Brown Adipose Tissue Ca\(^{2+}\)-ATPase

UNCOPLED ATP HYDROLYSIS AND THERMOCENIC ACTIVITY*

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In this report a sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) was identified in rats brown adipose tissue. Electrophoretic analysis of brown fat microsomal protein yields a 110-kDa band that is reactive to SERCA 1 antibody but is not reactive to SERCA 2 antibodies. Nevertheless, the kinetics properties of the brown fat SERCA differ from the skeletal muscle SERCA 1 inasmuch they manifest a different Ca\(^{2+}\) affinity and a much higher degree of ATPase/Ca\(^{2+}\) uncoupling. A SERCA enzyme is not found in white fat. Fatty acids promote Ca\(^{2+}\) leakage from brown fat vesicles. The heat released during ATP hydrolysis was \(-24.7\) kcal/mol when a Ca\(^{2+}\) gradient was formed across the vesicles membrane and \(-14.4\) kcal/mol in the absence of a gradient. The data reported suggest that in storage Ca\(^{2+}\) inside the endoplasmic reticulum, the Ca\(^{2+}\)-ATPase may represent a source of heat production contributing to the thermogenic function of brown adipose tissue.

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 nonshivering heat production and mechanisms of energy wasting to control obesity. The thermogenic activity of BAT is mediated by \(\alpha\)- and \(\beta\)-adrenergic receptors. Activation of \(\alpha\)-adrenoreceptors is coupled to inositol 1,4,5-triphosphate production and release of Ca\(^{2+}\) from intracellular stores. \(\beta\)-Adrenergic receptors promote an increase of Ca\(^{2+}\) release from cytoplasm, release of free fatty acids, and uncoupling of mitochondria through activation of uncoupling protein 1 (UCP1) (1–10). In addition to these effects, \(\beta\)-adrenergic receptors also increase the effect of Ca\(^{2+}\) release promoted by \(\alpha\)-adrenoreceptors through a mechanism not yet known (9). Recent experiments (9, 10) performed with cultured brown adipocytes indicates that the endoplasmic reticulum is the main intracellular Ca\(^{2+}\) store, but as far as we know, a sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) isoform has not yet been characterized in BAT.

The amount of heat released during ATP hydrolysis varies depending on the SERCA isoform used (11–14). The total amount of energy released during ATP hydrolysis is always the same, but the heat produced varies depending on the rates of coupled and uncoupled ATPase activity (12, 13, 15–18). When coupled to Ca\(^{2+}\) transport a part of the chemical energy derived from ATP hydrolysis is used to pump Ca\(^{2+}\) across the membrane and a part is converted into heat (reactions 1–6 in Fig. 1). During the uncoupled ATPase activity (reaction 10 in Fig. 1), none of the energy derived from ATP hydrolysis is used to pump Ca\(^{2+}\) and more energy is left available to be converted into heat (12, 13, 17, 18). In vesicles derived from either red muscle (SERCA 2A) or human blood platelets (SERCA 2B and 3) practically all the hydrolysis of ATP is coupled to Ca\(^{2+}\) transport and the heat released during hydrolysis varies between 10 and 12 kcal/mol of ATP cleaved (11, 13, 14). However, in vesicles derived from white muscle (SERCA 1) between 70 and 80% of the ATP cleaved is not coupled to Ca\(^{2+}\) transport (reaction 10 in Fig. 1), and the amount of heat released raises to the range of 20–30 kcal/mol of ATP cleaved (12, 17, 18).

In this report we identified a SERCA 1 in vesicles derived from BAT endoplasmic reticulum which has several kinetics properties that are different from those of white muscle SERCA 1.

