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Topical Review

Ca^{2+}-ATPases (SERCA): Energy Transduction and Heat Production in Transport ATPases

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Abstract. The sarcoplasmic reticulum Ca^{2+}-ATPase is able to cleave ATP through two different catalytic routes. In one of them, a part of the chemical energy derived from ATP hydrolysis is used to transport Ca^{2+} across the membrane and part is dissipated as heat. In the second route, the hydrolysis of ATP is completed *before* Ca^{2+} transport and all the energy derived from ATP hydrolysis is converted into heat. The second route is activated by the rise of the Ca^{2+} concentration in the vesicle lumen. In vesicles derived from white skeletal muscle the rate of the uncoupled ATPase is several-fold faster than the rate of the ATPase coupled to Ca^{2+} transport, and this accounts for both the low Ca^{2+}/ATP ratio usually measured during transport and for the difference of heat produced during the hydrolysis of ATP by intact and leaky vesicles. Different drugs were found to selectively inhibit the uncoupled ATPase activity without modifying the activity coupled to Ca^{2+} transport. When the vesicles are actively loaded, part of the Ca^{2+} accumulated leaks to the medium through the ATPase. Heat is either produced or released during the leakage, depending on whether or not the Ca^{2+} efflux is coupled to the synthesis of ATP from ADP and P_i.

Key words: Ca^{2+}-ATPase — Thermogenesis — Ca^{2+} transport — Heat production — ATP hydrolysis — ATP synthesis

Introduction

The sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCA) are a family of membrane-bound enzymes that are able to interconvert different forms of energy. The basic reaction sequence of the Ca^{2+}-ATPase (reactions 1 to 6 in Fig. 1) was first proposed in 1976 using vesicles derived from fast skeletal muscle and since then has been widely confirmed in different laboratories [5, 9, 28, 37, 67, 68]. During transport, a part of the chemical energy derived from ATP hydrolysis is used to transport Ca^{2+} across the membrane and it is therefore converted into work. After Ca^{2+} accumulation, a gradient is formed across the sarco/endoplasmic reticulum membrane and part of the energy used for transport is conserved in the form of osmotic energy [28, 35, 36]. During catalysis, a significant part of the energy derived from ATP hydrolysis is dissipated into the surrounding medium as heat. 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 into two non-interchangeable parts, one of which would be used for Ca^{2+} transport and the other converted into heat. In recent reports [14–18, 58, 62] it was found that depending on the conditions used, the amount of heat released during the hydrolysis of ATP may vary between 7 and 32 kcal/mol. This finding indicates that SERCA ATPases are able to handle the energy derived from ATP hydrolysis in such a way as to determine the parcel that is used for Ca^{2+} transport and the fraction that is dissipated as heat. In this view, the total amount of energy released during ATP hydrolysis is always the same, but the fraction of the total energy that is converted into work or heat seems to be modulated by the Ca^{2+}-ATPase. The general interest in heat production and thermogenesis has increased during the past decade due to their implications in health and disease. Heat generation plays a key role in the regulation of the energy balance of the cell and alterations of thermogenesis are noted in several diseases, such as adiposity and thyroid-hormone alterations [8, 42, 46, 48, 60, 64–66]. Skeletal muscle is by far the most abundant tissue of the human body, and accounts for over 50% of the total oxygen con-

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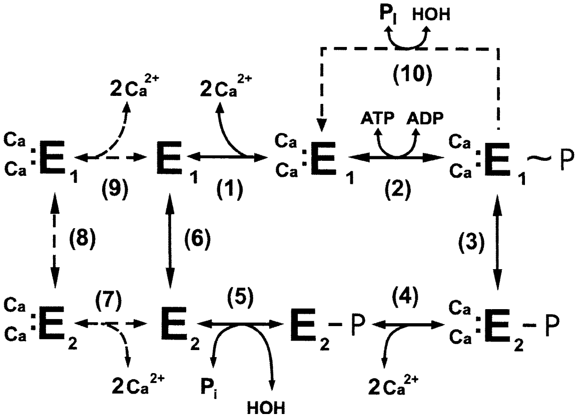

