Different sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPases isoforms are found in blood platelets and in skeletal muscle. The amount of heat produced during ATP hydrolysis by vesicles derived from the endoplasmic reticulum of blood platelets was the same in the absence and presence of a transmembrane Ca\(^{2+}\) gradient. Addition of platelets activating factor (PAF) to the medium promoted both a Ca\(^{2+}\) efflux that was arrested by thapsigargin and an increase of the yield of heat produced during ATP hydrolysis. The calorimetric enthalpy of ATP hydrolysis (\(\Delta H^{\text{cal}}\)) measured during Ca\(^{2+}\) transport varied between -10 and -12 kcal/mol without PAF and between -20 and -24 kcal/mol with 4 \(\mu\)M PAF. Different from platelets, in skeletal muscle vesicles a thapsigargin-sensitive Ca\(^{2+}\) efflux and a high heat production during ATP hydrolysis were measured without PAF and \(\Delta H^{\text{cal}}\) varied between -10 and -12 kcal/mol in the absence of Ca\(^{2+}\) and between -22 up to -32 kcal/mol after formation of a transmembrane Ca\(^{2+}\) gradient. PAF did not enhance the rate of thapsigargin-sensitive Ca\(^{2+}\) efflux nor increase the yield of heat produced during ATP hydrolysis. These findings indicate that the platelets of Ca\(^{2+}\)-ATPase isoforms are only able to convert osmotic energy into heat in the presence of PAF.

Heat generation plays a key role in the regulation of the metabolic activity and energy balance of the cell. In animals lacking brown adipose tissue, the principal source of heat during nonshivering thermogenesis is derived from the hydrolysis of ATP by the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase of skeletal muscles (1–6). This enzyme translocates Ca\(^{2+}\) from the cytosol to the lumen of the sarcoplasmic/endoplasmic vesicles by using the chemical energy derived from ATP hydrolysis (7–10). Calorimetric measurements of rat soleus muscle (2) indicate that 25 to 45% of heat produced in resting muscle is related to Ca\(^{2+}\) transport, and Ca\(^{2+}\) efflux that was arrested by thapsigargin is formed across the vesicles membrane (22–24). The extra heat produced during ATP hydrolysis seems to be promoted by the uncoupled Ca\(^{2+}\) efflux during which the energy derived from the Ca\(^{2+}\) gradient is converted by the SERCA 1 into heat.

In the present study we have measured the heat production during ATP hydrolysis, Ca\(^{2+}\) transport, and Ca\(^{2+}\) efflux in vesicles derived from the sarco/endoplasmic reticulum of skeletal muscle and blood platelets, both in the absence and presence of PAF.

**EXPERIMENTAL PROCEDURES**

**Vesicle Preparations**—Vesicles derived from the dense tubules of human blood platelets and the light fraction of rabbit skeletal muscle sarcoplasmic reticulum were prepared as described previously (25, 26). The muscle vesicle preparation does not contain significant amounts of ryanodine/caffeine-sensitive Ca\(^{2+}\)-channels nor does it exhibit the phenomenon of activation of Ca\(^{2+}\) efflux by external Ca\(^{2+}\), i.e. Ca\(^{2+}\)-induced Ca\(^{2+}\) release found in the heavy fraction of the sarcoplasmic reticulum (16). Both the muscle and blood platelet vesicles were stored in liquid nitrogen until use.

**Ca\(^{2+}\) Uptake and Ca\(^{2+}\) Efflux**—This was measured by the filtration method using \(^{45}\)Ca and Millipore filters (27). After filtration, the filters were washed five times with 5 ml of 3 mM La(NO\(_3\))\(_3\) and the radioactivity remaining on the filters was counted on a liquid scintillation counter. The free Ca\(^{2+}\) concentration was calculated using the association constants of Schwartzzenbach et al. (28) in a computer program described by Fabião and Fabião (29) and modified by Sorensen et al. (30). For Ca\(^{2+}\) efflux experiments, the vesicles were preloaded with \(^{45}\)Ca in a medium containing 50 mM MOPS-Tria (pH 7.0), 10 mM MgCl\(_2\), 100 mM KCl, 20 mM P, 0.3 mM CaCl\(_2\), 3 mM ATP, and 60 \(\mu\)g of vesicles protein/ml. After 30 (muscle vesicles) or 60 (platelet vesicles) min incubation at 35 °C, the vesicles were centrifuged at 40,000 × g for 30 min, the supernatant was discarded, and the walls of the tubes were blotted to minimize the volume of residual leading medium. The pellet was kept on ice and resuspended in ice-cold water immediately before use. The efflux was arrested as described above for the Ca\(^{2+}\) uptake.