MATERIALS AND METHODS

Vesicles Derived from Rat BAT, White Adipose Tissue, and from Rabbit Skeletal Muscle—Interscapular BAT was dissected from 20 rats and frozen at \(-72^\circ\)C overnight. After thawing, BAT was homogenized in a mixture containing 10 mM MOPS/Tris buffer, pH 7.0, 1 mM EDTA, and 10 g% sucrose. The homogenate centrifuged at 10,000 \(\times\) g during 20 min, and the pellet and the thick fat upper layer were discarded. Bovine serum albumin was added to the supernatant to a final concentration of 2.5 g%, and the mixture was centrifuged at 65,000 \(\times\) g during 40 min. The pellet was resuspended in a small volume solution containing 50 mM MOPS/Tris buffer, pH 7.0, 100 mM KCl, and 2.5 g% bovine serum albumin and centrifuged at 10,000 \(\times\) g during 20 min; the pellet was discarded, and the supernatant was centrifuged at 65,000 \(\times\) g during 40 min. The sample was resuspended in a small volume solution containing 50 mM MOPS/Tris buffer, pH 7.0, 0.8 M sucrose, and 5 mM NaN\(_3\) and frozen at \(-72^\circ\)C until used. A large excess of albumin was needed for the isolation of active BAT vesicles. When albumin was not used the vesicles obtained at the end of the fractionation process had a low Ca\(^{2+}\)-dependent ATPase activity and were not able to accumulate Ca\(^{2+}\). The rates of Ca\(^{2+}\) uptake and of Ca\(^{2+}\)-dependent ATPase activity of vesicles isolated as described above were not altered when more albumin was added to the assay medium in concentrations varying from 0.1 up to 2.5 g%, thus indicating that the amount of albumin used during the isolation procedure was sufficient for the protection of the vesicles. The activity of the vesicles was stable during the initial 3–4 days storage at \(-72^\circ\)C but decreased progressively during the subsequent days, and after 10 days storage at \(-72^\circ\)C it no longer able to either accumulate Ca\(^{2+}\) or to hydrolyze ATP. In six different groups of rats, the amount of microsome protein obtained from each gram of BAT used was 0.86 \pm 0.17 mg (mean \pm S.E.). Vesicles derived from rat epididymis white adipose tissue were prepared as described above for BAT. In three different groups of rats, the amount of microsome protein obtained from each gram of white adipose tissue was 0.12 \pm 0.21 mg (mean \pm S.E.).
Vesicles derived from rabbit white and red muscles were prepared as described previously (14).

**Results**

**Heat Production by BAT Ca\(^{2+}\)-ATPase**

The catalytic cycle of the Ca\(^{2+}\)-ATPase is shown. The sequence includes two distinct enzymes: Ec and E\(_p\). The Ca\(^{2+}\)-binding sites in the Ec form face the external surface of the vesicle and have a high affinity for Ca\(^{2+}\) (K\(_d\) = 2 \times 10^{-7} \text{ M at pH 7}). In the Ec form the Ca\(^{2+}\)-binding sites face the vesicle lumen and have a low affinity for Ca\(^{2+}\) (K\(_d\) = 10^{-3} \text{ M at pH 7}). The enzyme form Ec is phosphorylated by ATP but not by P, and there the enzyme form Ec \(_p\) is phosphorylated by P, but not by ATP. When the Ca\(^{2+}\) concentration on the two sites of the membrane is inferior to 50 \text{ M}, reaction 4 is irreversible, and this forces the sequence to flow forward from reaction 1 to 6. This is observed when leaky vesicles are used. With intact vesicles, the Ca\(^{2+}\)-pumped by the ATPase is retained in the vesicle lumen. The high intravesicular Ca\(^{2+}\) concentration (\(-10 \text{ M}) permits the reversal of the catalytic cycle (reactions 5 to 1 backwards) during which a part of the Ca\(^{2+}\) accumulated leaves the vesicles in a process coupled with the synthesis of ATP from ADP and P. For vesicles derived from white muscle (SERCA 1), the rise of the intravesicular Ca\(^{2+}\) concentration leads to ramifications of the catalytic cycle, the uncoupled Ca\(^{2+}\)-efflux mediated by reactions 7-9, and the uncoupled ATPase activity mediated by reaction 10. The amount of heat released during ATP hydrolysis varies depending on the phosphoenzyme cleaved and on whether or not the Ca\(^{2+}\) is translocated through the membrane. The total amount of energy released during ATP hydrolysis is always the same, but the heat produced varies depending on how much of this energy is used to pump Ca\(^{2+}\). When coupled to Ca\(^{2+}\) transport (reactions 1-6) a part of the chemical energy derived from ATP hydrolysis is used to translocate Ca\(^{2+}\) across the membrane and a part is converted into heat. In this case the hydrolysis of ATP is completed after the cleavage of the low energy phosphoenzyme Ec-P, and 10-12 kcal are released during the hydrolysis of each ATP molecule. During the uncoupled ATPase activity the high energy phosphoenzyme is cleaved (reaction 10), there is no Ca\(^{2+}\) translocation, and all the energy derived from ATP hydrolysis is converted into heat. In this case the amount of heat released during the hydrolysis of 1 mol of ATP raises to the range of 20-30 kcal (12, 15, 15, 22, 23, 32, 35, 39-41).