Fig. 1. The catalytic cycle of the Ca^{2+}-ATPase. The sequence includes two distinct enzyme conformations, E_1 and E_2. The Ca^{2+}-binding sites in the E_1 form face the external surface of the vesicle and have a high affinity for Ca^{2+} ($K_a = 10^{-6}$ M at pH 7). In the E_2 form the Ca^{2+}-binding sites face the vesicle lumen and have a low affinity for Ca^{2+} ($K_a = 10^{-3}$ M at pH 7). The enzyme form E_1 is phosphorylated by ATP but not by P_i, forming the high-energy phosphoenzyme $2Ca{:}E_1{\sim}P$ (K_{eq} hydrolysis $\sim 10^6$, $\Delta G^0 \sim -8.4$ kcal/mol). The enzyme form E_2 is phosphorylated by P_i but not by ATP, forming the low-energy phosphoenzyme $2Ca{:}E_2$-P (K_{eq} hydrolysis ~ 1, $\Delta G^0 \sim 0$ kcal/mol). When the Ca^{2+} concentration on the two sites of the membrane is inferior to 50 μM (leaky vesicles), reaction 4 is irreversible and this forces the sequence to flow forward from reaction 1 to 6. Reactions 7 to 10 (dashed lines) are only detected when intact vesicles are used and during transport the Ca^{2+} concentration in the vesicle lumen rises to a value higher than 1 mM. This permits the reversal of reactions 4 and 3, a condition that triggers simultaneously (*i*) the reversal of the catalytic cycle (reactions *5* to *1* backwards) during which the Ca^{2+} efflux is coupled with ATP synthesis; (*ii*) the uncoupled Ca^{2+} efflux mediated by reactions *7* to *9* flowing forward and (*iii*) accumulation of the phosphoenzyme form $2Ca{:}E_1{\sim}P$ and activation of the uncoupled ATPase activity mediated by reaction 10. For details, *see* ref. 5, 10,17, 32, 37, 40, 62, 67, 68, 71.

sumption in a resting human being and up to 90% during very active muscular work. Different studies indicate that the hydrolysis of ATP by the sarcoplasmic reticulum Ca^{2+}-ATPase is one of the heat sources contributing to the thermogenesis of animals without brown adipose tissue [8, 42]. These include measurements of heat release in whole muscle [1, 6], adaptation of fishes and birds to cold environments [1, 30], estimates of the fraction of the total muscle ATP cleaved by the Ca^{2+}-ATPase [8] and the effects of thyroid hormone 3,5,3′-triiodo L-thyronine (T_3) injection [60, 66].

Conversion of Chemical Energy into Work, Osmotic Energy and Heat

This is usually measured using vesicles derived from the sarcoplasmic reticulum of rabbit white skeletal muscle (SERCA1) and assay media containing Ca^{2+} concentrations similar to those found in the cytosol, ranging from 0.1 up to 10 μM [9, 28, 35–37, 40, 67]. When intact vesicles are used, a Ca^{2+} gradient is formed across the vesicles membrane and a part of the chemical energy derived from ATP cleavage is conserved in the form of osmotic energy. If the vesicles are permeabilized (leaky vesicles), then the Ca^{2+} transported through the membrane diffuses back to the assay medium and the chemical energy derived from ATP hydrolysis is continuously converted into work, none being conserved as osmotic energy. In this situation the catalytic cycle involves only reactions 1 to 6 flowing forward (Fig. 1). Heat is produced during transport both in presence and absence of a Ca^{2+} gradient (leaky vesicles), and in the two conditions the amount of heat produced is proportional to the amount of ATP cleaved. However, the amount of heat released during the hydrolysis of each ATP molecule varies depending on whether or not a gradient is formed. In the presence of gradient, between 20 and 24 kcal are released per mol of ATP cleaved and in the absence of gradient, the heat released decreases to the range of 8 to 12 kcal per mol of ATP cleaved [14, 16–18, 58, 62]. This difference is promoted by the uncoupled Ca^{2+} efflux represented by reactions 7 to 9 in Fig. 1 [11, 22, 26, 34, 38] and by the uncoupled ATPase activity (reaction 10) recently discovered [17, 33, 47, 62, 71]. These two activities can only be detected when the vesicles are able to accumulate Ca^{2+} (Tables 1 and 2). From the two activities, the uncoupled ATPase plays a predominant role in establishing the difference of heat measured in presence and absence of a gradient [17, 62]. During transport the hydrolysis of one ATP molecule leads to the translocation of two Ca^{2+} ions across the membrane [9, 32, 35–37, 40, 67, 68]. This value for the Ca^{2+}/ATP stoichiometry however, can only be measured when the experimental conditions used ensure the maintenance of a very low Ca^{2+} concentration in the vesicle lumen, as, for instance, by using a large amount of vesicles in presence of oxalate and a small amount of Ca^{2+}. In this condition, practically all the Ca^{2+} available in the medium is rapidly taken up and stored inside the vesicles as calcium-oxalate crystal, thus maintaining the free lumenal Ca^{2+} concentration in the micromolar range [32, 35–37, 47]. A Ca^{2+}/ATP stoichiometry of two was also clearly demonstrated in transient kinetics experiments, during the first catalytic cycle of the enzyme [9, 39–41]. When the Ca^{2+} concentration inside the vesicles rises to the millimolar range, as it is observed in the cell, then the Ca^{2+}/ATP stoichiometry measured varies between 0.3 and 0.6 [17, 37, 40, 56, 62, 71]. After formation of a gradient, a part of the Ca^{2+} accumulated leaks through the Ca^{2+}-ATPase without synthesizing ATP (Table 1). This leakage is referred to as uncoupled Ca^{2+} efflux [11, 22, 26, 34, 38] and is best measured diluting vesicles previously loaded with Ca^{2+} in a medium containing excess EGTA.

