Uncoupled ATP Hydrolysis and Thermogenic Activity of the Sarcoplasmic Reticulum Ca\textsuperscript{2+}-ATPase

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The sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase transports Ca\textsuperscript{2+} using the energy derived from ATP hydrolysis. During catalysis, part of the energy is used to translocate Ca\textsuperscript{2+} across the membrane, and part is dissipated as heat. At 35 °C the heat released during the hydrolysis of each ATP molecule varies depending on the formation of a Ca\textsuperscript{2+} gradient across the membrane. With leaky vesicles (no gradient) the heat released varies between 9 and 12 kcal/mol of ATP cleaved, and with intact vesicles (gradient), the heat released increases to 20–24 kcal/mol of ATP. After Ca\textsuperscript{2+} accumulation, 82% of the Ca\textsuperscript{2+}-ATPase activity is not coupled to Ca\textsuperscript{2+} transport, and the ratio between Ca\textsuperscript{2+} transported and ATP cleaved is 0.3. The addition of 20% dimethyl sulfoxide (v/v) to the medium or decreasing the temperature from 35 to 20 °C abolishes the difference of heat produced during ATP hydrolysis in the presence and absence of a gradient. This is accompanied by a simultaneous inhibition of the uncoupled ATPase activity and an increase of the Ca\textsuperscript{2+}/ATP ratio from 0.3 to 1.3–1.4. It is concluded that the uncoupled Ca\textsuperscript{2+}-ATPase is responsible for both the low Ca\textsuperscript{2+}/ATP ratio measured during transport and the difference of heat produced during ATP hydrolysis in the presence and absence of a gradient.

Vesicles derived from the sarcoplasmic reticulum of rabbit white skeletal muscle retain a membrane-bound Ca\textsuperscript{2+}-ATPase, which is able to pump Ca\textsuperscript{2+} across the vesicle membrane using the chemical energy derived from ATP hydrolysis. During Ca\textsuperscript{2+} transport chemical and osmotic energy is interconverted by the ATPase (1, 2). This is represented in Fig. 1 as reactions 1–6 flowing forward and backwards (3–6). A part of the energy released during ATP hydrolysis is not used for transport and is converted into heat. Recently (7–12) it was found that at physiological temperature (35 °C), the heat derived from ATP hydrolysis varies depending on whether or not a Ca\textsuperscript{2+} gradient is formed across the vesicle membrane. With leaky vesicles (no gradient), 8–12 kcal are released during the hydrolysis of 1 mol of ATP. After formation of a Ca\textsuperscript{2+} gradient (intact vesicles) the heat released increased to the range of 20 to 24 kcal/mol of ATP cleaved. These findings indicate that Ca\textsuperscript{2+}-ATPase is able to handle the energy derived from ATP hydrolysis in such a way as to determine the parcel that is used for Ca\textsuperscript{2+} accumulation and the fraction that is dissipated as heat. The difference in heat measured in the presence and absence of a Ca\textsuperscript{2+} gradient is abolished when Me\textsubscript{2}SO\textsuperscript{(20% v/v)} is added to the medium or when the temperature of the assay medium is decreased from 35 to 20 °C (7, 8, 11). At present, we do not know why these two conditions decrease the heat production measured with Ca\textsuperscript{2+}-loaded vesicles.

