Control of energy fluxes by the sarcoplasmic reticulum Ca^{2+}-ATPase: ATP hydrolysis, ATP synthesis and heat production

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Abstract The experiments described indicate that heat is released when Ca^{2+} leaks through the Ca^{2+}-ATPase of sarcoplasmic reticulum vesicles. In the presence of a transmembrane Ca^{2+} concentration gradient, agents that modify the amount of ATP synthesized from ADP and P_i also modify the amount of heat produced by the hydrolysis of each ATP molecule. Thus, in the presence of heparin, less ATP is synthesized and more heat is produced. Conversely, with dimethyl sulfoxide more ATP is synthesized and less heat is produced. The data indicate that between limits (−10 to −30 kcal/mol) the Ca^{2+}-ATPase can regulate the interconversion of energy in such a way as to vary the fraction of energy derived from ATP hydrolysis which is converted into heat and that which is converted into other forms of energy.

Key words: Heat; Nonshivering thermogenesis; ATP hydrolysis; Ca^{2+}-ATPase; Sarcoplasmic reticulum

1. Introduction

The Ca^{2+}-ATPase found in the membrane of the sarcoplasmic reticulum (SR) of skeletal muscle is able to interconvert different forms of energy. This enzyme translocates Ca^{2+} from the cytoplasm to the lumen of the SR using the chemical energy derived from ATP hydrolysis. After Ca^{2+} has accumulated inside the SR, a Ca^{2+} gradient is formed across the membrane and this promotes the reversal of the catalytic cycle of the enzyme. During this reversal Ca^{2+} leaves the SR through the Ca^{2+}-ATPase and this is coupled with the synthesis of ATP from ADP and P_i. In this process, the osmotic energy derived from the gradient is transformed by the enzyme back into chemical energy. In the literature, the simultaneous synthesis and hydrolysis of ATP is referred to as the ATP ↔ P_i exchange reaction [1–6]. In conditions similar to those found in the living cell, the Ca^{2+} concentration is high inside the SR and low on the outside, and the pump operates continuously forward and backwards, cleaving and synthesizing ATP continuously. Recently [7–15] it has been shown that during the ATP ↔ P_i exchange only part of the Ca^{2+} efflux is coupled to the synthesis of ATP. The other part leaks through the Ca^{2+}-ATPase without promoting the synthesis of ATP, a process referred to as an uncoupled efflux. The rates of the coupled and the uncoupled Ca^{2+} effluxes can be modified by different drugs, including dimethyl sulfoxide and heparin.

Only a part of the chemical energy released during the hydrolysis of ATP by the Ca^{2+}-ATPase is converted into osmotic energy. The other part is converted into heat, and this is used by the cell to maintain a constant and high body temperature. Nonshivering thermogenesis is a key component of temperature regulation in animals that have little or no brown adipose tissue. During nonshivering thermogenesis most of the heat is derived from resting muscle but the mechanism of heat production is still unclear [16]. It has been proposed that Ca^{2+} leaks from the SR and heat would then be derived from the hydrolysis of the extra amount of ATP needed to maintain a low myoplasmic Ca^{2+} concentration [16–20]. In this formulation it is assumed that the amount of heat produced during the hydrolysis of an ATP molecule is always the same and is not modified by the formation of the gradient, as if the energy released by ATP hydrolysis were to be divided in two non-interchangeable parts: one would be converted into heat, and the other used for Ca^{2+} transport [16–20]. As far as we know there is no published evidence showing that the osmotic energy derived from the Ca^{2+} gradient can also be converted into heat by the SR ATPase.

The data presented in this report show that heat is produced during the uncoupled Ca^{2+} efflux and that it is possible to vary the amount of heat produced during ATP hydrolysis using drugs that change the rates of the coupled and uncoupled Ca^{2+} effluxes.