Table 1. Energy transduction during thapsigargin-sensitive Ca^{2+} efflux

Addition to efflux medium	Ca^{2+} efflux μmol/mg·min^{-1}	ATP synthesis μmol/mg·min^{-1}	Heat μcal/mg·min^{-1}	ΔH^{cal} kcal/mol Ca^{2+}
ADP, P_i, Mg^{2+}, K^+	86 ± 10	69 ± 7	+429 ± 112	+5.0 ± 1.0
ADP, P_i, EDTA, K^+	12 ± 3	None	−367 ± 53	−30.0 ± 1.6
EDTA	70 ± 3	None	+129 ± 60	+1.8 ± 0.4

Vesicles were previously loaded with Ca^{2+} and then diluted in a medium containing 50 mM MOPS/Tris buffer pH 7.0, 5 mM EGTA and 5 mM sodium azide. When added to the medium, the concentrations of ADP, P_i, Mg^{2+}, K^+ and EDTA were 0.1, 10, 4, 100, and 5 mM respectively. The values of Ca^{2+} effluxes are the difference between the rates measured with and without 1 μM thapsigargin. The rate of Ca^{2+} efflux measured in presence of thapsigargin was 29 ± 6 nmol/mg·min^{-1}. With thapsigargin there was neither ATP synthesis nor heat release. The assay temperature was 35°C. Values are mean ± SE of 11 to 14 experiments. The calorimetric enthalpy (ΔH^{cal}) was calculated by dividing the rate of heat released by the rate of Ca^{2+} efflux. A negative value indicates that the reaction is exothermic and a positive value indicates that it is endothermic. Data are from ref. 15 and 17.

Table 2. Efficiency of Ca^{2+} transport at steady state: Ca^{2+}/ ATP ratio measured in different experimental conditions

Additions	Ca^{2+} uptake ($Ca^{2+} \Longleftrightarrow Ca^{2+}$ exchange) nmol/mg·min^{-1}	Coupled ATPase nmol/mg·min^{-1}	Uncoupled ATPase nmol/mg·min^{-1}	Ca^{2+}/ATP ratio
ATP 1 mM	285 ± 20	142 ± 10	312 ± 56	0.65 ± 0.08
ATP 1 mM + NaF*	390 ± 30	210 ± 10	10 ± 1	1.84 ± 0.08
0.05 mM ADP + 2 mM PEP + PK	173 ± 37	89 ± 18	737 ± 98	0.26 ± 0.06
0.05 mM ADP + 5 mM Fru-1,6-P + PPK	273 ± 37	129 ± 25	1 ± 1	2.33 ± 0.22
0.05 mM ADP + 5 mM gluc-6-P + HK	237 ± 25	122 ± 8	22 ± 15	2.31 ± 0.43

The assay medium composition was 50 mM MOPS-Tris buffer (pH 7.0), 2 mM $MgCl_2$, 120 μM $CaCl_2$, 100 μM EGTA, 10 mM P_i, 100 mM KCl, 5 mM NaN_3, 10 μM P^1,P^5-di(adenosine 5′) pentaphosphate and the additions shown in the Table. The experiments were performed at 35°C. In the Table, PEP, PK, Fru-l,6-P, PPK, gluc-6-P and HK refers to phosphoenolpyruvate, pyruvate kinase (10 U/ml), fructose 1, 6-diphosphate, phosphofructokinase (10 U/ml), glucose 6 phosphate and hexokinase (10 U/ml); (*) vesicles were preincubated in media containing 2 mM EGTA and 20 mM NaF and then diluted 75-fold in the assay medium. The Ca^{2+}/ATP ratio was calculated dividing the rates of $Ca^{2+} \Longleftrightarrow Ca^{2+}$ exchange by total ATPase activity (coupled + uncoupled). At steady state, the rate of efflux is the same as that of Ca^{2+} uptake, and by measuring the rate of $Ca_{in}{}^{2+} \Longleftrightarrow Ca_{out}{}^{2+}$exchange it is possible to determine the value of the two rates. The exchange represents the fraction of Ca^{2+} that leaves the vesicles and is pumped back inside the vesicles by the ATPase. The values are mean ± SE of 5 to 6 experiments. For further details, see ref. 17 and 62.