During catalysis the hydrolysis of one ATP molecule leads to the translocation of two Ca\textsuperscript{2+} ions across the membrane (Fig. 1). This was measured using large amounts of vesicles, oxalate, and a small amount of Ca\textsuperscript{2+}. In this condition, practically all of the Ca\textsuperscript{2+} available in the medium is rapidly stored inside the vesicles as calcium oxalate crystals, which ensures the maintenance of a low luminal free Ca\textsuperscript{2+} concentration (100 µM) (1, 2). A stoichiometry of two Ca\textsuperscript{2+} ions transported for each ATP cleaved was also clearly demonstrated in transient kinetics experiments, where the transport was measured during the first catalytic cycle of the enzyme, i.e. before the luminal Ca\textsuperscript{2+} concentration raised to a high level (4, 6, 13, 14). Oxalate is not available in muscles, and during transport the Ca\textsuperscript{2+} concentration inside the reticulum increased from 5 to 20 mM (4). When this is reproduced in vitro, the Ca\textsuperscript{2+}/ATP stoichiometry measured varies between 0.3 and 0.6 (6, 10, 12, 15, 16). A high luminal Ca\textsuperscript{2+} concentration leads to leakage of Ca\textsuperscript{2+} through the ATPase (reactions 7–9 in Fig. 1) referred to as uncoupled Ca\textsuperscript{2+} efflux (10–12, 15, 17, 18). In earlier reports the low values of Ca\textsuperscript{2+}/ATP measured during transport were attributed to the leakage of Ca\textsuperscript{2+} from the vesicle. In 1995 Yu and Inesi (19) observed that the progressive rise of the intravesicular Ca\textsuperscript{2+} concentration promotes the hydrolysis of ATP without concomitant Ca\textsuperscript{2+} translocation through the membrane (reaction 10 in Fig. 1). This was confirmed in different laboratories (10, 12, 20, 21) and was referred to as uncoupled ATPase activity. In conditions similar to those found in the cell, the rate of the uncoupled ATPase activity is 2–8 times faster than the activity coupled to Ca\textsuperscript{2+} transport (10, 12). The uncoupled Ca\textsuperscript{2+} efflux and uncoupled ATPase activity account for the low Ca\textsuperscript{2+}/ATP stoichiometry measured during transport. Recent reports indicate that the extra amount of heat measured during ATP hydrolysis with Ca\textsuperscript{2+}-loaded vesicles could be derived from the uncoupled ATPase activity (10, 12).

In this report, we measured the rates of uncoupled Ca\textsuperscript{2+} efflux and uncoupled ATPase activity and heat production at 20 °C and at 35 °C in the presence and absence of dimethyl sulfoxide. The aim was to establish whether or not there is a converter the energy derived from ATP hydrolysis in such a way as to determine the parcel that is used for Ca\textsuperscript{2+} accumulation and the fraction that is dissipated as heat. The difference in heat measured in the presence and absence of a Ca\textsuperscript{2+} gradient is abolished when Me\textsubscript{2}SO\textsuperscript{(20% v/v)} is added to the medium or when the temperature of the assay medium is decreased from 35 to 20 °C (7, 8, 11). At present, we do not know why these two conditions decrease the heat production measured with Ca\textsuperscript{2+}-loaded vesicles.

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In this report, we measured the rates of uncoupled Ca\textsuperscript{2+} efflux and uncoupled ATPase activity and heat production at 20 °C and at 35 °C in the presence and absence of dimethyl sulfoxide. The aim was to establish whether or not there is a
correlation between the uncoupled activities of the Ca\(^{2+}\)-ATPase and the differences of heat production measured in the presence and absence of a Ca\(^{2+}\) gradient. In the discussion, the relevance of these data to thermogenesis is debated.

**RESULTS**

**Ca\(^{2+}\) Transport, ATP Hydrolysis, and ATP Synthesis**—Both the initial velocities of Ca\(^{2+}\) uptake and the amount of Ca\(^{2+}\) retained by the vesicles were found to vary depending on the conditions used (Figs. 2 and 3). The Ca\(^{2+}\) concentration in the lumen of intact vesicles reaches the millimolar range a few seconds after the transport is initiated (2–6). This triggers the reversal of the catalytic cycle of the ATPase (2–6, 25, 26) during which Ca\(^{2+}\) leaves the vesicles through the ATPase, and ATP is synthesized from ADP and P\(_i\). In control experiments, the ATPase activity was determined at 35 °C (Table I). The addition of Me\(_2\)SO promoted both a decrease of ATP hydrolysis and a large increase of ATP synthesis rates. As a result, about 30% of the ATP cleaved during transport is synthesized back. On the other hand, there was practically no ATP synthesis at 20 °C (Table I).

