A Ca\(^{2+}\) transporting ATPase has been shown to be an integral component of skeletal muscle sarcolemma. The enzyme is stimulated by calmodulin and inhibited by micromolar concentrations of vanadate. The presence of calmodulin results in the transition of the ATPase to a high Ca\(^{2+}\) affinity state; removal of calmodulin reverses this effect. The Ca\(^{2+}\)-ATPase and the Ca\(^{2+}\) uptake associated with it are also regulated by a specific cyclic AMP-dependent phosphorylation system. The phosphorylation of the membrane enhances the Ca\(^{2+}\)-ATPase activity. The effect is reversible. Three sarcotendinous proteins (Mr 35,000, 28,000, and 26,000) were identified as specific substrates of the Ca\(^{2+}\)-dependent phosphorylation system. The sarcotendinous Ca\(^{2+}\)-ATPase was isolated by means of a calmodulin affinity chromatography column as a protein band of Mr 140,000, which could be eluted from the column by EDTA. The band possesses Ca\(^{2+}\)-ATPase activity and is phosphorylated with \([\gamma-\text{P}]\text{ATP}\) in a Ca\(^{2+}\)-dependent manner.

The membrane of the transverse tubules does not contain the calmodulin-sensitive Ca\(^{2+}\)-ATPase. It contains only one cyclic AMP-dependent phosphoprotein (Mr 30,000).

The skeletal muscle cell presents a highly differentiated membrane system with at least two morphologically identifiable portions in contact with the extracellular fluid, the sarcolemma, and the 'T-tubule' system. At present, information concerning Ca\(^{2+}\) transport mechanisms across these membrane systems is limited, but the availability of highly purified skeletal muscle sarcolemma preparations (1) is indeed a component of the sarcolemma membrane. The Ca\(^{2+}\)-ATPase activity of the 'T-tubule' membrane is very low. The very limited degree of purification of these sarcolemma preparations and the lack of criteria for safely attributing the pumping of Ca\(^{2+}\) to a sarcolemma ATPase have left open the possibility that the activity is due to contaminating sarcoplasmic reticulum. Studies by Seiler and Fleischer (1) have shown that well-defined plasma membranes isolated from rabbit skeletal muscle have very low Ca\(^{2+}\)-ATPase activity. They have suggested that the activity is not a plasma membrane enzyme but reflects the contamination by the sarcoplasmic reticulum membrane.

Limited attention has been devoted to the second Ca\(^{2+}\)-transporting system in muscle plasma membranes, the Na\(^+\)/Ca\(^{2+}\) exchanger. Physiological studies with intact muscle cells (14) and biochemical studies with isolated sarcolemma (15) have indicated the presence of the Na\(^+\)/Ca\(^{2+}\) exchanger also in skeletal muscle.

The present work shows that the Ca\(^{2+}\)-ATPase found in skeletal muscle sarcolemma preparations (1) is indeed a component of the sarcolemma membrane. By contrast, the Ca\(^{2+}\)-ATPase activity of the 'T-tubule' membrane is very low. The Ca\(^{2+}\)-ATPase of sarcolemma membrane is stimulated by calmodulin and markedly and reversibly affected by the phosphorylation of membrane proteins. Cyclic AMP-dependent phosphorylation of sarcolemma stimulates the activity of the enzyme, whereas its dephosphorylation markedly inhibits it. A preliminary report of this work has appeared elsewhere (40).

