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Heat production by skeletal muscles of rats and rabbits and utilization of glucose 6-phosphate as ATP regenerative system by rats and rabbits heart Ca^{2+}-ATPase

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Abstract

This report is divided in two parts. The first section shows that vesicles derived from the sarcoplasmic reticulum of rats skeletal muscle can cleave ATP at a faster rate and produce more heat that the vesicles derived from rabbit skeletal muscle. In the second part, we compared the rates of Ca^{2+} transport and ATP hydrolysis by rats and rabbits heart sarcoplasmic reticulum. It is shown that the two vesicles preparations are able to use glucose 6-phosphate and hexokinase as an ATP regenerative system. The rates of Ca^{2+}-uptake and ATP hydrolysis measured with glucose 6-phosphate and hexokinase is four to six times slower than that measured with phosphoenolpyruvate and pyruvate kinase as ATP regenerative system.

Keywords: Heat; Skeletal muscle; Heart; SERCA; Glucose 6-phosphate; ATP regenerating system

There are great masters who are able to unveil from Nature seminal new findings which inspire and teach many in different lands. That is the case of Prof. Ebashi. The contribution of Master Ebashi in the discovery of the endo/sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and his revolutionary discovery of proteins that regulates physiological events when it binds Ca^{2+}, was to the starting point of more than one generation of young scientists.

Until recently it was assumed that the amount of heat produced during the hydrolysis of an ATP molecule is always the same, as if the energy released during ATP cleavage were divided in two non-interchangeable parts, one would be used for Ca^{2+} transport (work) and the other converted into heat. In recent reports [1–7] it was found that depending on the conditions used, the amount of heat released during the ATP hydrolysis and Ca^{2+} transport may vary between 7 and 32 kcal/mol. This finding indicated that SERCA are able to handle the energy derived from ATP hydrolysis in such a way as to determine the parcel which is used for Ca^{2+} transport and the parcel of energy that is used for the heat production. Heat generation and burning calories are implicated in the regulation of several physiological processes including body temperature, metabolism, body weight, energy balance and cold acclimation [8–10]. The heat derived from SERCA activity may play an important role in the regulation of non-shivering thermogenesis and obesity control [4,9,21–27].

The various SERCA isoforms have a high affinity for ATP, the apparent K_m being $\sim 10^{-6}$ M. In previous works [11,12] it was shown that brain and skeletal muscle SERCA are able to use glucose 6-phosphate and hexokinase as an ATP regenerative system. This has not been tested previously in cardiac SERCA. The importance of using a low energy phosphate compound as an ATP regenerating system is that it may represent a salvage route used at early stages of cardiac ischemia.

In this report, we will compare the transport and the SERCA thermogenic activity of two animal species (rat and rabbit) and from two different tissues, white skeletal

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muscle and heart. In rats, most of the heat needed for non-shivering thermogenesis is derived from brown adipose tissue (BAT). However, in rabbits that have no significant amount of BAT, the principal source of heat during non-shivering thermogenesis seems to be derived from the ATP hydrolysis catalyzed by SERCA of skeletal muscles [8–10].

Materials and methods

Sarcoplasmic reticulum vesicles. These were derived from the longitudinal sarcoplasmic reticulum of rabbit or Wistar rat muscles and were prepared as described previously [13]. White skeletal muscle was dissected from hind limb and cardiac muscle correspond to ventricle and atria. These vesicles were stored at −80 °C until use.

Ca^{2+}-uptake. These were measured by the filtration method [14]. For ^{45}Ca-uptake, trace amounts of ^{45}Ca were included in the assay medium. The reaction was arrested by filtering samples of the assay medium through Millipore filters. After filtration, the filters were washed five times with 5 ml of 3 mM $La(NO_3)_3$ and the radioactivity remaining on the filters was counted using a liquid scintillation counter.

ATPase activity. ATPase activity was assayed in white muscle vesicles by measuring the release of ^{32}Pi from [γ-^{32}P]ATP. The [γ-^{32}P]ATP not hydrolyzed during the reaction was extracted with activated charcoal as described previously [37]. In cardiac muscle vesicles, ATPase activity was assayed by colorimetric method that measure the inorganic phosphate (P_i) released in medium [15]. The reaction was arrested with trichloroacetic acid (final concentration, 5%, w/v). Two different ATPase activities can be distinguished in sarcoplasmic reticulum vesicles derived from heart [16–20]. The Mg^{2+}-dependent activity requires only Mg^{2+} for its activation and is measured in the presence of 10 mM EGTA to remove contaminant Ca^{2+} from the medium. The Ca^{2+}-dependent ATPase activity, which is correlated with Ca^{2+} transport, is determined by subtracting the Mg^{2+}-dependent activity from the activity measured in the presence of both Mg^{2+} and Ca^{2+}.