2. Materials and methods

2.1. Preparation of intact and permeated vesicles

Vesicles derived from the SR of rabbit skeletal muscle were prepared as described by Eletr and Inesi [21]. This preparation has practically no junctional protein and the efflux of Ca^{2+} measured in these vesicles is not altered by ryanodine. Permeated vesicles were prepared by incubating SR vesicles at pH 9.0 in the presence of 2 mM EGTA at room temperature for 20 min. After that the pH was readjusted to 7.0 the ATPase activity of the vesicles is maintained but the permeability of the membrane is increased and the vesicles are no longer able to accumulate Ca^{2+} [22]. In order to ensure that the vesicles did not retain any Ca^{2+} in the lumen, the divalent cation ionophore A-23187 (20 μM) was included in the assay medium in addition to the treatment with EGTA at pH 9.0.

2.2. ATPase activity, Ca^{2+} uptake, ATP synthesis and heats of reaction

The methods for measuring the ATPase activity using (γ-^{32}P) ATP, calcium uptake using ^{45}Ca and ATP synthesis from ADP and $^{32}P_i$ are described elsewhere [23]. Heats of reaction were measured using an OMEGA Isothermal Titration Calorimeter from Microcal Inc. (Northampton, MA) [24]. The calorimeter cell was filled with a reaction medium containing ATP, and the reference cell was filled with Milli-Q water. After equilibration at 35°C, the reaction was started by injecting the enzyme into the reaction cell and the heat change due to the ATP hydrolysis was recorded up to a maximum of 40 min.

2.3. Calculation of ΔH^{cal}

The calorimetric enthalpy of ATP hydrolysis (ΔH^{cal}) was calculated by dividing the amount of heat released by the amount of ATP hydrolyzed. The units used were moles for ATP hydrolyzed and kcal for the

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heat released. A negative value indicates that the reaction was exothermic and a positive value indicate that it was endothermic.

2.4. Chemicals

Heparin from porcine intestinal mucosa was obtained from Sigma Chemical (St. Louis, MO). All other reagents were of analytical grade.

3. Results and discussion

A transmembrane Ca^{2+} gradient is formed when intact SR vesicles are incubated in a medium containing ATP. This is not observed with permeated vesicles because the membrane was disrupted. Both in the presence and absence of a Ca^{2+} gradient, the amount of heat produced during the hydrolysis of ATP was found to be proportional to the amount of ATP hydrolyzed. However, in the presence of the gradient, the amount of heat produced by the hydrolysis of each ATP molecule was larger than that measured with permeated vesicles (Fig. 1 and Table 1). This difference suggests that the SR vesicles were able to convert a part of the osmotic energy derived from the gradient into heat. This raises the possibility that this conversion could reflect the uncoupled leakage of Ca^{2+} through the ATPase. Thus, the coupled and the uncoupled Ca^{2+} effluxes could represent two distinct routes of energy conversion, both mediated by the Ca^{2+}-ATPase: one route in which the osmotic energy derived from the Ca^{2+} gradient is used to synthesize ATP (coupled Ca^{2+} efflux), and one route in which the osmotic energy is converted into heat (uncoupled Ca^{2+} efflux). According to this reasoning it would be expected that drugs that change the rates of the coupled and uncoupled Ca^{2+} effluxes should also change the amount of heat produced and the amount of ATP synthesized during the ATP ↔ P_i exchange reaction. In previous reports [9,11,25,26] it was shown that the Ca^{2+}-ATPase is coupled more tightly by dimethyl sulfoxide and uncoupled by heparin [14,15,27]. Different amounts of Ca^{2+} are accumulated in the presence of these two drugs (Fig. 2). The amount of Ca^{2+} retained by the vesicles is determined by both the rates of Ca^{2+} uptake and the rate of Ca^{2+} leakage from the vesicles. Thus when more enzyme units in the membrane are coupled, less Ca^{2+} leaks from the vesicles during transport and more Ca^{2+} is retained inside the vesicles [9–11]. This was observed when dimethyl sulfoxide was included in the assay medium (Fig. 2). Conversely, when more enzyme units are uncoupled, there is more Ca^{2+} leakage and less Ca^{2+} is retained by the vesicles. Accordingly, less Ca^{2+} was accumulated by the vesicles after the addition of heparin to the medium (Fig. 2). In spite of the fact that the total Ca^{2+} accumulated varied in presence of heparin and dimethyl sulfoxide, the free Ca^{2+} concentration inside the vesicles and the gradient formed across the membrane were practically the same in the three conditions shown in Fig. 2. During transport, the P_i available in the assay medium diffuses through the membrane to form calcium phosphate crystals inside the vesicles. These crystals operate as a Ca^{2+} buffer that maintains the free Ca^{2+} concentration constant (~5 mM) inside the vesicles at the level of the solubility product of calcium phosphate [4,5,28,29].