When the vesicles are still being filled the rate of Ca\(^{2+}\) uptake measured represents a balance between the Ca\(^{2+}\)-pumped inside the vesicles by the ATPase and the rate of Ca\(^{2+}\) that leaves the vesicles driven by the gradient formed across the membrane. During the initial minutes of incubation these two rates are different and cannot be measured separately. Thus, the stoichiometry between the fluxes of Ca\(^{2+}\) through the membrane and the rates of either ATP cleavage or ATP synthesis cannot be evaluated with precision. After the steady state is reached, the rate of efflux is the same as that of Ca\(^{2+}\) uptake, and by measuring the rate of Ca\(^{2+}\) efflux measured by filterations (2). For \(^{45}\)Ca\(^{2+}\) uptake, trace amounts of \(^{45}\)Ca were included in the assay medium. The reaction was arrested by filtering samples of the assay medium in Millipore filters. After filtration, the filters were washed five times with 5 ml of 3 mol La(NO\(_3\))\(_3\) and the radioactivity remaining on the filters was counted using a liquid scintillation counter. For the Ca\(^{2+}\) in \(\rightleftharpoons\) Ca\(^{2+}\) out exchange the assay medium was divided into two samples. Trace amounts of \(^{45}\)Ca\(^{2+}\) were added to only one of the samples, and the reaction was started by the simultaneous addition of vesicles to the two media. The sample containing the radioactive Ca\(^{2+}\) was used to determine the incubation time where the vesicles were filled and the steady state was reached by adding a trace amount of \(^{45}\)Ca\(^{2+}\) to the second sample containing vesicles loaded with non-radioactive Ca\(^{2+}\). The exchange of the radioactive Ca\(^{2+}\) from the medium with the non-radioactive Ca\(^{2+}\) contained inside the vesicles was measured by filtering samples of the assay medium in Millipore filters at different intervals after the addition of \(^{45}\)Ca\(^{2+}\). ATPase Activity ATP Synthesis—These were assayed using either \(\left[\gamma^{32}\right]\)ATP or \(\left[\gamma^{32}\right]P\) as previously described (24).

Heat of Reaction—These were measured using an OMEGA isothermal titration calorimeter from Microlab Inc. (Northampton, MA) (7–12). The calorimeter cell (1.5 ml) was filled with reaction medium, and the reference cell was filled with Milli-Q water. After equilibration at the desired temperature, the reaction was started by injecting vesicles into the reaction cell, and the heat change during ATP hydrolysis was recorded for 20–100 min. The volume of vesicle suspension injected in the cell varied between 0.02 and 0.03 ml. The heat change measured during the initial 2 min after vesicle injection was discarded to avoid artifacts such as the heat derived from the dilution of the loaded vesicles into the reaction medium and the binding of ions to the Ca\(^{2+}\)-ATPase. The duration of these events is less than 1 min. The calorimetric enthalpy (\(\Delta H^{\text{cal}}\)) 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.
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Fig. 2. Ca\(^{2+}\) uptake, Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange (A), ATP hydrolysis (B), and ATP synthesis (C) at 35 °C in the presence and absence of Me\(_2\)SO. The assay medium composition was 50 mM MOPS-Tris buffer (pH 7.0), 1 mM ATP, 4 mM MgCl\(_2\), 0.21 mM CaCl\(_2\), 0.20 mM EGTA, 10 mM P\(_2\), 100 mM KCl, and 5 mM Na\(_2\)PO\(_4\) without Me\(_2\)SO (closed symbols) and with 20% (v/v) Me\(_2\)SO (open symbols). The reaction was started by the addition of vesicles, 10 μg of protein/ml; A, Ca\(^{2+}\) uptake (○, ○) and Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange (■, □). The rate of Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange was measured as described under “Experimental Procedures,” and the arrow indicates the addition of trace amounts of \(^{45}\)Ca\(^{2+}\) to the tube containing vesicles loaded with non-radioactive Ca\(^{2+}\). The calculated free Ca\(^{2+}\) concentration in the media was 10.1 μM (12). The figure shows a representative experiment.