**ATPase Activity**—This was assayed measuring the release of \(^{32}\)P from [\(\gamma\)-\(^{32}\)P]ATP. The \(^{32}\)P, produced was extracted from the medium with ammonium molybdate and a mixture of isobutyl alcohol and benzoic acid.

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§ The abbreviations used are: SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase; PAF, platelet activating factor; MOPS, 4-morpholinepropanesulfonic acid.
The Mg2+ dependent activity was measured in the presence of 5 mM EGTA. The Ca2+-ATPase activity was determined by subtracting the Mg2+ dependent activity from the activity measured in the presence of both Mg2+ and Ca2+.

Synthesis of [γ-32P]ATP from ADP and 32P—This was measured as described previously (31). [32P]Pi was obtained from the Brazilian Institute of Atomic Energy.

Heats of Reaction—This was measured using an OMEGA Isothermal Titration Calorimeter from Microcal Inc. (Northampton, MA) (22, 24). The calorimeter cell was filled with a reaction medium, and the reference cell was filled with Milli-Q water. After equilibration at the desired temperature, the reaction was started by injecting sarcoplasmic reticulum vesicles into the reaction cell and the heat change due to ATP hydrolysis was recorded starting from 2 min after the injection up to a maximum of 30 min. The calorimetric enthalpy of hydrolysis (ΔHcal) was calculated by dividing the amount of heat released by the net amount of ATP hydrolyzed. The units used were moles for ATP hydrolyzed and kcal for the heat released. A negative value indicates that the reaction was exothermic and a positive value indicates that it was endothermic.

Chemicals—The PAF used was 11-O-phosphatidylcholine β-acetyl-γ-O-hexadecyl (1-O-hexadecyl-2-acetyl-rac-glycero-3-phosphocholine), obtained from Sigma. PAF was dissolved in ethanol. Thapsigargin (LC Sciences, Woburn, MA) was dissolved in dimethyl sulfoxide. After dilution to the assay medium composition and experimental conditions were as described in the legend to Fig. 1 using 5 mM EGTA (zero Ca2+), different mixtures of EGTA and CaCl2 where zero (●), 0.1 mM EGTA and 0.029, 0.063, 0.082 or 0.112 mM CaCl2. The calculated free Ca2+ concentrations with these different mixtures of EGTA and CaCl2 where zero (Δ), 10 μM (○), or 1 μM (●). The medium was divided into 3 samples. One was used for heat measurements (C). To the other 2 samples, trace amounts of either 45Ca or [γ-32P]ATP were added for measurements for Ca2+ uptake (A) and ATPase activity (B). The three reactions were started simultaneously by addition of vesicles to a final protein concentrations of 40 μg/ml. The assay temperature was 35 °C.

![Fig. 1. Platelet vesicles: Ca2+ uptake (A), ATPase activity (B), and heat released (C).](image)