The uncoupled Ca^{2+} efflux is inhibited by ligands of the enzyme, which favor the formation of the forms 2Ca:E_1 and E_2-P. These include K^+, a mixture of P_i and Mg^{2+} and the presence of Ca^{2+} in the assay medium [11, 15]. The uncoupled efflux is also impaired by thapsigargin, a specific inhibitor of the Ca^{2+}-ATPase [22, 63], by polyamines, ruthenium red, and dimethyl sulfoxide and it is activated by local anesthetics, phenothyazines, fatty acids, ethanol, heparin and diacylglycerol analogues [4, 19, 25, 29, 31, 57, 69]. The effect of heparin is antagonized by K^+ [14, 25] and that of diacylglycerol analogues is antagonized by cyclosporin A [4]. Until the discovery of the uncoupled ATPase activity, the low values of Ca^{2+}/ATP measured were attributed to the uncoupled Ca^{2+} efflux. In 1995 Yu and Inesi [71] observed that the progressive rise in the Ca^{2+} concentration in the vesicle lumen promotes the hydrolysis of the phosphoenzyme form 2Ca:E_2~P (reaction *10* in Fig. 1) and as a result, the cleavage of ATP is processed without a concomitant Ca^{2+} translocation through the membrane. This was confirmed in different laboratories [17, 33, 47, 62] and was referred to as uncoupled ATPase activity. During transport, a substantial amount of ATP is cleaved through the uncoupled route and depending on the conditions used, the rate of the uncoupled ATPase activity can be 2- to 8-fold faster than the ATPase activity coupled to Ca^{2+} transport (Table 2). When the uncoupled ATPase activity is inhibited, there is both an increase of the Ca^{2+}/ATP ratio (Table 2) and a decrease in the yield of heat produced during ATP cleavage [14, 17, 18, 62], thus abolishing the difference of ΔH^{cal} for ATP hydrolysis measured with intact and leaky vesicles (Table 3). Both the coupled and uncoupled ATPase activity have the same Ca^{2+} dependence [62]. The uncoupled ATPase activity can be specifically inhibited by fluoride [62] and by curcumin, an inhibitor of carcinogenesis. An inhibition of the uncoupled ATPase activity is also observed in presence of a low ATP and high ADP concentration or when the assay medium temperature is decreased from 35°C to 25°C [14, 17, 18].

The substances and conditions that were found to inhibit the uncoupled ATPase activity had no ef-

Table 3. Heat released during the hydrolysis of ATP in presence and absence of a Ca^{2+} gradient.

Additions	ΔH^{cal}, kcal /mol ATP cleaved	
	Intact vesicles (gradient)	Leaky vesicles (without gradient)
ATP 1 mM	-22.9 ± 1.3	-12.2 ± 1.3
ATP 1 mM at low temperature (25°C)	-11.5 ± 0.5	-11.9 ± 0.4
ATP 1 mM + DMSO 20% (v/v)	-13.2 ± 0.7	-12.1 ± 1.0
ATP 1 mM + NaF*	-8.6 ± 2.5	-8.4 ± 1.0
0.05 mM ADP + 2 mM PEP + PK	-15.5 ± 1.3	-7.1 ± 0.7
0.05 mM ADP + 5 mM Fru-1,6-P + PPK	$+7.0 \pm 0.7$	$+1.0 \pm 0.1$
0.05 mM ADP + 5 mM gluc-6-P + HK	$+10.2 \pm 1.1$	$+0.5 \pm 0.1$

Except where indicated (25°C), all assays were performed at 35°C. Other conditions were as described in Tables 1 and 2. The calorimetric enthalpy (ΔH^{cal}) was calculated by dividing the amount of heat released by the amount of ATP hydrolyzed. The units used were moles for substrate hydrolyzed and kcal for the heat released. A negative value indicates that the reaction is exothermic and a positive value indicates that it is endothermic. For details, *see* ref. 14, 17, 18, 58 and 62.

*Vesicles were preincubated in media containing 2 mM EGTA and 20 mM NaF and then diluted 75-fold in the assay medium.

fect on the values of the ΔH^{cal} for ATP hydrolysis measured with leaky vesicles because the uncoupled Ca^{2+} efflux and ATPase activity are only activated when the Ca^{2+} concentration in the vesicle lumen rises to the millimolar range [33, 71].

