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Characterization of the sarcoplasmic reticulum Ca-ATPase from rabbit temporalis muscle

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ABSTRACT

Objective: The aim of this work was to isolate the sarcoplasmic reticulum (SR) Ca-ATPase from rabbit temporalis muscle and to determine the optimal conditions for calcium transport and enzymatic activity.

Design: SR vesicles were isolated from rabbit temporalis muscle by differential centrifugation, the protein composition analyzed by electrophoresis and compared to fast-twitch muscle membrane suspensions. ELISA was used to determine the sarcoendoplasmic reticulum Ca-ATPase (SERCA) isoform. Ca-ATPase activity was determined by a colorimetric method. Calcium-binding to the Ca-ATPase, calcium uptake, calcium efflux and phosphorylation by P_i were determined with radioisotopic techniques.

Results: Sixty five percent of the total protein concentration of SR membranes suspensions from rabbit temporalis corresponded to SERCA. Of the total SERCA protein, 64% was SERCA 2, 35% was SERCA 1 and less than 1% was SERCA 3. The optimal conditions of the SERCA isolated from rabbit temporalis muscle were: pH 7.2, 5 μM Ca^{2+}, 100 μM EGTA, 90 μM Mg^{2+}, 3 mM ATP and 100 mM KCl and did not differ from fast-twitch skeletal muscle. The temporalis maximal calcium uptake and Ca-ATPase activity were lower but the sensitivity to the specific Ca-ATPase inhibitor thapsigargin was higher. Calcium-binding to the enzyme and calcium efflux were similar while the phosphorylation of the enzyme by P_i was lower.

Conclusion: The lower enzymatic activity and calcium transport capability of the Ca-ATPase isolated from rabbit temporalis, and the higher sensitivity to inhibitory drugs are consistent with the presence of a substantial proportion of SERCA 2, which can be expected in other rabbit masticatory muscles.

1. Introduction

The sarcoplasmic reticulum (SR) Ca^{2+}-depedent adenosine triphosphatase (Ca-ATPase) is a membrane-bound protein responsible for active calcium transport from the myoplasm to the SR lumen at the expense of adenosine triphosphate (ATP) hydrolysis, leading to muscle relaxation.[1] Ca-ATPase exists in several isoforms. It has been reported that the specific sarcoendoplasmic reticulum Ca-ATPase (SERCA) isoform expressed in a muscle fibre is closely related to the myosin isoform in the same fibre, resulting in the appropriate matching of relaxation rate to the contraction speed of the fibre.[2] During the catalytic cycle the enzyme undergoes transitions between E1 and E2 conformational states.[3] In the forward reaction of the enzymatic cycle, the Ca-ATPase, in its E1 form, binds two calcium ions to form the E1Ca2 species. The phosphorylation of the enzyme by ATP yields the E1PCa2

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species and drives the movement of calcium to the lumenal sites, which favours the hydrolysis of the phosphoenzyme E2P to E2, completing the cycle. In the reverse process, the enzyme is phosphorylated by inorganic phosphate (P_i) and the energy derived from the calcium concentration gradient is used to synthesize ATP from adenosine diphosphate (ADP) and P_i SR membranes can be isolated as sealed vesicles from muscle homogenates by differential centrifugation.[4,5] Such membranes preparations have been widely used as an experimental model to study the Ca-ATPase activity and calcium transport.

Functional aspects of the SR Ca-ATPase, such as structure,[6,7] enzymatic activity, modulation of calcium transport, binding and efflux, phosphorylation by ATP and P_i[8,9] as well as the effects of different drugs[10–13] and cations[14,15] have been detailed studied in membranes isolated from fast-twitch skeletal muscles. However, few authors have reported on Ca-ATPase activity and calcium transport from SR membranes in masticatory muscles.[16,17] This is of interest because phenotypic properties of jaw-closing muscles are highly species-specific.[2] Fibre types in masticatory muscles of different species are extremely divergent. These muscles are closely adapted to feeding habits, diet and food types. They belong to a distinct muscle allotype, defined by their unique developmental origin, which enables them to express jaw-specific and other myofibrillar proteins different from those found in adult limb muscles. For instance, carnivores express 'superfast' or masticatory myosin, which is associated with high bite force,[2,18,19] while kangaroos express alpha-cardiac myosin.[20] The rabbit temporalis is a masticatory muscle which expresses fast, slow and alpha-cardiac myosins.[21] It is involved indeed in mastication, deglutition and mandible positioning.[22] This variety of functions includes finely graded and less precise tasks which demand different speed of muscle shortening in response to specific stimuli.[23] From this background, the SR Ca-ATPase from rabbit temporalis muscle could be thought to display a different calcium uptake or release capability compared to fast-twitch skeletal muscle. Since the enzymatic activity and calcium transport capability of the SR Ca-ATPase from rabbit temporalis muscle has not been characterized yet, we undertook this study to clarify some aspects of the differences between masticatory and fast-twitch muscles.