Table 1
Effect of the Ca^{2+} gradient and drugs on the ΔH^{cal} of ATP hydrolysis

Vesicles	Drug	ΔH^{cal} (kcal/mol)
Permeated	None	−12.2 ± 1.3 (16)
	Dimethyl sulfoxide (20%)	−12.1 ± 1.0 (7)
Intact	None	−22.3 ± 1.4 (27)*
	Dimethyl sulfoxide (20%)	−13.2 ± 0.7 (9)
	Heparin (3 μg/ml)	−30.2 ± 2.1 (4)**
	Heparin (10 μg/ml)	− 9.8 ± 2.3 (3)

The conditions were as in Fig. 1. The ΔH^{cal} values in the table are the average ± SE of the number of experiments shown in parentheses. The differences between the ΔH^{cal} of intact and permeated vesicles or between intact with and without 20% dimethyl sulfoxide (*) and between intact vesicles with and without heparin (**) were significant (*t* test) with $P < 0.001$ and $P < 0.05$, respectively.

The addition of dimethyl sulfoxide to the assay medium promoted both a decrease in the amount of heat produced during the hydrolysis of ATP and an increase in the amount of ATP resynthesized during the ATP ↔ P_i exchange reaction (Fig. 3). After the addition of dimethyl sulfoxide, the value of ΔH^{cal} measured in the presence of the Ca^{2+} gradient decreased to the same value as that measured with permeated vesicles (Table 1). Note that dimethyl sulfoxide did not change the ΔH^{cal} measured with permeated vesicles (Table 1). The oppo-

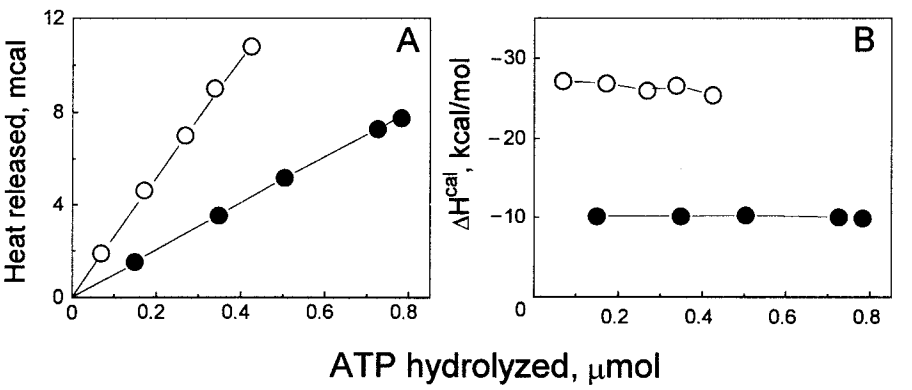

Fig. 1. Heat released during ATP hydrolysis in presence and absence of a transmembrane Ca^{2+} gradient. The reaction was performed at 35°C. The assay medium composition was 50 mM MOPS-Tris buffer, pH 7.0, 0.1 mM $CaCl_2$, 1 mM ATP, 4 mM $MgCl_2$, and 10 mM P_i. The medium was divided into four samples. One was used for heat measurements. To the other three samples trace amounts of either ^{45}Ca, [γ-^{32}P]ATP or $^{32}P_i$ were added for measurement of Ca^{2+} uptake, ATP hydrolysis and ATP synthesis, respectively. The four reactions were started simultaneously by the addition of vesicle protein (20 μg/ ml). For the intact vesicles (●), the time course of Ca^{2+} uptake is shown in Fig. 2 and the ratio between ATP cleaved and ATP synthesized is shown in Fig. 3. For the permeated vesicles (○), 5 μM A23187 was included in the assay medium. The permeated vesicles did not accumulate Ca^{2+} nor did they catalyze the synthesis of ATP from ADP and P_i.