**Gel Electrophoresis, Western Blot, and Autoradiography**

Samples were separated in a 7.5% polyacrylamide gel according to Laemmli (19). Electrophoresis of protein from the gel to nitrocellulose membrane was performed for 15 min at 250 mA per gel in 25 mm Tris, 192 mm glycine, and 20% methanol using a Mini Trans-Blot cell from Bio-Rad. Membranes were blocked with 3% nonfat dry milk in Tris-buffered saline with 0.1% Tween 20 for 1 hr at room temperature. Membranes were then washed and incubated for 1 hr with monoclonal antibodies anti-SERCA 1 or anti-SERCA 2 at room temperature. The membranes were washed and blots were revealed using ECL detection kit from Amersham BioSciences (14). Monoclonal antibodies for SERCA 1 (clone I1111) and SERCA 2 (clone I1112) were obtained from Affinity BioReagents, Inc.

**Ca\(^{2+}\) Uptake and Ca\(^{2+}\) \(_{\text{out}}\) Exchange**

These were measured by the filtration method (12, 17, 18, 20). For \(^{45}\text{Ca}\) uptake, trace amounts of \(^{45}\text{Ca}\) 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\(\text{NO}_3\) and the radioactivity remaining on the filters was counted using a liquid scintillation counter. For Ca\(^{2+}\) \(_{\text{in}}\) \(\rightarrow\) Ca\(^{2+}\) \(_{\text{out}}\) exchange, the assay medium was divided into two samples. Trace amount of \(^{45}\text{Ca}\) was 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 when the vesicles were filled, and the steady state \(^{45}\text{Ca}\) uptake was reached. The rate of Ca\(^{2+}\) \(_{\text{in}}\) \(\rightarrow\) Ca\(^{2+}\) \(_{\text{out}}\) exchange was measured after steady state was reached by adding trace amount of Ca\(^{2+}\) to the second sample containing vesicles loaded with non-radioactive Ca\(^{2+}\). The exchange between radioactive Ca\(^{2+}\) from the medium and the non-radioactive Ca\(^{2+}\) contained inside the vesicles was measured by filtering samples of the assay medium through Millipore filters 5, 10, 15, 20, and 25 s after the addition of \(^{45}\text{Ca}^{2+}\). This was assayed by measuring the release of \(^{32}\text{P}\) from \(\gamma\text{-}^{32}\text{P}\text{ATP}\). The reaction was arrested with trichloroacetic acid, final concentration 5% (v/v). The \(\gamma\text{-}^{32}\text{P}\text{ATP}\) not hydrolyzed during the reaction was extracted with activated charcoal as described previously (21).

**ATP Synthesis**

This was measured using an OMEGA Isothermal Titration Calorimeter from Microcal, Inc. (Northampton, MA). 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 20 min. The volume of vesicle suspension injected in the sample cell varied between 0.02 and 0.03 ml. The heat change measured during the initial 2 min after vesicles injection was discarded to avoid artifacts such as heat derived from the dilution of the vesicles suspension in the reaction medium and binding energy. The duration of the ATPase reaction was 35°C. The calorimetric enthalpy (ΔH) is calculated dividing the amount of heat released by the amount of ATP hydrolyzed. 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. The enthalpy of buffer protonation at 35°C was measured at pH 7.0. The reaction was started by the addition of known amounts of HCl to the assay medium and the value found was about 3.8 kcal/mol. The concentration of the different magnesium complexes and ionic species of ATP, ADP, and P, were calculated as described previously (25-27), and from these values the fraction of ATP cleaved that generates free protons at pH 7.0 was estimated to be less than 30%. Thus, the heat derived from buffer protonation during ATP cleaved was about 1 kcal/mol of ATP cleaved.