Fig. 3. Ca\(^{2+}\) uptake, Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange (A) and ATP hydrolysis (B) measured at 20°C. The assay medium composition was 50 mM MOPS-Tris buffer (pH 7.0), 1 mM ATP, 1 mM MgCl\(_2\), 0.21 mM CaCl\(_2\), 0.20 mM EGTA, 10 mM P\(_2\), 100 mM KCl, and 5 mM Na\(_2\)PO\(_4\). The reaction was started by the addition of vesicles, 40 μg of protein/ml; A, Ca\(^{2+}\) uptake (○) and Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange (○). The calculated free Ca\(^{2+}\) concentration in the medium was 10.1 μM. The figure shows a representative experiment.

1.4 and 1.3 when either the temperature is decreased to 20 °C or when Me\(_2\)SO is added to the medium, respectively. (iii) The rates of coupled and uncoupled Ca\(^{2+}\) efflux; in different laboratories it has been shown that during coupled efflux (reactions 5 to 1 flowing backwards), the release of two Ca\(^{2+}\) ions from the vesicles drives the synthesis of one ATP molecule (2–6, 10, 25, 26). The coupled Ca\(^{2+}\) efflux was therefore calculated by multiplying the rate of ATP synthesis by 2, and the difference between the rate of Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange, and the coupled Ca\(^{2+}\) efflux represents the uncoupled Ca\(^{2+}\) efflux (reactions 7–9 in Fig. 1). The coupled efflux increased 6-fold after the addition of Me\(_2\)SO and was abolished at 20 °C (Table II). On the other hand, the uncoupled Ca\(^{2+}\) efflux was decreased 2-fold after the addition of Me\(_2\)SO and 3-fold at 20°C. (iv) The rates of ATP hydrolysis coupled and uncoupled to the translocation of Ca\(^{2+}\), for the calculations of which we used the values of net ATP hydrolysis and the stoichiometry of two Ca\(^{2+}\) ions pumped for each ATP molecule cleaved. Thus, the rate of Ca\(^{2+}\)\(_{\text{in}}\) ⇔ Ca\(^{2+}\)\(_{\text{out}}\) exchange shown in Table I divided by 2 gives the rate of coupled ATP hydrolysis, i.e., the ATP cleaved to pump back the Ca\(^{2+}\) that leaves the vesicles during the exchange (reactions 1–5 in Fig. 1). The difference between the total net ATP hydrolysis and the coupled ATP hydrolysis gives the value of the uncoupled ATPase activity (reactions 2 and 10 in Fig. 1). At 35 °C the rate of the uncoupled ATPase activity was 4.6 times faster than the ATPase activity coupled to Ca\(^{2+}\) transport. Similar values have been found in previous reports (10, 12). We now show that there is a substantial decrease of both the uncoupled ATPase activity and the ratio between the uncoupled and coupled ATPase activity when the temperature is decreased from 35 °C to 20 °C or when Me\(_2\)SO is added to the medium (Table II).

