis.

Both young and old SR vesicles displayed strongly biphasic inactivation kinetics (Fig. 2) with about 25% of the original activity being resistant to prolonged incubation. This age-independent heterogeneity in heat stability may reflect a heterogeneity in the SR vesicles preparations as discussed above since these preparations were made from a mixture of different muscles and were not fractionated to yield their more homogeneous subfractions [40]. Also, the coupled and uncoupled vesicles which are apparently present in our preparation may be expected to possess different heat stabilities and may contribute to the biphasic inactivation kinetics.

A marked difference was found between SR vesicles from young and old muscle in the rate of the first step of inactivation of the ATPase, the old Ca^{2+} pump displaying reduced stability. In contrast, no difference was detected between the inactivation kinetics of young and old ATPases dissolved in Triton X-100. Moreover, the biphasicity of inactivation seen in intact vesicles disappears upon their solvation and no stable fraction of ATPase is apparent here down to below 6% of the original activity. These results clearly indicate that age-related modifications are present in the SR membrane. Changes, with aging, in various cellular membranes have been well documented [25] and may involve alterations in lipid composition due, among other reasons, to the accumulation of peroxidation products. A comparative study of the degree of oxidation in young and old SR membranes, as well as of the effects of aging on their viscosities, may indeed be very rewarding.

While the dissolved ATPase protein displayed no age-related modifications in its inactivation rate this should not be regarded as a proof that no such changes are present. Indeed, the inactivation of solubilized SR ATPase has been shown to proceed via the dissociation of the dimeric enzyme into (unstable) monomeric subunits, the latter losing activity relatively very rapidly [16]. Thus, while our inactivation data in Triton X-100 indicate that the degree of dissociation of the ATPase is independent of age, other properties of the protein may indeed be altered. A detailed,

comparative study of the ATPase purified from young and old rats is needed in order to satisfactorily resolve this problem.

ACKNOWLEDGEMENTS

This work was supported by a Grant in Aid from the American Heart Association of Michigan.

REFERENCES

1 M. Tada, T. Yamamoto and Y. Tonomura, Molecular mechanism of active calcium transport by sarcoplasmic reticulum. *Physiol. Rev., 58* (1978) 1—79.
2 G. Inesi and M. Kurzmack, The Ca^{2+} dependent ATPase of sarcoplasmic reticulum. In D. Chapman (ed.), *Biomembrane Structure and Function,* Verlag Chemie, Weinheim, 1984, pp. 355—410.
3 W.B. Van Winkle, The structure of striated muscle sacroplasmic reticulum. In M.L. Entman and W.B. Van Winkle (eds.), *Sarcoplasmic Reticulum in Muscle Physiology,* Vol. 1, CRC Press, Boca Raton, Florida, 1986, pp. 17—45.
4 W. Hasselbach, The reversibility of the sarcoplasmic calcium pump. *Biochim. Biophys. Acta, 515* (1978) 23—53.
5 C.M. Pickart and W.P. Jenks, Energetics of the calcium transporting ATPase. *J. Biol. Chem., 259* (1984) 1629—1643.
6 H. Ludi and W. Hasselbach, Excimer formation of ATPase from sarcoplasmic reticulum labeled with N-(3-pyrene) maleinimide. *Eur. J. Biochem., 130* (1983) 5—8.
7 J.M. Vanderkooi, A. Terokomas, H. Nakamura and A. Martonosi, Fluorescence energy transfer between Ca^{2+} transport ATPase molecules in artificial membranes. *Biochemistry, 16* (1977) 1262—1267.
8 J.P. Froehlich, E.G. Lakatta, E. Beard, H.A. Spurgeon, M.L. Weisfeldt and G. Gerstenblith, Studies of sarcoplasmic reticulum function and contraction duration in young adult and aged rat myocardium. *J. Mol. Cell Cardiol., 10* (1978) 427—438.
9 N. Narayanan, Differential alterations in ATP-supported calcium transport activities of sarcoplasmic reticulum and sarcolemma of aging myocardium. *Biochim. Biophys. Acta, 678* (1981) 442—459.
10 N. Narayanan, Comparison of ATP-dependent calcium transport and calcium-activated ATPase activities of cardiac sarcoplasmic reticulum and sarcolemma from rats of various ages. *Mech. Ageing Dev., 38* (1987) 127—143.
11 E.G. Lakatta, Cardiac muscle changes in senescence. *Ann. Rev. Physiol., 49* (1987) 519—531.
12 C.H. Orchard and E.G. Lakatta, Intracellular calcium transients and developed tensions in rat heart muscle. A mechanism for the negative interval-strength relationship. *J. Gen. Physiol., 86* (1985) 637—651.
13 L. deMeis and A.L. Vianna, Energy interconversion by the Ca^{2+}-dependent ATPase of the sarcoplasmic reticulum. *Ann. Rev. Biochem., 48* (1979) 275—292.
14 M. Kurzmack and G. Inesi, The initial phase of Ca^{2+} uptake and ATPase activity of sarcoplasmic reticulum vesicles. *FEBS Lett., 74* (1977) 35—37.
15 S. Meltzer and M.C. Berman, Effects of pH temperature and calcium concentration on the stoichiometry of the calcium pump of sarcoplasmic reticulum, *J. Biol. Chem., 259* (1984) 4244—4253.
16 A. Gafni and P.D. Boyer, Modulation of stoichiometry of the sarcoplasmic reticulum calcium pump may enhance thermodynamic efficiency. *Proc. Natl. Acad. Sci. USA, 82* (1985) 98—101.
17 M leMaire and J.V. Moller, Protein-protein and protein-lipid interactions of the sarcoplasmic reticulum Ca^{2+} ATPase. In M.L. Entman and W.B. Van Winkle (eds.), *Sarcoplasmic Reticulum in Muscle Physiology,* Vol. I, CRC Press, Boca Raton, Florida, 1986, pp. 101—126.