**Experimental Procedure**

All experiments were performed at 35°C. The vesicles were diluted in a medium containing 50 mM MOPS/Tris buffer, 100 mM KCl, 10 mM P, and 10 mM CaCl\(_2\). In a typical experiment the assay medium was divided in five samples, which were used for the simultaneous measurement of Ca\(^{2+}\) uptake, Ca\(^{2+}\) \(_{\text{in}}\) \(\rightarrow\) Ca\(^{2+}\) \(_{\text{out}}\) exchange, substrate hydrolysis, ATP synthesis, and heat release. The syringe of the calorimeter was filled with vesicles and the temperature difference between the syringe, and the reaction cell of the calorimeter was allowed to equilibrate, a process that usually took between 8 and 20 min. During equilibration, the vesicles were measured for uptake, Ca\(^{2+}\) \(_{\text{in}}\) \(\rightarrow\) Ca\(^{2+}\) \(_{\text{out}}\) exchange, ATP hydrolysis and ATP synthesis were kept at the same temperature, length of time and protein dilution as the vesicles kept in the calorimeter syringe. These different measurements were started simultaneously with vesicles to a final concentration of 0.01 mg/ml NaCl, (5 mM), an inhibitor of ATP synthase, was added to the assay medium to avoid interference from possible contamination of the sarcoplasmic reticulum vesicles with this enzyme.

The free Ca\(^{2+}\) concentration in the medium was calculated as described previously (17, 25-27).

**RESULTS**

**Gel Electrophoresis and Western Blot Analysis**

Vesicles derived from BAT rats endoplasmic reticulum revealed a 110 kDa band characteristic of SERCA that reacted with monoclonal antibodies anti-SERCA 1 of white skeletal muscle but did not react with antibodies anti-SERCA 2 (Fig. 2). A 110-kDa band that reacted with SERCA antibodies was not found in vesicles derived from rat white adipose tissue (data not shown).

**Ca\(^{2+}\) Uptake and Ca\(^{2+}\) \(_{\text{dependent}}\) ATP Hydrolysis**

BAT vesicles were able to accumulate Ca\(^{2+}\) using the energy derived from ATP hydrolysis. The rates of Ca\(^{2+}\) transport and ATP...
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significant Ca$^{2+}$-dependent ATPase activity (Fig. 5). The discrepancy between the rates of Ca$^{2+}$ uptake and ATP hydrolysis decreased as the Ca$^{2+}$ concentrations in the medium was raised to higher values. The Ca$^{2+}$ concentration needed for half-maximal activation (apparent $K_m$) of Ca$^{2+}$ transport and Ca$^{2+}$-dependent ATPase activity were different (Fig. 5C), 0.45 ± 0.04 and 0.15 ± 0.03 μM respectively. These values constitute the mean ± S.E. of six experiments, and the difference between them was statistically significant (t test, $p < 0.001$). The data for Ca$^{2+}$ dependence of enzyme activity were best fit by a model requiring two highly cooperative Ca$^{2+}$-binding sites for the activity with Hill coefficient of 1.74 ± 0.19 for Ca$^{2+}$ uptake and 1.85 ± 0.07 for the Ca$^{2+}$-dependent ATPase activity. In vesicles derived from rabbit white muscle (SERCA 1), the raise of the Ca$^{2+}$ concentration in the vesicles lumen promotes an inhibition of the ATPase activity. In the bibliography (22, 23, 29) this inhibition is referred to as to as “back inhibition” and is abolished when the Ca$^{2+}$ accumulation in the vesicles lumen is prevented by the addition of the Ca$^{2+}$ ionophore A23187 to the medium. The back inhibition was also observed in vesicles derived from BAT but the degree of inhibition was found to vary depending on the free Ca$^{2+}$ concentration in the assay medium (Fig. 6). In presence of 0.15 μM free Ca$^{2+}$, the rates of ATP hydrolysis in intact and leaky vesicles were practically the same, but in presence of saturating Ca$^{2+}$ concentrations the ATPase activity of leaky vesicles was ~2–3-fold faster than that of intact vesicles. Similar to the difference between Ca$^{2+}$ transport and ATPase activity noted in Fig. 5, the difference of ATPase activity between intact and leaky vesicles was related to a disparity between the apparent $K_m$ for Ca$^{2+}$. In four experiments the $K_m$ value measured for the Ca$^{2+}$-dependent ATPase activity of leaky vesicles was 0.41 ± 0.03 μM, a value that was practically the same as that measured for Ca$^{2+}$ transport and significantly higher ($t$ test, $p < 0.001$) than the $K_m$ for ATP hydrolysis measured with intact vesicles (Fig. 5).