**RESULTS**

Ca2+ Transport and Heat Production by Muscle and Platelet Vesicles—In agreement with previous reports, we found that the vesicles derived from blood platelets (Fig. 1A and Table I) were able to accumulate a smaller amount of Ca2+ than the vesicles derived from muscle (Fig. 2A and Table II). During transport the two vesicle preparations catalyze simultaneously the hydrolysis (Figs. 1B and 2B) and the synthesis of ATP from ADP and Pi. The rate of synthesis was severalfold slower than the rate of hydrolysis. Using the same experimental conditions as those described in Tables I and II and in the presence of 1 μM free Ca2+, the rates of ATP synthesis for platelets and muscle vesicles were 0.08 ± 0.01 (6) and 2.57 ± 0.22 (4) μmol of ATP/mg protein·30 min⁻¹, respectively. These values are the average ± S.E. of the number of experiments shown in parentheses. The kinetics of Ca2+ transport and ATP synthesis have been analyzed in detail in previous reports (7–10). In this study we focused on heat produced during transport. The overall reaction of Ca2+ transport was exothermic regardless of whether muscle or platelet vesicles were used (Figs. 1C and 2C). The amount of heat released during the different incubation intervals was proportional to the amount of ATP cleaved. This could be visualized by either plotting the heat released as a function of the amount of ATP hydrolyzed (Fig. 3) or calculating the ΔHcal using the values of heat released and Pi produced at different incubation intervals (Fig. 4). Two different ATPase activities can be distinguished in both platelet and muscle vesicles. The Mg2+ dependent activity requires only Mg2+ for its activation and is measured in the presence of EGTA to remove contaminant Ca2+ from the assay medium. The ATPase activity which is correlated with Ca2+ transport requires both Ca2+ and Mg2+ for full activity. In both vesicle preparations, the Mg2+ dependent ATPase activity represents a small fraction of the total ATPase activity measured in presence of Mg2+ and Ca2+ (Figs. 1B and 2B). The amount of heat produced during the hydrolysis of ATP by the Mg2+ dependent ATPase was the same regardless of whether muscle or platelet vesicles were used (Fig. 3C) and the ΔHcal value (Fig. 4 and Tables I and II) calculated in the two conditions was the same as that previously measured with soluble F1 mitochondrial ATPase (23) and soluble myosin at pH 7.2 (32). For the vesicles derived from muscle (SERCA 1) the formation of a Ca2+ gradient increased the yield of heat production during ATP hy-

![Fig. 2. Skeletal muscle vesicles, Ca2+ uptake (A), ATPase activity (B), and heat released (C).](image)

**Table 1**

Heat production during Ca2+ transport and ATP hydrolysis by the platelets Ca2+-ATPase

| [Ca2+] | [Ca2+] uptake | ATPase activity | Heat released | ΔHcal |
|--------|---------------|-----------------|---------------|-------|
| μM     | μmol/mg, 30 min | μmol/mg, 30 min | kcal/mg, 30 min | kcal/mol |
| 0      | 0.35 ± 0.08 (7) | 4.17 ± 0.87 (7) | -12.30 ± 0.71 (7) |
| 0.1    | 0.80 ± 0.11 (6) | 9.80 ± 1.30 (6) | -13.40 ± 2.27 (6) |
| 0.4    | 1.45 ± 0.28 (9) | 14.34 ± 1.67 (9) | -10.73 ± 0.86 (9) |
| 1.0    | 1.77 ± 0.29 (4) | 15.93 ± 0.75 (4) | -9.91 ± 1.93 (4) |
| 10.0   | 1.61 ± 0.23 (15)| 17.11 ± 2.59 (15)| -10.99 ± 1.09 (15)|

The assay medium composition and experimental conditions were as described in Fig. 1 using 5 mM EGTA (zero Ca2+) or 0.1 mM EGTA and 0.029, 0.063, 0.082 or 0.112 mM CaCl2. The free Ca2+ concentrations calculated with the different EGTA and CaCl2 concentrations used are shown. Values are mean ± S.E. of the number of experiments shown in parentheses.
Heat Production by the Ca\textsuperscript{2+}-ATPase