Conversion of Osmotic Energy into Chemical Energy and Heat

The reversal of the catalytic cycle of the Ca^{2+}-ATPase and conversion of osmotic energy into chemical energy (reactions 6 to 1 flowing backwards in Fig. 1) were first demonstrated by Makinose and Hasselbach in 1971 [52, 54] and afterwards confirmed in different laboratories [28, 32, 36, 37, 67, 68]. When the vesicles are previously loaded with Ca^{2+} and then diluted in a medium containing ADP, P_i, Mg^{2+} and excess EGTA, the Ca^{2+} retained by the vesicles is released into the medium at a fast rate and coupled with the efflux of two Ca^{2+} ions, and one ATP molecule is synthesized from ADP and P_i (Table 2). The process of ATP synthesis is endergonic and 11.3 kcal are absorbed from the medium during the synthesis of one ATP mole [15, 17]. Ca^{2+} is released from the vesicles at a slower rate if one of the reactants needed for ATP synthesis (ADP, P_i or Mg^{2+}) is omitted from the medium. In this case the Ca^{2+} efflux is exergonic, and 16.3 kcal are released for each mol of Ca^{2+} released. The rate of Ca^{2+} efflux can be increased to the same level as that measured during the synthesis of ATP if all reactants of the enzyme are removed form the medium i.e., the efflux is measured in presence of excess EGTA and in absence of ADP, P_i, Mg^{2+} and K^+. In this condition, however, during the Ca^{2+} efflux there is neither ATP synthesis nor heat production [15, 17]. These experiments indicate that the Ca^{2+}-ATPase can function in at least three different states (Table 2). In the first state it uses the energy derived from the gradient to synthesize ATP; in the second state, the energy derived from the gradient is converted into heat and in the third state, the Ca^{2+}-ATPase is uncoupled and cannot convert the energy derived from the gradient into either chemical energy or into heat. The Ca^{2+} efflux measured in the three states as well as the synthesis of ATP measured in state 1 and the heat released measured in state 2 are all impaired by thapsigargin, a specific inhibitor of the Ca^{2+}-ATPase [63].

Conversion of Thermal Energy into either Osmotic or Chemical Energy

The SERCA are able to use glucose 6-P and hexokinase or fructose 1,6-P and phosphofructokinase as ATP-regenerating system [17, 59, 61]. The affinity of the different SERCA for ATP is sufficiently high ($K_a \sim 10^{-6}$ M) to permit the formation of the enzyme-substrate complex even in the presence of the very low concentration of ATP formed from ADP and either glucose-6-P or fructose-1,6-P. During Ca^{2+} uptake, the ADP formed after ATP hydrolysis is phosphorylated by the sugar phosphate in order to maintain the equilibrium concentration of ATP. Thus, in steady-state conditions, the Ca^{2+} transport proceeds as if it were supported by the cleavage of the sugar phosphate, as shown for glucose-6-P by the addition of the reactions

$$\text{glucose-6-P} + \text{ADP} \Leftrightarrow \text{glucose} + \text{ATP} \tag{1}$$

$$\underline{\text{ATP} + \text{HOH} \Leftrightarrow \text{ADP} + P_i} \tag{2}$$

$$\text{glucose-6-P} + \text{HOH} \Leftrightarrow \text{glucose} + P_i \tag{3}$$

The difference between the use of sugar phosphate and either phosphoenolpyruvate or phosphocreatine as ATP-regenerating systems is the amount of ADP available in the medium during the reaction. While with phosphoenolpyruvate or phosphocreatine there is practically no ADP available, with the sugar phosphate most of the nucleotide in the medium is in the form of ADP. Curiously, with the use of sugar phosphate, the uncoupled ATPase activity is practically abolished and the transport is no longer exergonic, but on the contrary, it is endothermic, and the amount of heat absorbed from the environment with intact vesicles is larger than that measured with leaky vesicles (Tables 2 and 3). At present it is not clear why the Ca^{2+} transport is endothermic when sugar phosphates are used. One possibility is the different values of ΔG^0 and ΔG of the phosphate compounds used. The ΔG^0 reported in the bibliography for ATP hydrolysis varies between −7.0 and −8.0 kcal/mol and for phosphoenolpyruvate, fructose-1,6-P or glucose-6-P is −14.0, −2.8 and −2.5 kcal/mol respectively [17]. The ΔG values calculated taking into account the concentrations of reactants and products found in the medium during the experiments reported vary between −10.1 and −11.9 for 1 mᴍ ATP, between −18.0 and −18.6 for phosphoenolpyruvate, between −7.1 and −7.4 for fructose-l,6-P, and between −6.8 to −7.0 for glucose-6-P [17]. These values suggest that the Ca^{2+}-ATPase is able to use the thermal energy available in the medium to help the pumping of Ca^{2+} into the vesicles when sugar phosphates are used as ATP-regenerating system. This possibility is supported by early reports [21, 23], showing that the Ca^{2+}-ATPase is able to catalyze the synthesis of ATP from ADP and P_i after a rapid temperature transition. In these reports, synthesis was measured in the absence of a transmembrane Ca^{2+} gradient and led to the conclusion that the Ca^{2+}-ATPase can convert thermal energy into chemical energy.