Heat of reaction. This was measured using an OMEGA Isothermal Titration Calorimeter from Microcal (Northampton, MA, USA). The calorimeter sample cell (1.5 ml) was filled with reaction medium, and the reference cell was filled with Milli-Q water. After equilibration at 35 °C, the reaction was started by injecting vesicles into the sample cell and the heat change was recorded for 30 min. The volume of vesicle suspension injected into the sample cell varied between 0.03 and 0.06 ml. The heat change measured during the initial 2 min after vesicle injection was discarded to avoid artifacts such as heat derived from the dilution of the vesicle suspension in the reaction medium and binding of ions to the Ca^{2+}-ATPase. The duration of these events is less than 1 min. Calorimetric enthalpy (ΔH^{cal}) is calculated by dividing the amount of heat released by the amount of ATP hydrolyzed [1,2,4–6]. The units used are mol for substrate hydrolyzed and kcal for heat released. Negative values indicate that the reaction is exothermic and positive values indicate that it is endothermic.

Results and discussion

ATPase activity and heat production

Skeletal muscle is by far the most abundant tissue of human body and accounts for over 50% of the total oxygen consumption in a resting human and up to 90% during very active muscular work [10,28]. Brown adipose tissue (BAT) is capable of rapidly converting fat stores to heat and has been used as a model system for the understanding of non-shivering heat production and mechanisms of energy wasting to control obesity. In animals lacking BAT, the main source of heat during non-shivering thermogenesis is derived from the hydrolysis of ATP by the SERCA of skeletal muscle [8–10]. In this work, we compare the rates of Ca^{2+}-uptake, ATP hydrolysis and heat produced by SERCA of skeletal muscle of two different animals species, rats that do have a significant deposit of BAT and rabbits which do not have BAT.

Vesicles derived from rabbit skeletal muscle accumulated more Ca^{2+} (steady state) and at a faster rate than vesicles derived from rat skeletal muscle (Table 1). Surprisingly, we found that the rate of ATP cleavage and heat production by rat SERCA was two to three-folds faster than that of rabbit (Fig. 1). Fig. 1 shows a typical experiment and Table 1 shows the values measured in different vesicles preparations. Notice in Table 1 that the amount of heat produced during the hydrolysis of each ATP molecule (ΔH^{cal}) was the same in rats and rabbits. The relationship between body surface area and volume of rats is significantly larger than that of rabbit. Therefore, rats are prone to dissipate more heat to the environment than rabbit. Therefore, in order to maintain the body temperature, in addition to BAT, rats also produce more heat than rabbit at the level of muscle SERCA.

ATP regenerating systems

The reaction catalyzed by hexokinase is usually thought to be irreversible in physiological conditions:

$$\text{Glucose} + \text{ATP} \Longleftrightarrow \text{glucose 6-P} + \text{ADP} \qquad \text{(reaction1)}$$

When equilibrium of the reaction is reached, most of the ATP is converted into glucose 6-P but a small fraction of ATP remains available in the medium. Because its high affinity for ATP, SERCA are able to bind the small amount of ATP available in the medium and use it for

Table 1
Ca^{2+}-uptake, Ca^{2+}-ATPase activity, heat released and ΔH^{cal} in vesicles derived sarcoplasmic reticulum of rat and rabbit white muscle

	2 mM ATP	
	Rat	Rabbit
Ca^{2+}-uptake		
Initial velocity (μmol/mg protein.min)	0.40 ± 0.04 (7)	0.53 ± 0.04 (6)
Steady-state (μmol/mg protein)	2.80 ± 0.38 (7)	3.25 ± 0.41 (6)
Ca^{2+}-ATPase activity (μmol Pi/mg protein min)	3.54 ± 0.49 (7)	0.57 ± 0,06 (13)*
Heat released (mcal/mg protein min)	−70.2 ± 10,5 (7)	−12.84 ± 0.42 (13)*
ΔH^{cal} (mcal/μmol P_i hydrolyzed)	−21.32 ± 1.1 (7)	−22.85 ± 1.25 (13)

Values are means ± SE of the number of experiments (n) shown in the table. The difference of ATP hydrolysis and heat release between muscles rat and rabbit SERCA are statistically significant.

* $p < 0.001$.