**Uncoupled ATPase Activity**—This can be assessed by either measuring the ratio between the initial rates of Ca$^{2+}$ uptake and ATP hydrolysis (Table I) or by measuring the rates of Ca$^{2+}$ in ↔ Ca$^{2+}$ out exchange and ATP hydrolysis at steady state. In muscle vesicles the hydrolysis of one ATP molecule leads to the translocation of two Ca$^{2+}$ ions across the membrane. This was determined in transient kinetics experiments where the transport of Ca$^{2+}$ was measured during the first catalytic cycle of the ATPase (30–32). After a few seconds of reaction the Ca$^{2+}$ concentration inside the vesicles rises to the millimolar range, and this leads to Ca$^{2+}$ leakage (reactions 7–9 in Fig. 1) and uncoupled ATPase activity (reaction 10). In vesicles derived from rabbit white muscle the leakage and uncoupled hydrolysis
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**Table I**

| [Ca\(^{2+}\)](mM) | Ca\(^{2+}\) uptake, initial rate (A) | Ca\(^{2+}\) uptake, steady state | Ca\(^{2+}\)-ATPase (B) | ATP synthesis | Ca\(^{2+}\)/ATP, A/B |
|------------------|-----------------------------------|---------------------------------|----------------------|---------------|------------------|
| 0.1              | 0.8 \(\pm\) 0.6 (6)               | 3.0 \(\pm\) 1.5 (6)             | 89.2 \(\pm\) 12.2 (6) | 16 \(\pm\) 2 (3) | 0.009            |
| 1.7              | 31 \(\pm\) 4 (20)                | 257 \(\pm\) 25 (27)            | 325 \(\pm\) 39 (20)   |               | 0.005            |

The values in the table are mean \(\pm\) S.E., and the numbers in parentheses indicate the number of experiments.

**Table II**

| Vesicles and activity | ATPase | Heat released | \(\Delta H^{\circ}\) |
|-----------------------|--------|---------------|-------------------|
| Intact vesicles (gradient) | \(\mu\)mol/mg min\(^{-1}\) | mcal/mg min\(^{-1}\) | kcal/mol          |
| Mg\(^{2+}\)-dependent activity | 0.31 \(\pm\) 0.06 | -3.53 \(\pm\) 0.45 | -13.19 \(\pm\) 1.43* |
| Ca\(^{2+}\)-dependent activity | 0.37 \(\pm\) 0.07 | -9.13 \(\pm\) 1.84 | -24.67 \(\pm\) 2.17* |
| Leaky vesicles (A23187) | 0.68 \(\pm\) 0.25 | -9.81 \(\pm\) 3.55 (4) | -14.35 \(\pm\) 0.72* |

* \(p < 0.001\).

**Fig. 4. Inhibition by thapsigargin.** The assay medium composition was as described in the legend to Fig. 3. A, Ca\(^{2+}\) uptake; B, ATPase activity \(\mathbf{1}\), total; \(\Delta\), Ca\(^{2+}\)-dependent activity; and \(\mathbf{1}\), Mg\(^{2+}\)-dependent activity. The values of Ca\(^{2+}\) uptake \(\mathbf{1}\) and Ca\(^{2+}\)-dependent ATPase activity \(\mathbf{1}\) were expressed as percent of the activity measured in absence of thapsigargin.