Energy Conservation during Steady State

During Ca^{2+} transport the enzyme is able to re-synthesize a part of the ATP previously cleaved. In early reports [9, 28, 32, 36, 52, 67, 68] this was referred to as the ATP$\Longleftrightarrow$$P_i$ exchange reaction and can be best measured after the vesicles are filled with Ca^{2+} and when the steady state is reached. In this condition the Ca^{2+} concentrations inside the vesicles and in the assay medium remain constant but the enzyme operates simultaneously forward (ATP hydrolysis and Ca^{2+} uptake), and backwards (Ca^{2+} efflux and ATP synthesis) and chemical and osmotic energy are continuously interconverted by the ATPase. The ratio between the rates of ATP hydrolysis and ATP synthesis varies depending on the experimental conditions used [5, 9, 10, 12, 17, 62] and gives a measure of the degree of energy conservation of the system. The more ATP is synthesized, the smaller is the ratio between the rates of hydrolysis and synthesis, and the more energy is conserved by the system, i.e., the steady state can be conserved for a longer period of time because the net decline of the ATP concentration in the medium proceeds at a slower rate.

Energy Interconversion in Absence of a Ca^{2+} Gradient

The Ca^{2+}-ATPase is able to modulate the interconversion of different forms of energy even in the absence of a transmembrane Ca^{2+} gradient. Leaky vesicles can catalyze both the hydrolysis and the synthesis of ATP when the Ca^{2+} concentration in the medium is raised to the range of 2 to 10 mᴍ i.e., to a level similar to that found inside intact vesicles during transport [5, 20, 28, 37, 67, 68]. This promotes both a decrease of the rate of ATP hydrolysis and activation of the synthesis of ATP. The ratio between the rates of hydrolysis and synthesis measured with leaky vesicles is about one order of magnitude higher than that measured with intact vesicles, i.e., the degree of energy conservation attained with leaky vesicles is much smaller than that measured in presence of a Ca^{2+} gradient. The heat released after the hydrolysis of each ATP molecule decreased when the Ca^{2+} concentration in the medium was raised from 0.1 to 2.0 mᴍ. The decrease is noted at the same Ca^{2+} concentrations that activate the ATP$\Longleftrightarrow$$P_i$ exchange reaction. If one of the reactants needed for ATP synthesis, such as P_i, is omitted from the medium, then, when the Ca^{2+} concentration is raised from 0.1 to 2.0 mᴍ, there is neither ATP synthesis nor decrease of heat produced during ATP cleavage [13]. This finding suggests that the binding of Ca^{2+} to the low-affinity site of the enzyme will permit the ATPase to retain a part of the chemical energy derived from the hydrolysis of ATP previously cleaved in a form that permits the resynthesis of a new ATP molecule from ADP and P_i. This energy is not retained and it is dissipated as heat if Ca^{2+} does not bind to the low-affinity site of the enzyme.

Ca^{2+} Transport and Heat Production by Endothermic and Poikilothermic Animals—Effect of Temperature