**EXPERIMENTAL PROCEDURES**

**Materials**—CNBr-activated Sepharose 4B was obtained from Pharmacia, Uppsala, Sweden. Bovine brain calmodulin was purified according to Ref. 16. Asolectin, oligomycin, valinomycin, cAMP, cAMP-dependent protein kinase (type II), and protein kinase inhibitor (cAMP-dependent; type II) were from Sigma. Ouabain and arsenazo III were from Fluka AG, Buchs, Switzerland, sodium orthovanadate was from ICN Pharmaceuticals Inc., Plainview, NC, \(\gamma-\text{P}\)
\section*{The Ca\textsuperscript{2+}-ATPase of Skeletal Muscle Sarcolemma}

\textsuperscript{3}P-ATP was obtained from Amersham, England, and \textsuperscript{45}CaCl\textsubscript{2} was from EIR, Wuerlingen, Switzerland. Calmodulin (R-24571) was a kind gift of Dr. Van Belle (Janssen Pharmaceuticals, Beerse, Belgium). A-23187 was from Lilly. Protease inhibitors were from Merck, Darmstadt, FRG, except p-aminobenzamidine, which was from Sigma. Phosphoehoronypruvate, pyruvate kinase, lactate dehydrogenase, and NAD were obtained from Boehringer Mannheim. Parvalbumin from rat skeletal muscle was a generous gift of Dr. C. W. Heizmann. All chemicals used were of the highest quality commercially available.

\textbf{Preparation of Skeletal Muscle Sarcolemma and T-tubule Membranes—} Sarcolemmal membranes were isolated from rabbit white muscle according to the procedure of Seiler and Fleischer (1), starting from 500 g of fresh tissue. Sarcolemmal vesicles were collected at the 17/23% sucrose interface, suspended in 0.3 M sucrose, 5 mM imidazole, pH 7.4, and 0.1 mM EGTA, immediately frozen in liquid nitrogen, and stored at \(-80\) °C until used. Since the "dextran vesicles" described by Seiler and Fleischer (1) could be isolated only in relatively low yield, the studies presented here were carried out on vesicles purified on a sucrose gradient only ("sucrose vesicles" (1)). In addition, the procedure of sarcolemma isolation originally described by Seiler and Fleischer (1) has been modified by introducing 0.1 mM dithiotreitol and 0.1 mg of bovine serum albumin/ml of the homogenizing medium. These changes were apparently essential to increase the yield of sealed sarcolemmal vesicles.

The quality of the sarcolemma membrane preparation described by Seiler and Fleischer (1) has been verified by the authors by the following criteria. (i) The preparation had high activities of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity, adenylate cyclase, and acetylcholinesterase, and acetic acid low activities of cytochrome c reductase and monoamine oxidase. (ii) Thin sections and negative staining electron microscopy have confirmed the absence of significant sarcosomic reticulum and mitochondria contamination. The sarcolemma preparation used here had the same properties as the preparation described by Seiler and Fleischer (1).

T-tubule membranes were prepared according to Rosenblatt et al. (3). At the end of the procedure the vesicles were suspended in 0.3 M sucrose, 5 mM imidazole, pH 7.4, and 0.1 mM EGTA, frozen in liquid nitrogen, and stored at \(-80\) °C.

\textbf{Measurement of Ca\textsuperscript{2+} Transport—ATP-dependent Ca\textsuperscript{2+} uptake was measured isotopically as described by Caroni and Carafoli (6). Membrane vesicles (50 mg of protein) were incubated at 37 °C in a medium containing 130 mM KCl, 20 mM Tris, pH 7.4, 5 mM MgCl\textsubscript{2}, 2 mM ATP, 2 mM ruthenium red, and 40 \textmu M free Ca\textsuperscript{2+}. Aliquots were withdrawn at 10-s intervals, immediately filtered through Millipore filters (0.45 \textmu m pore size), and washed once with cold 130 mM KCl, 20 mM Tris, pH 7.4, and 1 mM LaCl\textsubscript{3}. When indicated, 3 \textmu M of purified bovine brain calmodulin, 5 \textmu M vanadate, or 3.3 mM oxalate were included. The Ca\textsuperscript{2+} uptake velocity was constant within the first 60 s.

The Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity was measured as described by Jones et al. (20) after preincubation of the membranes (0.5 mg/ml) at room temperature in the presence of SDS (0.3 mg/ml) (29). Estimates of the sidedness of the sarcolemmal preparation were carried out according to Caroni and Carafoli (17). The activity was measured in the absence and presence of detergent or in the presence of 1 \textmu M mepacrine.