| Additions | Ca\textsuperscript{2+} uptake \( \mu \text{mol/mg, 30 min} \) | ATPase activity | Heat released | \( \Delta F\text{cal} \) |
|-----------|---------------------------------|-----------------|---------------|------------------|
| Intact vesicles, ECTA, 2 mM | 2.11 ± 0.12 | 20.56 ± 2.3 | -10.20 ± 1.38 | |
| Leaky vesicles, Ca\textsuperscript{2+}, 10 \( \mu \text{M} \) | 0 | 42.60 ± 2.41 | 518.87 ± 27.8 | -12.18 ± 1.29 |
| Intact vesicles, Ca\textsuperscript{2+}, 1 \( \mu \text{M} \) | 1.85 ± 0.16 | 40.28 ± 2.51 | 1,270.40 ± 62.9 | -31.88 ± 1.22 |
| Intact vesicles, Ca\textsuperscript{2+}, 10 \( \mu \text{M} \) | 2.65 ± 0.43 | 46.11 ± 2.18 | 1,054.06 ± 147.39 | -22.67 ± 2.14 |

**Fig. 3.** Heat released during ATP hydrolysis by muscle and platelet vesicles. The values of heat released measured in Figs. 1 and 2 were plotted as a function of ATP hydrolyzed. A, platelets, or B, muscle vesicles in the absence of Ca\textsuperscript{2+} and 5 mM EGTA (A) and either 10 (C) or 1.0 (D) \( \mu \text{M} \) free Ca\textsuperscript{2+}. The data obtained with the use of 5 mM EGTA in A and B were plotted in C, where open symbols represent platelet vesicles and closed symbols represent muscle vesicles.

**Fig. 4.** Effect of Ca\textsuperscript{2+} gradient on the \( \Delta F\text{cal} \) values measured with platelet (A) and skeletal muscle (B) vesicles. The experimental conditions were the same as those of Figs. 1 and 2 with 1 \( \mu \text{M} \) free Ca\textsuperscript{2+} (●), 10 \( \mu \text{M} \) free Ca\textsuperscript{2+} (○), or 5 mM EGTA (△).

**Fig. 5.** Heat production during Ca\textsuperscript{2+} transport and ATP hydrolysis by the skeletal muscle Ca\textsuperscript{2+}-ATPase in the presence and absence of a transmembrane Ca\textsuperscript{2+} gradient.

The assay medium composition and experimental conditions were as described in Fig. 2. For the leaky vesicles, 4 \( \mu \text{M} \) of the divalent cation ionophore A23187 was included in the assay medium. Values are mean ± S.E. of the number of experiments shown in parentheses. With intact vesicles, the difference of \( \Delta F\text{cal} \) measured with EGTA and 10 \( \mu \text{M} \) Ca\textsuperscript{2+} and the difference between the values measured with 1 and 10 \( \mu \text{M} \) Ca\textsuperscript{2+} were significant (t test) with \( p < 0.001 \) and \( p < 0.005 \), respectively.

drolysis (Figs. 3B and 4B and Table II). This was not observed with the use of platelet vesicles (SERCA 2b and 3) where the yield of heat produced during ATP cleavage was the same in the presence and absence of a transmembrane Ca\textsuperscript{2+} gradient (Figs. 3A and 4A and Table I). For the muscle vesicles (Table II), there was no difference in the \( \Delta F\text{cal} \) value of the Mg\textsuperscript{2+}-dependent ATPase and the Ca\textsuperscript{2+}-ATPase when the vesicles were rendered leaky (no gradient). With intact vesicles, the \( \Delta F\text{cal} \) value was more negative, i.e. more heat was produced during the hydrolysis of each ATP molecule when the free Ca\textsuperscript{2+} concentration in the medium was decreased from 10 to 1 \( \mu \text{M} \) (Fig. 4B and Table II). During transport, the \( P_i \) available in the assay medium diffuses through the membrane to form Ca\textsuperscript{2+} phosphate crystals inside the vesicles. These crystals operate as a Ca\textsuperscript{2+} buffer that maintains the free Ca\textsuperscript{2+} concentration inside the vesicles constant (~5 mM) at the level of the solubility product of calcium phosphate (8, 33). The energy derived from the gradient depends on the difference between the Ca\textsuperscript{2+} concentrations inside and outside the vesicles. Thus, the different values of \( \Delta F\text{cal} \) measured with the muscle vesicles with 1 and 10 \( \mu \text{M} \) Ca\textsuperscript{2+} suggest that when the free Ca\textsuperscript{2+} concentration in the medium is lower, the gradient formed across the vesicles membrane is steeper; thus more heat was produced and a more negative value of the \( \Delta F\text{cal} \) for ATP hydrolysis was observed. With vesicles derived from blood platelets, there was no extra heat production during Ca\textsuperscript{2+} transport regardless of the free Ca\textsuperscript{2+} concentration in the medium (Figs. 3A and 4A and Table I). Similar to muscle, P\textsubscript{i} diffuses through the membrane of the platelet vesicles forming calcium phosphate crystals inside the vesicles that ensures the maintenance of the free Ca\textsuperscript{2+} concentration in the vesicles lumen at the same level as that of the muscle (~5 mM). Thus during transport, the Ca\textsuperscript{2+} gradient formed across the membrane in the presence of 1 and 10 \( \mu \text{M} \) Ca\textsuperscript{2+} should be the same in muscle and platelet vesicles. These findings indicate that different from the muscle, the Ca\textsuperscript{2+}-ATPase of platelets is not able to convert the osmotic energy derived from the gradient into heat.