**Fig. 5. Ca\(^{2+}\) dependence for Ca\(^{2+}\) uptake and Ca\(^{2+}\)-dependent ATPase activity.** The assay medium composition was 1 mM ATP, 2 mM MgCl\(_2\), 100 mM KCl, 10 mM P\(_2\), 5 mM Na\(_2\)HPO\(_4\), 0.10 mM CaCl\(_2\), and different EGTA concentrations to achieve the different free Ca\(^{2+}\) concentrations shown in the figure. The Mg\(^{2+}\)-dependent ATPase activity was measured as described in Fig. 2 and subtracted from the total ATPase activity measured in the presence of both Mg\(^{2+}\) and Ca\(^{2+}\). The Ca\(^{2+}\)-uptake \(\Delta\) and Ca\(^{2+}\)-dependent ATPase activity \(\mathbf{1}\) in the presence of either 0.08 \(\mathbf{1}\), 0.23 \(\Delta\), or 1.7 \(\mu\)M \(\mathbf{1}\) free Ca\(^{2+}\). The values of Ca\(^{2+}\) uptake \(\mathbf{1}\) and Ca\(^{2+}\)-dependent ATPase activity \(\mathbf{1}\) were expressed as percent of the activity measured in presence of 1.7 \(\mu\)M free Ca\(^{2+}\).

promote a decrease of the Ca\(^{2+}\)/ATP ratio to values varying between 0.3 and 0.7 (12–18, 30–35). The data of Fig. 5 and Table I show that in BAT vesicles the Ca\(^{2+}\)/ATP ratio measured after 1-min reaction varies depending on the Ca\(^{2+}\) concentration in the medium and that at all Ca\(^{2+}\) concentrations tested it is far smaller than that measured in muscle vesicles; in the presence of Ca\(^{2+}\) concentrations similar to that found in resting adipocytes, the Ca\(^{2+}\)/ATP ratio measured in BAT vesicles is 30–80 times smaller, and in presence of 1.7 \(\mu\)M Ca\(^{2+}\) it is 3–7 times smaller than the Ca\(^{2+}\)/ATP ratio reported for white muscle vesicles. During the first minute of incubation the vesicles are still being filled, and 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. Thus, the stoichiometry between the rates of 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 ATP uptake, and by measuring the rate of Ca\(^{2+}\) 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 (12, 13, 17, 18). In four experiments the rates of Ca\(^{2+}\) leakage and Ca\(^{2+}\)-dependent ATP hydrolysis measured with BAT vesicles at steady state in medium containing 1.7 \(\mu\)M free Ca\(^{2+}\) were 9 \(\pm\) 2 and 370 \(\pm\) 30 nmol/mg min\(^{-1}\), respectively. These values show that very little Ca\(^{2+}\) leaked from BAT vesicles after they were filled, and during steady state the energy derived from only 1 out of 365 ATP cleaved is used to pump back the Ca\(^{2+}\) that leaked from the vesicles. In white muscle vesicles at steady state 1 out of 3–6 ATP cleaved is used for Ca\(^{2+}\) transport (12, 17, 18). These values show that BAT vesicles cleave far more ATP through the uncoupled route that the vesicles derived from white muscle.

**Effect of Fatty Acids—The Ca\(^{2+}\) accumulation by BAT vesi-
cles was impaired by the addition of low fatty acids concentrations to the medium (Fig. 7). The fatty acids tested were arachidic (C20:0), stearic (C18:0), linoleic (C18:2), and linolenic (C18:3) acid. The inhibitory activity of these four fatty acids was the same, and in 18 experiments, the concentration needed for half-maximal inhibition of Ca2+ uptake was 2.9 ± 0.2 μM regardless of the acyl chain length and degree of saturation of the fatty acid used. The decrease of uptake was accompanied by a small, but significant, increase of the Ca2+ dependent ATPase activity. The different fatty acids tested increased the membrane permeability leading to a rapid leakage of Ca2+ from the vesicles (Fig. 8). In previous reports (36) it was found that arachidic (C20:0) and stearic (C18:0) acid inhibited the Ca2+ uptake and increased the rate of Ca2+ efflux of white muscle vesicles, but the concentration of fatty acids needed for a 50% inhibition was higher than 20 μM, i.e. more than one order of magnitude higher than that needed to inhibit the BAT vesicles. Another difference between the two types of vesicles is that in muscle vesicles, but not in BAT vesicles, the inhibitory activity varies depending on degree of saturation of the fatty acid used (36).