Correlation between Heat Production, Ca\(^{2+}\) Transport and ATP Hydrolysis—In these experiments the rate of heat release and substrate hydrolysis were measured simultaneously in leaky vesicles (no gradient) and intact vesicles (gradient). For intact vesicles, the values of hydrolysis were corrected for the ATP synthesized back at the different incubation intervals (net hydrolysis). Within the Ca\(^{2+}\) concentrations range used, there was no ATP synthesis when leaky vesicles were used (2–6), regardless of the temperature or the addition of Me\(_2\)SO to the medium. With intact vesicles (gradient) the rate of heat production during ATP hydrolysis and Ca\(^{2+}\) transport at 35 °C was several times faster than that measured either at 20 °C or in the presence of Me\(_2\)SO. The amount of heat released in the presence and absence of a Ca\(^{2+}\) gradient was proportional to the amount of ATP hydrolyzed, both during the initial incubation intervals of Ca\(^{2+}\) uptake, and after that the steady state was reached. This could be visualized by plotting the heat release as a function of the amount of ATP hydrolyzed (Figs. 4B and 5) or calculating the calorimetric enthalpy (ΔHTotal) of ATP hydrolysis (Table III). In earlier reports (7–12), it was found that at 35 °C the heat released for each ATP molecule hydrolyzed by intact vesicles (gradient) was double that measured with leaky vesicles (no gradient) and that this difference was abolished when either the temperature was decreased to 20 °C or when Me\(_2\)SO was added to the medium. This was confirmed in Figs. 4B and 5A and Table III using the same conditions as those used for the measurement of coupled and uncoupled Ca\(^{2+}\) efflux and ATP hydrolysis (Table II). The difference between the ΔHTotal of ATP hydrolysis measured in the presence and in the absence of gradient was no longer observed when the temperature was lowered to 20 °C or when Me\(_2\)SO was added to the medium (Table III). In addition to abolish the difference of ΔHTotal, these conditions also promoted a significant decrease of the uncoupled ATPase activity (Table II), thus suggesting that the uncoupled ATPase activity indeed contribute to the extra amount of heat produced when ATP is cleaved by the Ca\(^{2+}\)-loaded vesicles. In a previous report (10, 27) the heat produced during the unidirectional Ca\(^{2+}\) movement from the vesicle lumen to the medium was measured by diluting vesicles previously loaded with Ca\(^{2+}\) in efflux media containing ADP, P\(_i\), Mg\(^{2+}\), or K\(^+\). These experiments revealed that the Ca\(^{2+}\)-ATPase can function at least in two different forms: (i) it absorbs heat from the medium when the efflux is coupled to ATP synthesis (ΔHTotal + 5.7 kcal/mol of Ca\(^{2+}\) released); (ii) it
TABLE I
Rates of ATP hydrolysis, ATP synthesis, and Ca$^{2+}$\textit{in} $\Rightarrow$ Ca$^{2+}$\textit{out} exchange at steady state

| Conditions | $n$ | ATP hydrolysis (a) | ATP synthesis (b) | Net ATP hydrolysis (c) | Ca$^{2+}$\textit{in} $\Rightarrow$ Ca$^{2+}$\textit{out} exchange (d) | ATP hydrolysis\textit{/}ATP synthesis (a/b) | Ca$^{2+}$/ATP ratio (d/c) |
|------------|----|-------------------|-------------------|-----------------------|-------------------------------------------------|--------------------------------|--------------------------|
| 35°C       | 7  | 1,422 ± 125       | 29 ± 2            | 1,394 ± 126           | 438 ± 45                                         | 49.0                          | 0.3                      |
| 35°C, 20% Me$_2$SO (v/v) | 10 | 596 ± 38          | 176 ± 24          | 419 ± 55              | 561 ± 40                                         | 3.4                           | 1.3                      |
| 20°C       | 7  | 98 ± 9            | 2 ± 1             | 96 ± 9                | 130 ± 4                                          | 49.0                          | 1.4                      |

TABLE II
Rates of coupled and uncoupled ATPase activity and Ca$^{2+}$ efflux

| Conditions | $n$ | Ca$^{2+}$ efflux | Net Ca$^{2+}$-ATPase | Ratio uncoupled/coupled |
|------------|----|-----------------|----------------------|-------------------------|
|            |    | Coupled (a)     | Uncoupled (b)        |                         |
| 35°C       | 7  | 59 ± 5          | 390 ± 57             |                         |
| 35°C, 20% Me$_2$SO (v/v) | 10 | 352 ± 49       | 210 ± 58             |                         |
| 20°C       | 7  | 3 ± 1           | 126 ± 4              |                         |