\textbf{Dephosphorylation and Phosphorylation of Membrane Proteins—} The various membrane fractions were dephosphorylated at room temperature for 10 min by incubation in a medium containing 3 mM MgCl\textsubscript{2}, 0.3 M sucrose, 5 mM imidazole, pH 7.4, at the protein concentration of 1 mg/ml. They were immediately phosphorylated by the addition of ATP (0.1 mM) and terminated after 2 min by adding 3 mM EDTA and placing the tubes on ice. The tubes were immediately centrifuged in a Beckman Airfuge at an air pressure of 206.7 kilopascals for 15 min. When indicated, 2 \textmu M of cAMP-dependent protein kinase inhibitor (cAMP-dependent)/ml were included. The pellets obtained were suspended in 0.3 M sucrose, 5 mM imidazole, pH 7.4, and used immediately for the assay.

To analyze the phosphoprotein formed membrane vesicles (50 mg of protein) were phosphorylated with [\textgamma-\textsuperscript{32}P]ATP (0.5 mM/cell) at room temperature for the time indicated. The reaction was quenched by the addition of 2 volumes of a stopping solution containing 130 mM KCl, pH 6.8, 10% SDS, 2% mercaptoethanol, 20% glycerol, and 0.01% bromphenol blue. When indicated, the samples were boiled for 15 min. The peptides were separated by SDS-polyacrylamide gel electrophoresis using the discontinuous buffer system of Laemmli (21). The gels were fixed for 15 min in 10% trichloroacetic acid and 40% methanol, stained with Coomasie Blue, destained, dried, and exposed to an x-ray film using an intensifying screen for 2–3 days at \(-80\) °C. The relative level of radioactivity associated with the proteins was determined by cutting the radioactive protein bands and counting the radioactivity in a scintillation counter.

\textbf{Isolation of the Sarcolemmal Ca\textsuperscript{2+}-ATPase—} Skeletal muscle sarcolemmal vesicles (final protein concentration, 5 mg/ml) were solubilized with 1 mg of Triton X-100/mg of protein for 10 min at 4 °C (19) in a medium containing 20 mM HEPES, pH 7.4, 130 mM KCl, 0.05% Triton X-100, and 0.5 mM of various phosphatase inhibitors (22) added as a 200-fold concentrated stock solution in dimethyl sulfoxide: phenylmethylsulfonyl fluoride, and 0.5 mM of the following protease inhibitors (22) added as a 200-fold concentrated stock solution in dimethyl sulfoxide: phenylmethylsulfonyl fluoride, 1-(p-toluenesulfonyl)-amido-2-phenylethyl chloromethyl ketone, 1,5-amin-1-(p-toluenesulfonyl)-amidopenyl chloromethyl ketone, p-aminobenzenamine, and 1,10-phenanthroline. The protease inhibitors were used to prevent the proteolytic degradation of membrane proteins including the Ca\textsuperscript{2+}-ATPase. The mixture was centrifuged for 40 min at 150,000 \times g. Asolectin (0.5 mg/ml, final concentration) and CaCl\textsubscript{2} (0.1 mM, final concentration) were added to the supernatant fluid, which was immediately applied to a calmodulin affinity column (19). The size of the calmodulin affinity matrix was reduced to 10% of the original size with 2 mM EDTA instead of 0.1 mM CaCl\textsubscript{2}. To the fractions eluted with EDTA, MgCl\textsubscript{2} and CaCl\textsubscript{2} were added to a final concentration of 2 and 0.05 mM, respectively. All fractions were assayed for Ca\textsuperscript{2+} transport immediately after column chromatography. Polyacrylamide gel electrophoresis of the purified enzyme was carried out according to Laemmli (21). The gels were stained by a silver impregnation method (23). For the phosphorylation of the purified Ca\textsuperscript{2+}-ATPase with [\textgamma-\textsuperscript{32}P]ATP, the method described by Niggli et al. (19) was used.
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**Assays**—Protein was determined by the method of Lowry et al. (24) using bovine serum albumin as a standard.