Heat Production during ATP Hydrolysis—Heat was produced during ATP hydrolysis (Figs. 8 and 9). The amount of heat produced was proportional to the amount of ATP cleaved (Figs. 8C and 9C). Thus, the higher the free Ca2+ concentration in the medium, the more ATP was cleaved, and the more heat was produced by the BAT vesicles. The amount of heat released during the hydrolysis of each ATP molecule (ΔHcal values in Table II) varied depending on whether or not a gradient was formed across the vesicles membrane. In the presence of a Ca2+ gradient between 23 and 26 kcal were released per mol of ATP cleaved, and in the absence of gradient the heat release decreased to the range of 12–14 kcal/mol of ATP (Table II). Thus the ΔHcal values of ATP hydrolysis measured with BAT vesicles in the presence and absence of a gradient were practically the same as those previously measured with vesicles derived from white muscle and different from those measured with vesicles derived from blood platelets and red muscle (SERCA 2 and 3) where the ΔHcal varies between 10 and 12 kcal·mol⁻¹ both in the presence and absence of Ca2+ gradient (11–14, 24).

DISCUSSION

Ca2+ Affinity—Previous reports (9, 10) demonstrated that adrenergic stimulation promotes an increase of adipocytes cytosolic Ca2+ concentration from a basal level varying between 0.05 and 0.10 μM up to the range of 0.2–0.7 μM. When extended to the living cell, the values of Fig. 5 suggest that in resting cells the Ca2+-dependent ATPase activity of the brown adipocytes varies between 30 and 40% of its maximal activity and is fully active after adrenergic stimulation. The discrepancy between the Ca2+ dependencies for Ca2+ uptake and ATP hydrolysis noted in Fig. 5 seems to be a specific feature of the BAT SERCA 1-like isoform not found in the various SERCA isomorf studied so far (37, 38). In intact BAT vesicles, activation of both Ca2+ uptake and ATP hydrolysis is due to Ca2+ binding to the high affinity site of the enzyme, and the small difference between the apparent Ca2+ dependence for the two activities is probably related to elimination of some steps of the cycle due to slippage of the pump through reaction 10 in Fig. 1.

Effect of Fatty Acids—In brown adipocytes the activation of β3-adrenergic receptors leads to an increase of the lipolysis rate with release of free fatty acids, activation of mitochondrial UCP1 by fatty acids, uncoupling of mitochondrial respiration, and energy dissipation as heat (1–8). Perhaps, the fatty acid released during the β3-mediated response, in addition to activate UCP1, may also promote the release of Ca2+ from the endoplasmic reticulum as shown in Fig. 8. This would explain why the amount of Ca2+ released from the endoplasmic reticulum when both α1- and β3-adrenergic receptors are activated is larger than that measured when only the α1-receptor is activated (9).

Thermogenesis—Activation of BAT thermogenic activity is associated with and increase of the mitochondrial respiration rate (1–9). This has been attributed to the leakage of protons across the inner mitochondrial membrane promoted by activation of UCP1. To compensate for this leak, the cell would then increase the rate of oxygen consumption to maintain the proton gradient at a competent level for ATP synthesis (1–8). Heat is produced during the uncoupled ATPase activity of BAT vesicles (Figs. 8 and 9 and Table II), and to maintain the cytosolic ATP concentration, in the living cell the ADP produced by the uncoupled ATPase activity should also lead to an increase of the mitochondrial oxygen consumption. Perhaps, in addition to the
leakage of proton promoted by UCP1, the uncoupled ATPase activity of BAT SERCA may represent one of the routes of heat production that contributes to the thermogenic function of BAT cells.

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