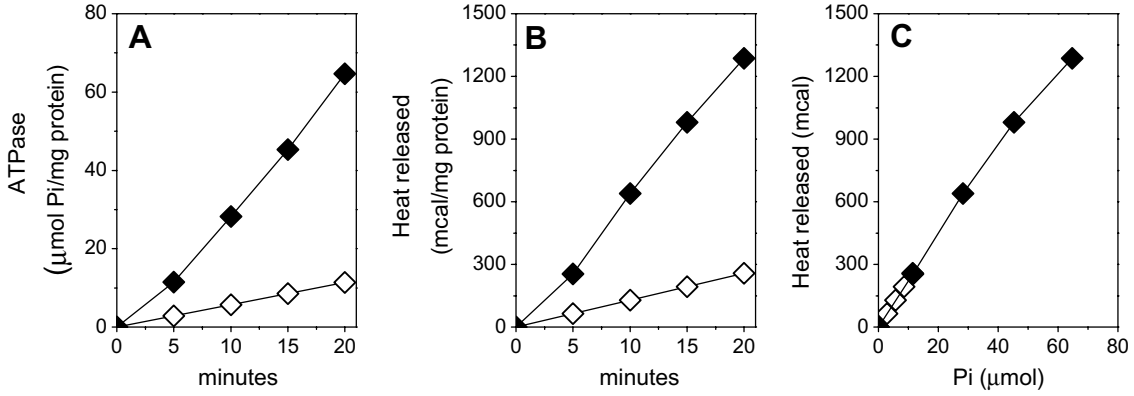

Fig. 1. ATPase activity (A) and heat released (B) in sarcoplasmic reticulum vesicles derived from rat (close symbol) or rabbit (open symbol) white skeletal muscle. In (C), data (A) and (B) were replotted. The assay medium composition was 2 mM ATP, 50 mM MOPS–Tris buffer (pH 7.0), 100 mM KCl, 10 mM Pi, 5 mM NaN_3, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 5 μg/ml vesicle protein.

Ca^{2+} transport. In order to maintain the equilibrium ATP concentration, the parcel of ATP hydrolyzed is regenerate by a "low energy" sugar phosphate.

(1) glucose 6-P +ADP ⇔ glucose + ATP

(2) ATP + HOH ⇔ ADP + P_i

(3) glucose 6-P + HOH ⇔ glucose + P_i

This has been previously described using vesicles derived form rabbit white muscle sarcoplasmic reticulum [11]. The sarcoplasmic reticulum of heart muscle is by far less developed than that of white skeletal muscle and the isoforms found embedded into the reticulum membrane is 2a in the heart and SERCA 1 in skeletal muscle. We now show that similar to white muscle SERCA 1, both rabbit and rat sarcoplasmic reticulum vesicles can also use glucose 6-phosphate and hexokinase as an ATP regenerating system to pump Ca^{2+} into the vesicles (Fig. 2). The difference between the use of sugar phosphate and phosphoenolpyruvate (high energy phosphate compound) as ATP regenerating systems is the amount of ADP available in the medium at steady state. While with phosphoenolpyruvate there is practically no ADP available, with glucose 6-phosphate, most of the nucleotide in the medium is in the form of ADP. In spite of the high ADP concentration, the high ATP affinity of the various SERCA allows the enzyme to "fish" the small amount of ATP available when reaction (reaction 1) above reaches equilibrium. The presence of an excess ADP in the medium, as observed with glucose 6-phosphate and hexokinase, slows down both the rate of Ca^{2+}-uptake and the rate of ATP hydrolysis (Fig. 3). Furthermore, the steady-state level of Ca^{2+} accumulation measured with glucose 6-phosphate is four to six-folds smaller than that measured using phosphoenolpyruvate as an ATP regenerative system (Table 2).

Intracellular Ca^{2+} overload often occurs during severe ischemia and intracellular acidosis, and is a key event leading to cellular injury. Activation of glycolysis during cellular ischemia and energy failure is usually associated with production of lactic acid and a decrease of the intracellular pH to values as low as 6.0 [29,30]. In cardiac muscle lactic acidosis leads to intracellular Ca^{2+} overload and cellular death [31]. Acidification of the medium promotes a decrease of both the Ca^{2+} affinity and the V_{max} of Ca^{2+} transport of the different Ca^{2+}-ATPase isoforms [32–39].