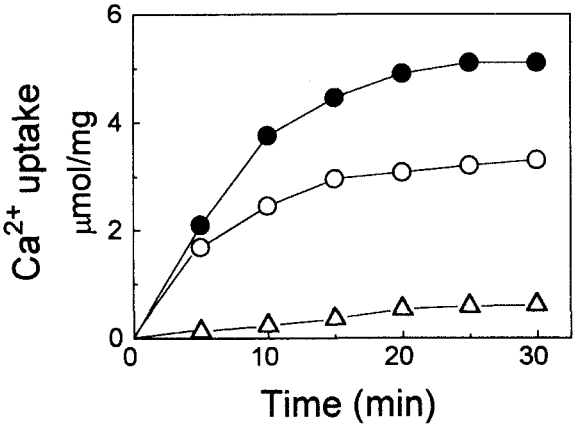

Fig. 2. Effects of drugs on Ca^{2+} uptake. The assay medium composition and other experimental conditions were as described in Fig. 1 for intact vesicles. (○) control; (●) 20% (v/v) dimethyl sulfoxide; (△) 3 μg/ml heparin.

site result was obtained with heparin. In the presence of 3 μg/ml heparin the vesicles were still able to accumulate Ca^{2+} (Fig. 2) but now the heat produced during ATP hydrolysis was higher than that measured in the control without drug, and this was accompanied by a decrease in the amount of ATP resynthesized during the ATP ↔ P_i exchange reaction (Fig. 3). The ΔH^{cal} measured with 3 μg/ml heparin was significantly more negative than that of the control (Table 1). The degree of leakage increased when the heparin concentration was raised to 10 μg/ml and although the vesicles were still able to hydrolyze ATP, they were no longer able to accumulate Ca^{2+}. This promoted a change in the ΔH^{cal} to the same value as that measured with permeated vesicles (Table 1).

The difference in heat production measured with intact and permeated vesicles was not related to the formation of calcium precipitates inside the vesicles, because the same ΔH^{cal} values for ATP hydrolysis were obtained when this was measured in the absence of Ca^{2+}-precipitating agents or in the presence of either 4 mM oxalate or 10 mM P_i (data not shown). The value of ΔH^{cal} was not dependent on the concentrations of ATP and ADP in the medium. Both in the presence and in the absence of a Ca^{2+} gradient, the values of ΔH^{cal} measured with either 0.1 or 1.0 mM ATP were practically the same (data not shown). During the hydrolysis of ATP, a variable amount of ADP is accumulated in the medium and in the conditions used, one molecule of ADP was formed for each ATP molecule hydrolyzed. As shown in Fig. 1B and 3C, essentially the same value of ΔH^{cal} for ATP hydrolysis was attained after the cleavage of different amounts of ATP.