**Uncoupled Ca\textsuperscript{2+} Efflux—Kinetics** evidence described in previous reports (22, 24) indicate that the extra heat measured in muscle vesicles was related to the uncoupled Ca\textsuperscript{2+} efflux mediated by the Ca\textsuperscript{2+}-ATPase. This can be measured arresting the pump by the addition of an excess EGTA to the medium (Fig. 5). In this condition, the free calcium available in the medium is chelated but Mg-ATP and other reagents remain at the same concentration as those used in the experiments of Figs. 1 and 2. For the muscle vesicles, the efflux promoted by EGTA decreased when thapsigargin, a specific inhibitor of the Ca\textsuperscript{2+}-ATPase (34, 35), was added to the medium simultaneously with EGTA. The difference between the total efflux and the efflux measured in the presence of thapsigargin represents the uncoupled efflux mediated by the Ca\textsuperscript{2+}-ATPase (19, 36) and in muscle vesicles it represents about 70% of the total Ca\textsuperscript{2+} efflux (Fig. 5 and Table III). The uncoupled efflux can also be measured diluting vesicles previously loaded with Ca\textsuperscript{2+} in a medium containing only buffer and EGTA (Fig. 6B and Tables III and IV). For the muscle vesicles, this leakage was also decreased by thapsigargin to the same extent as that measured in the conditions of Fig. 5. The Ca\textsuperscript{2+} efflux of platelet vesicles was slower than that of muscle and was not impaired by thapsigargin, regardless of the method used to measure the efflux.
Heat Production by the Ca\(^{2+}\)-ATPase

**Fig. 5. Ca\(^{2+}\) release from skeletal muscle vesicles.** The assay medium composition was 50 mM MOPS/Tris (pH 7.0), 2 mM MgCl\(_2\), 0.1 mM CaCl\(_2\), 20 mM P. The reaction was started by the addition of muscle vesicles to a final concentration of 0.050 mg/ml protein. ○, control without additions. The arrow indicates the addition of either 5 mM EGTA (△) or 5 mM EGTA plus 1 μM thapsigargin (▲).

**Table III**  
Ca\(^{2+}\) efflux from skeletal muscle vesicles

| Method used | n | Total (A) | 5 μM TG (B) | TG-sensitive (A-B) |
|-------------|---|-----------|-------------|-------------------|
| Without PAF |   |           |             |                   |
| 1) Preloaded and diluted | 8 | 206 ± 33 | 66 ± 14 | 139 ± 24 |
| 2) EGTA added during uptake | 7 | 203 ± 26 | 63 ± 19 | 140 ± 22 |
| With 4 μM PAF |   |           |             |                   |
| 3) Preloaded and diluted | 8 | 196 ± 34 | 109 ± 19 | 94 ± 23 |
| 4) PAF added during uptake | 4 | 228 ± 38 | 97 ± 20 | 130 ± 38 |

**Fig. 6. Ca\(^{2+}\) efflux from platelet (A) and skeletal muscle (B) vesicles.** The vesicles were preloaded with \(^{45}\)Ca as described under “Experimental Procedures” and diluted in a medium containing 50 mM MOPS/Tris (pH 7.0) and 0.1 mM EGTA either in the absence (●) or presence (○) of 1 μM thapsigargin.