TABLE III
Heat released during ATP hydrolysis

| Conditions | Δ$H^{\text{cat}}$/ATP |
|------------|----------------------|
|            | Gradient             | Leaky vesicles       |
| 35°C       | $-22.9 \pm 1.2$ (13) | $-12.2 \pm 1.3$ (16) |
| 20% Me$_2$SO (v/v), 35°C | $-10.3 \pm 1.3$ (10) | $-9.4 \pm 1.3$ (6) |
| 20°C       | $-10.5 \pm 0.9$ (12) | $-10.6 \pm 1.3$ (6) |

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The assay medium composition and experimental conditions were as in Figs. 2 and 3. The values of heat produced were plotted as a function of reaction time (Fig. 4). The numbers of experiments, and the values are means ± S.E.

![Diagram A](image1)

**FIG. 4.** Heat released during ATP hydrolysis. Heat release during ATP hydrolysis was measured using assay medium and experimental conditions of Figs. 2 and 3. The values of heat produced were plotted either as a function of reaction time (A) or as a function of ATP hydrolyzed (B). ●, 35°C; ▲, 20% Me$_2$SO at 35°C; and □, 20°C.

**FIG. 5.** Heat released during ATP hydrolysis in the presence (●) and absence (○) of a Ca$^{2+}$ gradient. In A, the assay medium and experimental conditions were as in Fig. 2 at 35°C and in B as in Fig. 3 at 20°C. Absence of gradient refers to the addition of 10 μM ionophore A23187 to the medium.

**DISCUSSION**

Coupling of the ATPase by Me$_2$SO and Low Temperature—In the two conditions there was an increase of the Ca$^{2+}$/ATP ratio from 0.3 to 1.3 and 1.4 (Table I). This was promoted by a decrease of both the uncoupled Ca$^{2+}$ efflux and uncoupled ATPase activity (Table II). From the two activities, the decrease of the uncoupled ATPase (7- and 32-fold) was more pronounced than that of the uncoupled efflux (2- and 3-fold). In parallel to the decrease of the uncoupled routes there was a decrease of the heat produced during ATP hydrolysis by Ca$^{2+}$-loaded vesicles (Table III). These observations support the proposal that the ramifications of the catalytic cycle are thermogenic routes that lead to an increase of the caloric yield of ATP hydrolysis, and the values of Table IV show that the uncoupled ATPase is the route that most contributes to the increase in the caloric yield of ATP hydrolysis noted when the vesicles accumulate Ca$^{2+}$. Although Me$_2$SO increased the rate of exchange (Table I), most of the Ca$^{2+}$ leaving the vesicles was coupled to the synthesis of ATP. An interesting new finding was that Me$_2$SO promoted a significant decrease of the ratio between the rate of ATP hydrolysis and ATP synthesis (Table I). This ratio gives a measure of the degree of energy conservation of the system (3, 4, 10). The more that ATP is synthesized, the smaller the ratio between the rates of hydrolysis and synthesis.

converts the energy derived from the gradient into heat when Mg$^{2+}$ is removed from the medium and the synthesis of ATP is impaired (Δ$H^{\text{cat}}$ = 14.9 kcal/mol of Ca$^{2+}$ released). Knowing the Δ$H^{\text{cat}}$ values for the coupled and uncoupled Ca$^{2+}$ efflux, it was possible to estimate the relative contribution of the efflux and of the substrate hydrolysis to the heat produced during steady state (Table IV). The values obtained clearly indicate that at 35°C, both in the presence and absence of Me$_2$SO, most of the heat produced was derived from the ATPase activity. At 20°C the amount of heat produced was small, and it was not possible to distinguish whether the main source of heat production was the uncoupled efflux or the ATPase activity.
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The heat derived from the coupled and uncoupled Ca\(^{2+}\) efflux was calculated using the \(\Delta H^{\text{act}}\) values of Ca\(^{2+}\) efflux of +5.7 and −14.9 kcal/mol of Ca\(^{2+}\) released, respectively (10, 27). The rates of coupled and uncoupled Ca\(^{2+}\) efflux are from Table II, and the values of heat measured are from Table III. The heat derived from the ATPase was calculated by subtracting the heat derived from the total Ca\(^{2+}\) efflux from the heat measured.