The experiments described suggest that from the total chemical energy released during ATP hydrolysis, about ~10 kcal/mol is converted into heat regardless of whether or not a Ca^{2+} gradient is formed across the membrane. This represents a minimum for the heat production and was measured using permeated vesicles and after abolishing the effect of the gradient with dimethyl sulfoxide (Table 1). The rest of the energy available from ATP hydrolysis (~20 kcal/mol) is probably used to translocate Ca^{2+} across the membrane (work). If the vesicles are permeated the Ca^{2+} translocated is not accumulated. In this case the Ca^{2+}-ATPase operates as if it were a motor pumping water between two interconnecting pools located at the same level. If the pump stops, none of the energy consumed by the motor can be recovered because the energy was not stored: a water column was not formed since the water pumped has already returned to the pool from were it was removed. However, if the membrane is intact, then the energy used for the translocation of Ca^{2+} is converted into osmotic energy (Ca^{2+} gradient) and the Ca^{2+}-ATPase can then use this energy to either synthesize a small part of the ATP previously cleaved or to produce heat. The balance between these two routes would be determined by the ratio between the coupled and uncoupled enzyme units. In one extreme (dimethyl sulfoxide), there is a high degree of energy conservation, most of the energy derived from the hydrolysis of ATP being conserved by the vesicles as osmotic energy (Fig. 2 and 3) and practically all the Ca^{2+} that leaves the vesicles is used to synthesize a part of the ATP previously cleaved. In the other extreme (3 μg/ml heparin), the SR operates as it was a heat source: a small amount of Ca^{2+} is retained by the vesicles (Fig. 2) and most of the energy derived from ATP hydrolysis is converted into heat (Fig. 3). In earlier reports (for review see [4–6,23,26,29]) it was shown that the intermediary steps involved in the hydrolysis of ATP are different in the four conditions shown in Table 1 (permeated vesicles, intact vesicles, intact vesicles with either dimethyl sulfoxide or heparin). It might be possible that the different ΔH^{cal} values found in Table 1 reflect the fact that in each condition, the ATP is cleaved through different paths during catalysis.

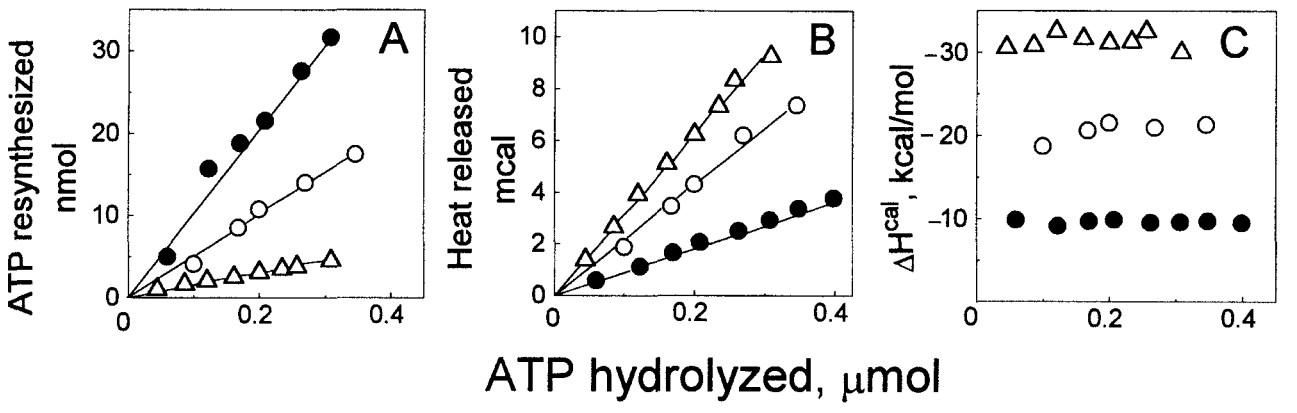

Fig. 3. Correlation between the amount of ATP synthesized (A), heat production (B), and ΔH^{cal} (C). The assay medium composition and other experimental conditions were as described in Fig. 1, using intact vesicles. (○) control; (●) 20% (v/v) dimethyl sulfoxide; (△) 3 μg/ml heparin.

In conclusion, the data presented show that the Ca^{2+}-ATPase can regulate the interconversion of energy in such a way as to vary the fraction of the energy derived from ATP hydrolysis which is dissipated as heat. Depending on the conditions used the ΔH^{cal} for ATP hydrolysis may vary from −10 to −30 kcal/mol P_i. This regulation may play a role in the mechanism of heat production in nonshivering thermogenesis.

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