**RESULTS**

In an initial series of experiments the basic characteristics of the purified plasma membrane were determined. The preparation used in the present study was enriched in sarcolemmal markers (Table I). It possessed high Na\textsuperscript{+}/K\textsuperscript{+}-ATPase and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activities. The latter was estimated by following the release of the Ca\textsuperscript{2+} accumulated into the vesicles by the ATP-dependent pump (see below) in response to the addition of NaCl. Over 85% of the Ca\textsuperscript{2+} accumulated by the preparations was released by 50 mM NaCl, a finding which is in excellent agreement with results on heart sarcolemmal vesicles, where the existence of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger is established (17, 25). The sidedness of the sarcolemmal vesicles was estimated from the ouabain inactivation of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity, assayed in the presence of the ionophore tubule vesicles (Table I). One other Ca\textsuperscript{2+}-binding protein was established (17, 25). The sidedness of the sarcolemmal vesicles number of preparations analyzed.

The sarcolemmal preparations used in these studies did not contain significant amounts of endogenous calmodulin. A hypotonic treatment of sarcolemmal vesicles, followed by a

**Table I**

Properties of the purified skeletal muscle sarcolemma preparation

<table>
<thead>
<tr>
<th>Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity*</th>
<th>40.0 ± 5.5 nmol P/min × mg (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger</td>
<td>24.0 ± 1.7 nmol Ca\textsuperscript{2+}/min × mg (5)</td>
</tr>
<tr>
<td>Initial rate</td>
<td>43.3 ± 3.5 nmol Ca\textsuperscript{2+}/min × mg (5)</td>
</tr>
<tr>
<td>Total uptake (after 3 min)</td>
<td>3.0 ± 0.2 nmol Ca\textsuperscript{2+}/mg protein (5)</td>
</tr>
</tbody>
</table>

* The Na\textsuperscript{+}/K\textsuperscript{+}-ATPase is presented here as the ouabain-sensitive activity obtained by subtracting the values in the presence of ouabain from the total values.

**Table II**

Ca\textsuperscript{2+}-ATPase activity of skeletal muscle sarcolemma and T-tubule membranes

<table>
<thead>
<tr>
<th>Additions</th>
<th>Sarcolemma\textsuperscript{a}</th>
<th>T-tubules\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>70.0 ± 5.9 (10)</td>
<td>24.0 ± 1.2 (5)</td>
</tr>
<tr>
<td>Calmodulin (3 pg)</td>
<td>127.0 ± 10.1 (10)</td>
<td>24.0 ± 1.5 (5)</td>
</tr>
<tr>
<td>Oligomycin (10 pg)</td>
<td>68.0 ± 5.1 (5)</td>
<td>25.0 ± 1.9 (5)</td>
</tr>
<tr>
<td>Ouabain (2 mM)</td>
<td>70.0 ± 4.2 (5)</td>
<td>23.0 ± 1.7 (5)</td>
</tr>
<tr>
<td>Potassium cyanide (0.5 mM)</td>
<td>65.0 ± 4.5 (5)</td>
<td>24.0 ± 1.3 (5)</td>
</tr>
<tr>
<td>Calmodizolium (2 mM)</td>
<td>66.0 ± 4.6 (5)</td>
<td>23.0 ± 1.8 (5)</td>
</tr>
<tr>
<td>Sarcolemma treated with a hypotonic solution followed by a hypertonic wash in the presence of EGTA</td>
<td>66.0 ± 5.0 (3)</td>
<td>ND\textsuperscript{4}</td>
</tr>
</tbody>
</table>

* The ATPase activity in the presence of Mg\textsuperscript{2+} (about 10.0 ± 1.0 nmol P/min × mg) was subtracted to obtain the values of the Ca\textsuperscript{2+}-ATPase activity of the sarcolemmal vesicles.

\textsuperscript{4} ND, not determined.