The aim of this work was to isolate the sarcoplasmic reticulum Ca-ATPase from rabbit temporalis muscle and to determine the optimal conditions for calcium transport and enzymatic activity.

2. Materials and methods

2.1. SR vesicles preparation

Temporalis and fast-type back muscles were dissected from adult New Zealand rabbits (6 months old, male, 2 kg). Animal care was provided according to the ''European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes'' (ETS No. 170). SR membranes fragments were isolated as sealed vesicles as described by Champeil et al.[5] The protein concentration was measured by the colorimetric procedure of Lowry et al.,[24] using bovine serum albumin as standard.

2.2. Electrophoresis

Sodium dodecyl sulphate (SDS) gel electrophoresis of the SR membrane suspension was carried out by the method of Laemmli.[25] The separation gel was made up of 10% polyacrylamide, 1.5 M Tris(hydroxymethyl)aminomethane (Tris)–HCl (pH 8.8), 10% ammonium persulphate and N,N,N′,N′-tetramethylethylenediamine (TEMED). The buffer for electrophoresis was made up of 0.025 mM Tris, 0.2 M aminoacetic acid and 0.1% SDS. After running gel electrophoresis at 150 V for 45 min, the gels were stained with 0.2% Coomassie blue R250 in 7.5% acetic acid and 30% methanol for 2 h followed by distaining in 7% acetic acid and 20% methanol.[26]

2.3. ELISA

The sandwich ELISA was used for quantifying the various antigens in SR membrane preparations as described by Leberer and Pette.[27] Microtitre plates were coated with anti-SERCA 1/2/3 IgG (10 μg/ml). Samples prepared in incubation buffer (phosphate-buffered saline/Tween containing 1% (w/v) milk powder, 10% (v/v) glycerol, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 1 mM phenylmethanesulphonylfluoride and Trasylol (100 k.i.u./ml) were introduced into wells and incubated for 2 h at room temperature. For blanks, the samples were introduced into wells coated with control IgG. After being rinsed three times with phosphate-buffered saline/Tween, the plates were incubated for 3 h at room temperature and later rinsed three times with phosphate-buffered saline/Tween and once with 0.01 M citrate buffer, pH 5.0. Staining was performed with a substrate solution containing 55 mg of phenylene-1,2-diamine dihydrochloride and 0.03 ml 30% H_2O_2 in 100 ml of 0.1 M citrate buffer, pH 5.0, for 30 min at 37 °C. The staining reaction was stopped by adding 0.05 ml of 2 M H_2SO_4. The optical density of each well was determined within 30 min, using a microplate reader set to 450 nm. Blanks were subtracted from the sample values.

2.4. Ca-ATPase activity

The Ca-ATPase activity was assayed in media containing 3-(n-morpholino) propanesulphonic acid (MOPS)–Tris or 2(N-morpholino) ethanesulphonic acid (MES)–Tris buffer (with variable pH depending on the experiment), calcimycin (calcium ionophore A 23187), ATP, KCl, $MgCl_2$, $CaCl_2$, and ethyleneglycolbis (β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA) at various concentrations. The free Ca^{2+} and Mg^{2+} concentrations were calculated according to Fabiato and Fabiato.[28] The incubations were at 37 °C, and the reaction times were adjusted to yield an ATP hydrolysis ranging between 10 and 20%. The reactions were started by addition of SR membranes (0.1 mg/ml) to the media and stopped with cold 5% trichloroacetic acid (final concentration). The denatured membranes were precipitated by centrifugation, and P_i was measured in the supernatants according to the method of Baginski et al.[29] and taken as an index of the ATPase activity. Blanks without SR vesicles were run in parallel and subtracted from the