**Table IV**  
Ca\(^{2+}\) efflux from blood platelets vesicles

| Vesicles | n | Total (A) | 5 μM TG (B) | TG-sensitive (A-B) |
|----------|---|-----------|-------------|-------------------|
| 1) Preloaded and diluted | 6 | 40 ± 3 | 41 ± 6 | 0 |
| 2) EGTA added during uptake | 4 | 44 ± 12 | 79 ± 25 | 0 |
| 3) PAF added during uptake | 4 | 273 ± 9 | 61 ± 3 | >212 ± 10 |

**Fig. 7. Effect of PAF on the Ca\(^{2+}\) uptake (●) and ATP hydrolysis (○) by platelet (A) and skeletal muscle (B) vesicles.** The assay medium composition was the same as described in the legend to Figs. 1 and 2. The values represent the average ± S.E. of five experiments.

(reticulum (37). We therefore tested different lipids in platelet vesicles in search of a compound that could promote a thapsigargin-sensitive Ca\(^{2+}\) efflux. The reasoning was that if we could promote the leakage of Ca\(^{2+}\) through the platelet Ca\(^{2+}\)-ATPase then, similar to the muscle vesicles, the platelet vesicles should become able to convert osmotic energy into heat. In the course of these experiments we found that N-acetyl-\(\beta\)-phosphatidylcholine \(\beta\)-acetyl-\(\gamma\)-O-hexadecyl could promote such an efflux in platelets but not in muscle vesicles. This phospholipid belongs to a family of acetylated phospholipids known as PAF which are produced when cells involved in inflammatory process are activated. PAF was found to inhibit the Ca\(^{2+}\) uptake of both platelets and muscle vesicles (Figs. 7 and 8 and Table V). With the two vesicles, half-maximal inhibition was obtained with 4 to 6 μM PAF. In contrast with the Ca\(^{2+}\) uptake, the ATPase activity of the two vesicle preparations was not inhibited by PAF (Fig. 7). The discrepancy between Ca\(^{2+}\) uptake and ATPase activity suggests that the decrease of Ca\(^{2+}\) accumulation was promoted by an increase of Ca\(^{2+}\) efflux and not by an inhibition of the ATPase. The amount of Ca\(^{2+}\) retained by the vesicles is determined by the differences between the rates of Ca\(^{2+}\) uptake and Ca\(^{2+}\) efflux. The higher the efflux, the smaller the amount of Ca\(^{2+}\) retained by the vesicles. The addition of PAF during the course of Ca\(^{2+}\) uptake promoted the release of Ca\(^{2+}\) until a new steady state level of Ca\(^{2+}\) retention was achieved (Fig. 8 and Table V). With both preparations, when the higher concentration of PAF was added, the lower the new steady state level of Ca\(^{2+}\) was (Figs. 7 and 8). The release of Ca\(^{2+}\) promoted by PAF was not accompanied by a burst of ATP synthesis. On the contrary, PAF inhibited the synthesis of ATP driven by the coupled Ca\(^{2+}\) efflux (Fig. 9). This indicates that Ca\(^{2+}\) release promoted by PAF was not promoted by an increase of the reversal of the pump. A major difference between
the muscle and platelet vesicles was found when thapsigargin was added to the medium together with PAF. For platelet vesicles, the rate of Ca\(^{2+}\) release measured after the addition of PAF was greatly decreased in the presence of thapsigargin (Fig. 8A and Table IV) indicating that most of the Ca\(^{2+}\) left the vesicles through the ATPase as an uncoupled Ca\(^{2+}\) efflux. This could be better seen after the initial minute of incubation. In fact, the rate of release in platelet vesicles was so fast that we could not measure the initial velocity of release with the method available in our laboratory. Thus, the values with PAF in Table III differ from the other values in that it does not reflect a true rate, but only the parcel of Ca\(^{2+}\) released during the first incubation minute. In muscle, the rate of Ca\(^{2+}\) efflux measured after the addition of PAF was slower than that measured with platelet vesicles (compare Fig. 8, A and B) and the proportion between the Ca\(^{2+}\) effusive sensitive and insensitive to thapsigargin measured with PAF was practically the same as that measured when the pump was arrested with EGTA (Table IV).