This subject was explored using vesicles derived from the sarcoplasmic reticulum vesicles of rabbit white muscle, trout muscle and smooth muscle of the sea cucumber *Ludwigothurea grisea* [7, 14, 16, 45]. The rates of Ca^{2+} uptake of the rabbit (endothermic) and trout (poikilothermic) vesicles increase with the temperature and when the steady state is reached, the amounts of Ca^{2+} retained at 25°C by the two vesicle preparations are practically the same. The trout Ca^{2+}-ATPase is unstable at temperatures higher than 25°C and is inactivated after a few minutes incubation at 35°C [7]. The rabbit ATPase, however, is stable for more than one hour at 35°C. The trout used for the preparation of the vesicles were adapted to a temperature varying between 20 and 25°C, whereas the rabbit physiological body temperature is 37°C. Thus, in spite of the fact that the two enzymes can pump similar amounts of Ca^{2+} at 25°C, at the physiological body temperature of the rabbit, rabbit sarcoplasmic reticulum is able to pump more Ca^{2+} and at a faster rate than the reticulum of the trout. After formation of the gradient, both the rabbit and the trout Ca^{2+}-ATPases are able to synthesize a small amount of ATP and in all conditions tested, the rate of synthesis is 25 to 45 times smaller than the rate of ATP hydrolysis. The heat released for each ATP molecule hydrolyzed varies depending on the temperature of the assay and the source of the vesicles used. For the rabbit, the value of ΔH^{cal} measured at 35°C with intact vesicles is double that measured with leaky vesicles (Table 3). This difference is no longer detected when the temperature is decreased to 25°C, as if in the rabbit the mechanism responsible for the gradient-dependent heat production were turned off when the temperature is decreased to a level far away from the physiologic body temperature. For the trout vesicles (poikilotherm), formation of a transmembrane Ca^{2+} gradient at 25°C leads to a change of the ΔH^{cal} for ATP hydrolysis to a value similar to that measured with the rabbit vesicles at 35°C. The difference of ΔH^{cal} values measured with trout vesicles in the presence and absence of a Ca^{2+} gradient is also abolished when the temperature of the medium is decreased, but in this case, to a value below 17°C. The ΔH^{cal} measured with leaky vesicles does not vary with the temperature nor with the source of the vesicles used. These data indicate that the gradient-dependent heat production is arrested when the temperature of the medium is decreased more than 5°C below the physiological body temperature, i.e., below 30°C for the rabbit and below 20°C for the trout [14]. The enhancement of heat production associated with the gradient could therefore play a physiological role in the maintenance of the body temperature but would not be a good emergency system to raise the temperature after rapid cooling of the animal to an extreme point that leads to a large variation of the body temperature. Similar to the rabbit and trout, vesicles derived from the reticulum of the sea cucumber smooth muscle are able to accumulate Ca^{2+} and during transport, the sea cucumber vesicles catalyze simultaneously the hydrolysis and the synthesis of ATP. Formation of a transmembrane Ca^{2+} gradient across the sea cucumber vesicles also leads to a change of the ΔH^{cal} for ATP hydrolysis to a value similar to that measured with the trout and rabbit vesicles [45].

Energy Transduction by Different Ca^{2+}-ATPase Isoforms

In addition to muscle cells, Ca^{2+}-ATPase isoforms are found in practically all animal tissues so far studied. Three distinct genes encode the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) isoforms, but the physiological meaning of isoform diversity is not clear. The SERCA 1 gene is expressed exclusively in fast skeletal muscle, whereas blood platelets express SERCA 3 and SERCA 2b genes [49–51, 70]. Vesicles derived from blood platelet endoplasmic reticulum are able to accumulate a smaller amount of Ca^{2+} than the vesicles derived from fast skeletal muscle [25, 57, 58]. During transport, the two vesicle preparations catalyze simultaneously the hydrolysis and the synthesis of ATP from ADP and P_i. However, different from the muscle, the yield of heat produced during ATP cleavage by the platelet vesicles is the same in presence and absence of a transmembrane Ca^{2+} gradient [58]. The situation is modified when DL-α-phosphatidylcholine β-acetyl-γ-*O*-hexadecyl is added to the assay medium. This phospholipid belongs to a family of acetylated phospholipids known as platelet activating factors (PAF) that are produced when cells involved in an inflammatory process are activated. In blood platelet vesicles, PAF increase the uncoupled Ca^{2+} efflux and enhance the amount of heat produced during the hydrolysis of ATP, reaching the same ΔH^{cal} values as those measured with vesicles of fast skeletal muscle [58]. This effect is specific for blood platelet vesicles. In muscle vesicles, PAF also promotes an increase of Ca^{2+} efflux but this effect is less pronounced than that measured with platelet vesicles and is not accompanied by an increase in the amount of heat produced during ATP hydrolysis.