18 J. Navarro, M. Toivio-Kinnucan and E. Racker, Effect of lipid composition on the calcium/adenosine 5'-triphosphate coupling ratio of the Ca^{2+}-ATPase of sarcoplasmic reticulum, *Biochemistry, 23* (1984) 130—135.

19 P. Champeil, S. Buschlen-Bouchy, F. Bastside and C. Gary-Bobo, Sarcoplasmic reticulum ATPase. Spin labeling detection of ligand-induced changes in the relative reactivities of certain sulfhydryl groups, *J. Biol. Chem., 253* (1978) 1179—1186.

20 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with phenol reagent. *J. Biol. Chem., 193* (1951) 265—275.

21 S.T. Ohnishi and R.S. Gall, Characterization of the catalyzed phosphate assay. *Anal. Biochem., 88* (1978) 347—356.

22 H.S. Penefski, Reversible binding of Pi by beef heart mitochondrial adenosine triphosphatase *J. Biol. Chem., 252* (1977) 2891—2899.

23 L. deMeis. *The Sarcoplasmic Reticulum,* Wiley, New York, 1981.

24 H.A. Bertrand, B.P. Yu and E.J. Masoro, The effect of rat age on the composition and functional activities of skeletal muscle sarcoplasmic reticulum preparations *Mech. Ageing Dev., 4* (1975) 7—17.

25 M. Rothstein, *Biochemical Approaches to Aging,* Academic Press, London, 1983.

26 A. Gafni, Age-related modifications in a muscle enzyme. In R.C. Adelman and E.E. Dekker (eds.), *Modification of Proteins During Aging,* Alan R. Liss, Inc., New York, 1985, pp. 19—38.

27 M. Rothstein, Age-related changes in enzyme levels and enzyme properties. In M Rothstein (ed.), *Rev. Biol. Res. Aging,* Vol. 2, Alan R. Liss, Inc., New York, 1985, pp. 421—433.

28 V. Dulic and A. Gafni, Mechanism of aging of rat muscle glyceraldehyde-3-phosphate dehydrogenase studied by selective enzyme oxidation. *Mech. Ageing Dev., 40* (1987) 289—306.

29 A. Gafni and N. Noy, Age-related effects in enzyme catalysis Mol. Cell, Biochem., 59 (1984) 113—128.

30 A. Zuniga and A. Gafni, Age-related modifications in rat cardiac phosphoglyceratekinase. Rejuvenation of the old enzyme by unfolding-refolding *Biochim. Biophys. Acta, 955* (1988) 50—57.

31 R. McCarter, Effects of age on contraction of mammalian skeletal muscle. In G. Kaldor and W.J DiBattista (eds.), *Aging in Muscle,* Raven Press, New York, New York, 1978, pp. 1—21.

32 R.E.P. Sica, O.P. Sanz and A. Colombi, The effects of aging upon the human soleus muscle. An electrophysiological study. *Medicina (Buenos Aires), 36* (1976) 443—446.

33 K. Fujisawa, Some observations on the skeletal musculature of aged rats. I. *J. Neurol. Sci., 22* (1974) 353—366.

34 K. Fujisawa, Some observations on the skeletal musculature of old rats. II. *J. Neurol. Sci., 24* (1974) 447—469.

35 M.J. Campbell, A.J. McComas and F. Petito, Physiological changes in aging muscle, *J. Neurol. Meurosurg. Psychiatr, 36* (1973) 174—183.

36 J.P. Bonnet, M. Galante, D. Brethes, J.C. Dedieu and J. Chevallier, Purification of sarcoplasmic reticulum vesicles through their loading with calcium phosphate, *Arch. Biochem. Biophys., 191* (1978) 32—41.

37 A.J. Murphy, M. Pepitone and S. Highsmith, Detergent solubilized sarcoplasmic reticulum ATPase. *J. Biol. Chem., 257* (1982) 3551—3554.

38 F. Sreter, N. Ikemoto and J, Gergerly, The effect of lyophilization and dithiotheitol on vesicles of skeletal and cardiac muscle sarcoplasmic reticulum, *Biochim. Biophys. Acta, 203* (1970) 354—357.

39 N. Noy, H. Schwartz and A. Gafni, Age-related changes in the redox status of rat muscle cells and their role in enzyme aging. *Mech. Ageing Dev., 29* (1985) 63—69.

40 G, Meissner, Isolation and characterization of two types of sarcoplasmic reticulum vesicles, *Biochim. Biophys, Acta 389* (1975) 51—68.