| Preincubation | Heat derived from Ca\(^{2+}\) efflux | Heat measured | Heat derived from ATPase |
|---------------|------------------------------------|--------------|-------------------------|
|               | Coupled (a)            | Uncoupled (b) | total (a + b)            |                           |
| 35 °C         | +0.34                 | −5.81        | −5.47                   | −18.94                   | −13.47                  |
| 20% Me\(_2\)SO (v/v), 35 °C | +2.01                 | −2.99        | −0.98                   | −4.32                    | −3.34                   |
| 20 °C         | +0.02                 | −1.86        | −1.86                   | −1.01                    | +0.85                   |

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 during the Catalytic Cycle—Earlier reports (3–6) demonstrated that during catalysis binding energy and chemical energy are interconverted by the Ca\(^{2+}\)-ATPase. With leaky vesicles, the low Ca\(^{2+}\) concentration (10 \(\mu\)M) available on the two sides of the membrane is not sufficient to permit a significant binding of Ca\(^{2+}\) to the enzyme forms \(E_{-P}\) and \(E_{p}\), and as a result reactions 3 and 4 are irreversible, the catalytic cycle flows continuously forward without branching, two Ca\(^{2+}\) ions are transported across the membrane, and the cleavage of each ATP molecule is completed with the hydrolysis of the low energy phosphoenzyme \(E_{-P}\) (2–5). Thus, during catalysis, a part of the energy derived from ATP hydrolysis is used to transport Ca\(^{2+}\) across the membrane (work), and a part is dissipated as heat. This sequence is altered after formation of the gradient. The high intravesicular Ca\(^{2+}\) concentration leads to the reversal of reactions 4 and 3 and the accumulation and hydrolysis of the high energy phosphoenzyme 2Ca\(^{2+}\)−\(E_{-P}\) (19, 20). Consequently, the cleavage of ATP is no longer coupled to the sequential binding and dissociation of Ca\(^{2+}\) from the enzyme, there is no conversion of chemical energy into work, and more energy is available to be converted into heat.

Recently Sumbilla et al. (28) observed that the Ca\(^{2+}\)/ATP coupling ratio is improved by the reduction of nucleotide concentration in the presence of the ATP regenerating system and/or complexation of luminal Ca\(^{2+}\) with phosphate or oxa-late. In this work (28), the authors determine the kinetic constant of the catalytic cycle partial reactions.

Thermogenesis—The general interest in heat production and thermogenesis has increased during the past decade due to its implications in health and disease. Alterations of thermogenesis are noted in several diseases, such as obesity and thyroid-hormone alterations. Different studies indicate that the hydrolysis of ATP by the Ca\(^{2+}\)-ATPase found in muscle sarcoplasmic reticulum is one of the heat sources contributing to the thermogenesis of animals lacking brown adipose tissue (29–31).

The data presented in this and previous reports (10, 12) suggest that the uncoupled ATPase activity may represent an important route of heat production that contributes to the cell thermogenesis. In intact vesicles, the rate of the uncoupled ATPase activity is 4.6 times faster than the coupled ATPase, i.e. 82% of the total ATP cleaved by Ca\(^{2+}\)-loaded vesicles is processed through the route that leads to a higher heat production (reaction 10). The uncoupled Ca\(^{2+}\) efflux also contributes with a small, but significant heat production. Table IV shows that from the total heat released during transport, 28.9% is derived from Ca\(^{2+}\) efflux and the remaining 71.1% from the ATPase activity. The heat produced in the cell is ultimately related to the turnover of ATP (32). Heat is produced during mitochondria respiration. Thus, in the living cell the Ca\(^{2+}\)-ATPase would account for two sources of heat: (i) the heat produced during ATP hydrolysis and Ca\(^{2+}\) efflux and (ii) the heat derived from the increase of mitochondria activity promoted by the raise in ADP concentration generated by the ATPase activity.

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