Having found a compound that induces the release of Ca\(^{2+}\) through the pump, we then measured the heat produced during ATP hydrolysis in the presence and absence of PAF. The PAF concentrations selected were sufficient to enhance the rate of efflux without completely abolishing the retention of Ca\(^{2+}\) by the vesicles, i.e. without abolishing the formation of a Ca\(^{2+}\) gradient through the vesicles membrane (Fig. 8). In such conditions PAF was found to enhance the amount of heat produced during the hydrolysis of ATP by blood platelets (Fig. 10 and Table V). In muscle vesicles, however, PAF was found to decrease the amount of heat produced during ATP hydrolysis. The ΔH\(^{\text{cal}}\) values measured with PAF and muscle vesicles were less negative than those measured in the absence of PAF, but still more negative than the values measured in the absence of Ca\(^{2+}\) gradient.

**DISCUSSION**

**Heat Production**—It is generally assumed that the energy released during the hydrolysis of ATP by Ca\(^{2+}\)-ATPase can be divided in two non-interchangeable parts, one is converted into heat and the other is used to pump Ca\(^{2+}\) across the membrane (1–6). Here, this was observed with the platelet vesicles before the addition of PAF (Table I). The recent finding (22–24) that the SERCA 1 can convert osmotic energy into heat revealed an alternative route that increases 2–3-fold the amount of heat produced during ATP hydrolysis, therefore permitting the maintenance of the cell temperature with a smaller consumption of ATP (Table II). By this route, a part of the energy released during ATP hydrolysis is dissipated into the surrounding medium as heat. The other part is used to pump Ca\(^{2+}\) across the membrane. During uptake, a fraction of the Ca\(^{2+}\) accumulated flows back through the Ca\(^{2+}\)-ATPase from the vesicles lumen to the medium driven by the Ca\(^{2+}\) gradient. This efflux is coupled with the production of heat (thapsigargin-sensitive efflux). Thus, for the muscle vesicles, heat would be produced at least in two different steps of the energy interconversion cycle: (i) during the hydrolysis of ATP, where a part of the chemical energy released was directly converted into heat and (ii) during the leakage of Ca\(^{2+}\) through the ATPase where part of the energy derived from ATP hydrolysis used to pump Ca\(^{2+}\) is first converted into osmotic energy, and then converted by the enzyme into heat (22, 24). The data reported show that not all SERCA isoforms are able to readily convert osmotic energy into heat. The vesicles of blood platelets, as obtained after cell fractionation, are not able to promote this conversion. These vesicles, however, can be converted by PAF into a system capable of increasing the heat production during ATP hydrolysis, suggesting that the mechanism capable of providing additional heat production can be turned on and off and this could represent a mechanism of thermoregulation specific of the cells expressing SERCA 2b and 3. Both in muscle and platelet vesicles there is a Ca\(^{2+}\) efflux which is not inhibited by thapsigargin. We do not know through which membrane structure this Ca\(^{2+}\) flows, but the data obtained with platelets before the addition of PAF indicate that during this efflux, osmotic energy is not converted into heat. In platelets, PAF promoted simultaneously the appearance of thapsigargin-sensitive efflux and extra heat production during ATP hydrolysis (Tables IV and V). These observations corroborate with the

**TABLE V**

*Effect of PAF on the Ca\(^{2+}\) uptake and the ΔH\(^{\text{cal}}\) of ATP hydrolysis*