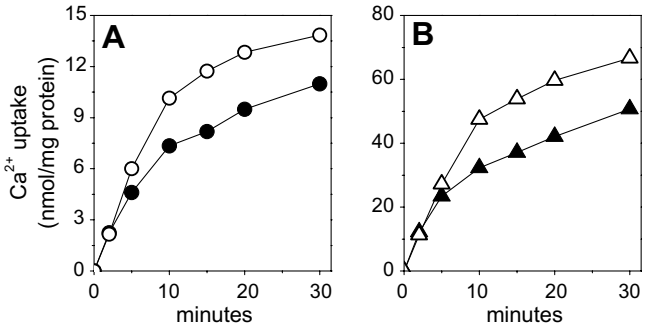

Fig. 2. Ca^{2+}-uptake in sarcoplasmic reticulum vesicles derived from rat (close symbol) or rabbit (open symbol) cardiac muscle. The assay medium composition was 50 mM MOPS–Tris buffer (pH 7.0), 100 mM KCl, 5 mM oxalate, 5 mM NaN_3, 4 mM $MgCl_2$, 0.05 mM $^{45}CaCl_2$ and 100 μg/ml vesicle protein. Ca^{2+}-uptake was supported by 10 U/ml hexokinase, 5 mM glucose 6-phosphate and 50 μM ADP (A) or 10 U/ml pyruvate kinase, 2 mM phosphoenolpyruvate and 50 μM ADP (B).

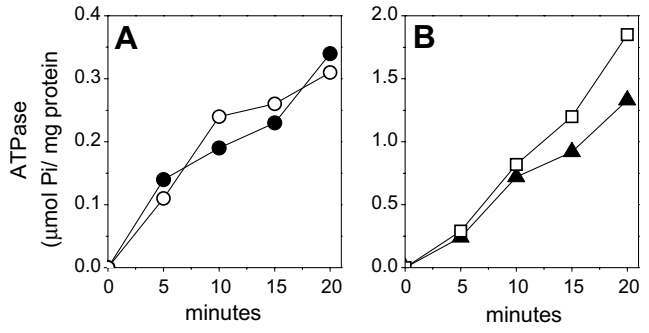

Fig. 3. Ca^{2+}-ATPase activity in sarcoplasmic reticulum vesicles derived from rat (close symbol) or rabbit (open symbol) cardiac muscle. The assay medium composition was 50 mM MOPS–Tris buffer (pH 7.0), 100 mM KCl, 5 mM oxalate, 5 mM NaN_3, 4 mM $MgCl_2$, 0,05 mM $CaCl_2$ and 100 μg/ml vesicle protein. Ca^{2+}-ATPase activity was supported by 10 U/ml hexokinase, 5 mM glucose 6-phosphate and 50 μM ADP (A) or 10 U/ml pyruvate kinase, 2 mM phosphoenolpyruvate and 50 μM ADP (B).

Table 2
Ca^{2+}-uptake and Ca^{2+}-ATPase activity in vesicles derived sarcoplasmic reticulum of rat and rabbit cardiac muscle using ATP-regenerate systems

	Hexokinase		Pyruvate kinase	
	Rat	Rabbit	Rat	Rabbit
Ca^{2+}-uptake				
Initial velocity (nmol/mg protein min)	1.02 ± 0.22 (7)	1.14 ± 0.23 (6)	5.40 ± 0.84 (8)*	5.55 ± 0.70 (10)*
Steady-state (nmol/mg protein)	10.23 ± 2.50 (7)	13.34 ± 2.81 (6)	46.39 ± 3.33 (8)*	63.14 ± 2.07 (10)*
Ca^{2+}-ATPase activity (μmol P_i/mg protein min)	0.02 ± 0.01 (7)	0.02 ± 0.01 (6)	0.07 ± 0.01 (8)*	0.09 ± 0.01 (10)*

Values are means ± SE of the number of experiments (n) shown in the table. The difference between hexokinase and pyruvate kinase are statistically significant.
* $p < 0.001$.

These changes of the enzyme properties greatly decrease the ability of the SERCA to drain Ca^{2+} from the cytosol into the reticulum. Glucose 6-phosphate can be formed from glycogen and Pi without the need for consuming ATP. The data presented in this report show that the reversal of the reactions catalyzed by hexokinase might be used for the draining of Ca^{2+} from the cytosol during energy failure. The advantage of this system is that during O_2 deprivation, only the initial steps of glycolysis would be activated and the cytosolic Ca^{2+} overload using glucose 6-phosphate an hexokinase as an ATP regenerating system. This would impair the subsequent reactions of glycolysis, thus avoiding the accumulation of lactic acid in the cytosol. The disadvantage is that less ATP would become available to the cell. After the formation of glucose 6-phosphate from glycogen and Pi, three ATP molecules are formed during degradation of glucose 6-phosphate to lactic acid. If glucose 6-phosphate is used to regenerate ATP, then two molecules of ATP that could be formed in the subsequent steps of glycolysis are not synthesized. Perhaps, at the early stages of anoxia and before the energy failure, the use of glucose 6-phosphate might represent a salvage route whereby the cardiac cells may energize the Ca^{2+}-ATPase, maintaining a low cytosolic Ca^{2+} concentration and avoiding the deleterious effect derived from Ca^{2+} overload and intracellular acidosis.

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