Energy Transduction during the Catalytic Cycle

The key feature of the cycle shown in Fig. 1 is the mechanism by which energy is transduced. The sequence of events proposed in earlier models of active transport assumed that energy was released at the moment of hydrolysis of the phosphate compound. The energy would then be absorbed by the enzyme and used to perform work. In this view, the energy of hydrolysis of phosphate compounds would be the same regardless of whether the phosphate compound was in solution in the cytosol or bound to the enzyme surface [10, 12, 53]. In aqueous solutions an acyl phosphate residue has a high energy of hydrolysis, the equilibrium constant for the hydrolysis (K_{eq}) being practically the same as that of ATP. In 1973 it was found that the ATPase could be spontaneously phosphorylated by P_i forming a phosphoaspartate without the need of energy input into the system (reaction *5* backward, $K_{\mathrm{eq}} \sim 1$; $\Delta G^0 \sim 0$). This was only observed in absence of Ca^{2+}, i.e., when the enzyme was stabilized in the E_2 form [5, 24, 28, 32, 36, 37, 43, 55, 67, 68]. The low-energy E_2-P could be converted back into the high-energy form $2Ca{:}E_1{\sim}P$ (reactions *4* and *3* backwards) when the Ca^{2+} concentration in the medium was suddenly raised to the range of 1 to 3 mM [20, 21, 27, 28, 32, 37, 44, 67], i.e., to a range similar to that found in the vesicle lumen after formation of a transmembrane Ca^{2+} gradient (~5 mM at pH 7.0). The 2Ca:E_1~P form (but not the E_2-P) can transfer its phosphate to ADP (reaction *2* backwards), forming soluble ATP [20, 27, 44]. The finding that the energy of hydrolysis of the phosphoenzyme varies during the catalytic cycle indicates that the energy needed for the translocation of Ca^{2+} through the membrane is used *before* the cleavage of the phosphoenzyme (reaction *3*), i.e., it is during the conversion of 2Ca:E_1~P into 2Ca:E_2-P that the chemical energy is used to perform work. Yu and Inesi [33, 71] showed that the uncoupled ATP hydrolysis is derived from the cleavage of the high-energy phosphoenzyme form 2Ca:E_1~P, while the coupled ATPase activity involves the cleavage of the low-energy phosphoenzyme *E_2-P* and according to the cycle of Fig. 1, chemical energy is converted into either work (leaky vesicles) or osmotic energy during the transition of the high-energy into low-energy phosphoenzyme (reactions *3* to *5*). Simultaneously with the discovery of the low-energy phosphoenzyme E_2-P [24, 55] in Paul Boyer's laboratory [2, 3] it was found that ATP could be spontaneously formed at the catalytic site of mitochondrial F_1 ATPase, and in the subsequent years it has become apparent that the conversion of a phosphate compound from "high" into "low" energy is a general feature found in different enzymes involved in processes of energy transduction such as the Na^+/K^+-ATPase, myosin, inorganic pyrophosphate and hexokinase [10, 12, 68]. After the description of the catalytic cycle of the Ca^{2+}-ATPase it was proposed that the energy change of the phosphoenzyme (reaction *3*) was related to a change in water activity in the microenvironment of the catalytic site [23]. The energy of hydrolysis of different phosphate compounds is determined by the differences in solvation energy between the reactant and products. Thus, a change of water activity in the catalytic site of the enzyme leads to a change in solvation energy of the phosphoenzyme and to a change in its energy of hydrolysis. Experimental evidences supporting this possibility were obtained in various laboratories and were described in reviews previously published [9, 10, 12, 68]. As for the phosphoaspartate at the catalytic site of the Ca^{2+}-ATPase, the conversion of the "low energy" tightly-bound ATP into the "high-energy" loosely-bound ATP seems to be promoted by a change of water activity at the catalytic site of the mitochondrial F_1 ATPase [2, 10, 12].

Heat Production during Futile Cycles of ATP Hydrolysis

In vesicles derived from white skeletal muscle (SERCA 1) the rate of the uncoupled ATPase activity is several-fold faster than the rate of the coupled ATPase activity (Table 3). When extended to the living cell, the cleavage of ATP through reaction *10* could be considered an apparently futile cycle without a physiological purpose; ATP is cleaved without apparent work and then the ADP produced is phosphorylated by the mitochondria leading to an increase in oxygen consumption. The data discussed above suggest that the uncoupled ATPase activity may represent an important route of heat production that contributes to the thermogenic control of the cell. Not only does the yield of heat produced during the hydrolysis of ATP double (Table 3), but the ADP produced leads to an increase in the mitochondrial respiration with more heat production. To a smaller degree, the same reasoning can be extended for the uncoupled Ca^{2+} efflux. The Ca^{2+} pumped at the expense of ATP cleaved leaks to the medium through reactions *7* to *9* and as a result, there is no net Ca^{2+} uptake during ATP cleavage and the ADP produced should also lead to an increase of mitochondrial respiration. In conclusion, the discovery of the uncoupled ATPase [71] and the finding that heat produced during ATP hydrolysis varies depending on the catalytic route selected [17, 18, 62] suggest that the SERCA 1 may be involved in two different physiological processes, one related to the regulation of the cytosolic Ca^{2+} concentration, and a second related to thermogenesis and regulation of the energy balance of the cell.

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