The assay medium and experimental conditions were as in Figs. 1 and 2. The values in the table are the average ± S.E. of the number of experiments shown in parentheses. The differences between the ΔH\(^{\text{cal}}\) values measured in the absence and in the presence of PAF with skeletal muscle were significant (t test) with p < 0.05 both with 1 and 10 μM Ca\(^{2+}\) and, with blood platelets were significant with p < 0.005 (1 μM Ca\(^{2+}\)) and p < 0.001 (10 μM Ca\(^{2+}\)).

| Ca\(^{2+}\) | PAF | Skeletal muscle vesicles | Blood platelet vesicles |
|---|---|---|---|
| | μM | | ΔH\(^{\text{cal}}\)| | μM | | ΔH\(^{\text{cal}}\)| |
| | | μmol/mg, 30 min | Kcal/mol | μmol/mg | Kcal/mol |
| 1 | 0 | 1.83 ± 0.21 | -32.99 ± 2.90 | 0.11 ± 0.03 | -12.58 ± 1.29 |
| 4 | 0.45 ± 0.19 | -25.69 ± 1.71 | 0.03 ± 0.01 | -20.04 ± 0.37 |
| 10 | 0 | 2.66 ± 0.44 | -22.92 ± 2.24 | 0.20 ± 0.04 | -10.70 ± 1.01 |
| 6 | 0.68 ± 0.35 | -16.91 ± 1.50 | 0.06 ± 0.01 | -23.90 ± 1.06 |
same as described in the legends to Figs. 2 and 3 in the absence (●) or presence (○) of 6 μM PAF. In the figure r is the correlation coefficients of fitted regression lines.

notion that the conversion of osmotic energy into heat cannot be promoted by any kind of Ca$^{2+}$ leakage and that a device is needed for this conversion (24). For the endoplasmic/sarcoplasmic reticulum, this device seems to be the Ca$^{2+}$-ATPase itself, which in addition to interconvert chemical into osmotic energy, could also convert osmotic energy into heat.

Platelets Activating Factor—Regardless of its possible physiological implication, in this study the use of PAF as an experimental tool permitted us to show that the platelet vesicles can be converted from an inactive into an active system capable of converting osmotic energy into heat. Heat generation is implicated in the regulation of several physiological processes including metabolism and energy balance of the cell. The V$_{\text{max}}$ of most enzymes varies significantly after a discrete temperature change leading to a substantial change of the metabolic activity of the cell (1–3). PAF was originally described as a soluble factor in blood, so it is apparent that some cells secrete it following synthesis. Subsequent experimentation revealed that the secretion of PAF varies greatly depending on the cell type. In some cells, for instance, endothelial cells, the PAF synthesized is not secreted and is used by the cell itself (for reviews, see Refs. 38 and 39). The synthesis of PAF is initiated by phospholipase A$_2$. The subsequent steps of synthesis are catalyzed by enzymes located in the endoplasmic reticulum. In metabolic labeling experiments, PAF appears first in the endoplasmic reticulum and then in the plasma membrane. PAF causes an elevation of the cytosolic-free Ca$^{2+}$-promoted by the release of Ca$^{2+}$ from both the plasma membrane and from intracellular stores. This was shown in various cells that express SERCA 2B and 3 including platelets, neutrophils, macrophage, endothelial cells, and neuronal cells (38, 39). PAF can therefore act in two different manners, through a receptor in the plasma membrane or as an intracellular messenger. The affinity for PAF of the cell membrane receptor is very high, and the PAF concentration needed for cell activation varies between 10$^{-12}$ and 10$^{-9}$ M, a concentration much smaller than that needed in this report to activate the thapsigargin-sensitive Ca$^{2+}$ efflux in platelet vesicles (38, 39). The only possibility that the effect of PAF observed in this report could have some physiological implication is if the concentration of PAF reaches the micromolar range in the microenvironment surrounding the endoplasmic reticulum membrane, where the Ca$^{2+}$-ATPase is located and PAF is synthesized. In this case, the local effect of PAF not only would promote the release of Ca$^{2+}$ from the endoplasmic reticulum (Fig. 8A) but also enhance the amount of heat produced during hydrolysis of ATP (Tables IV and V).

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