**FIG. 1.** Activation of the Ca\textsuperscript{2+}-ATPase of skeletal muscle sarcolemma by calmodulin. The Ca\textsuperscript{2+}-ATPase was assayed using the coupled enzyme assay described under “Experimental Procedures.” The amounts of purified bovine brain calmodulin indicated were added to the assay mixture. The results are expressed as the average of 5 experiments having a standard error of less than 10%.
The Ca\(^{2+}\)-ATPase of Skeletal Muscle Sarcolemma

![Graph](image)

**Fig. 2.** Effects of calmodulin on the apparent \(K_m\) (Ca\(^{2+}\)) and the \(V_{\text{max}}\) of the Ca\(^{2+}\)-ATPase of skeletal muscle sarcolemma. Experimental conditions were as in the experiment in Fig. 1. The free Ca\(^{2+}\) was calculated with the help of a computer program. The values are the average of 4 experiments having a standard error of about 10%. 0, untreated sarcolemmal vesicles; 1, sarcolemmal vesicles plus 3 \(\mu\)g of calmodulin.

**Table III**

ATP-driven Ca\(^{2+}\) uptake by skeletal muscle sarcolemma

<table>
<thead>
<tr>
<th>Additions</th>
<th>ATP-driven Ca(^{2+}) uptake (nmol Ca(^{2+})/mg protein in 1 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>18.0 ± 1.0 (5)</td>
</tr>
<tr>
<td>Calmodulin (3 (\mu)g)</td>
<td>28.0 ± 1.6 (5)</td>
</tr>
<tr>
<td>Oxalate (3.3 mM)</td>
<td>20.0 ± 1.1 (5)</td>
</tr>
</tbody>
</table>

TABLE IV also shows the effects of the phosphorylation of sarcolemma with added cAMP- or Ca\(^{2+}\)/calmodulin-dependent systems. Clearly, only the cAMP-dependent phosphorylation of the membrane was effective in restoring the original activity of Ca\(^{2+}\) transport (Ca\(^{2+}\)-ATPase activity and Ca\(^{2+}\) uptake). The addition of protein kinase inhibitor (cAMP dependent) to the phosphorylation mixture abolished the effects, indicating the cAMP dependence of the phosphorylation process. Cyclic AMP alone was effective in the activation of Ca\(^{2+}\) transport suggesting that the sarcolemmal vesicles contain an endogenous cAMP-dependent protein kinase. No effects were observed when cGMP was substituted for cAMP (Table IV). To make the effects of membrane phosphorylation on the Ca\(^{2+}\)-ATPase activity visible it was essential to dephosphorylate the vesicles. Phosphorylation of the native, i.e. not dephosphorylated sarcolemmal vesicles, failed to produce significant changes in the enzyme activity (data not shown). Dephosphorylation and phosphorylation had no influence on the apparent \(K_m\) (Ca\(^{2+}\)) of the Ca\(^{2+}\)-ATPase data (not shown). They only influenced the apparent \(V_{\text{max}}\) of the enzyme. No effects of membrane phosphorylation and dephosphorylation were observed on the Ca\(^{2+}\)-ATPase activity of the T-tubule membranes.

**Fig. 3.** Vanadate inhibition of the Ca\(^{2+}\)-ATPase activity of skeletal muscle sarcolemma. The activity was measured as described under "Experimental Procedures." 100% activity corresponding to 65 nmol P/\(\mu\)g protein, 50% and 25% were measured. The values are the average of 3 experiments, having a standard error of about 8%.

Effects of Vanadate on the Sarcolemmal Ca\(^{2+}\)-ATPase—Vanadate inhibits different transport ATPases (28) at concentrations that vary somewhat depending on the ATPase; the Ca\(^{2+}\)-ATPases of cardiac sarcolemma and erythrocyte membrane require less than 1 \(\mu\)M vanadate for half-maximal inhibition (8), whereas the sarcoplasmic reticulum ATPase may require up to 50 \(\mu\)M (29). The Ca\(^{2+}\)-ATPase activity of skeletal muscle sarcolemma was inhibited by vanadate (Fig. 3), half-maximal inhibition being produced by 1.7 \(\mu\)M. Inhibitions in excess of 90% were observed at 5 \(\mu\)M vanadate. On the other hand, vanadate had no effect on the T-tubule Ca\(^{2+}\)-ATPase, in agreement with what was described by Sabbadini and Okamoto (30). Concentrations as high as 500 \(\mu\)M were tested but failed to produce inhibition (data not shown).

Effects of Deoxyribonuclease and Phosphorylation of the Sarcolemmal Vesicles on the Ca\(^{2+}\)-ATPase—It has been shown earlier that freshly prepared cardiac sarcolemma vesicles are heavily phosphorylated and might contain endogenous phosphatase(s) (9, 31). Treatment of cardiac sarcolemma vesicles with Mg\(^{2+}\) alone was effective in dephosphorylating the membrane (9). The sarcolemma preparations used in this study were evidently also phosphorylated at the end of the isolation procedure, since their incubation in a medium containing 3 mM Mg\(^{2+}\) led to marked inhibition of the Ca\(^{2+}\)-ATPase and of Ca\(^{2+}\) transport (Table IV). Both activities were still stimulated by calmodulin to the same extent as observed in the native membrane.
Ca\(^{2+}\)-ATPase activity and ATP-driven Ca\(^{2+}\) uptake were measured as described under "Experimental Procedures." The membrane vesicles were dephosphorylated by incubation in the presence of 3 mM MgCl\(_2\), for 10 min at room temperature, immediately phosphorylated, centrifuged, and assayed. The numbers in parentheses represent the number of preparations analyzed.
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**FIG. 4.** Phosphorylation of skeletal muscle sarcolemma (A) and T-tubule (B) proteins. Polyacrylamide gel electrophoresis (15% acrylamide) of phosphorylated sarcolemma and T-tubule vesicles was performed as indicated under "Experimental Procedures." The phosphorylation was performed in the presence of: 1, 1 \(\mu\)M cAMP, 3 \(\mu\)g of cAMP-dependent protein kinase, 1 mM EGTA; 2, 1 \(\mu\)M cAMP, 1 mM EGTA; 3, 0.1 mM CaCl\(_2\), 3 \(\mu\)g of bovine brain purified calmodulin; 4, 1 mM EGTA; 5, 0.1 mM CaCl\(_2\). The reaction was carried out for 2 min at room temperature in 5 mM imidazole, pH 7.4, 2 mM MgCl\(_2\), 0.1 mM [\(\gamma\)-\(^{32}\)P]ATP, 1 mg of membrane protein/ml. Fifty \(\mu\)g of membrane protein were used/lane. a, Coomassie Blue stain; b, autoradiogram. Standard proteins for the estimation of \(M_r\) (O): phosphorylase b (92,500), bovine serum albumin (66,500), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500).

with EDTA was tested for the formation of the \(^{32}\)P-phosphoenzyme after incubation with [\(\gamma\)-\(^{32}\)P]ATP in the presence and absence of Ca\(^{2+}\). The results in Fig. 7B, lane 2, show that the radioactivity peak had an \(R_f\) value corresponding to a \(M_r\) of 140,000. In the presence of Mg\(^{2+}\) and EGTA or 5 \(\mu\)M vanadate, the amount of radioactivity associated with the 140,000-Da protein was reduced by more than 90%.

**DISCUSSION**

The results described in this report demonstrate that an ATP-energized Ca\(^{2+}\) pump is an intrinsic component of the sarcolemma of skeletal muscle. That the ATP-dependent Ca\(^{2+}\) uptake of the sarcolemmal preparation used is indeed present in the sarcolemmal vesicles is shown by the Na\(^{+}\)-dependent release of the Ca\(^{2+}\) accumulated; the Na\(^{+}\)/Ca\(^{2+}\) exchange system is normally considered to be a functional marker of sarcolemma. The skeletal muscle sarcolemmal Ca\(^{2+}\)-ATPase and the associated ATP-dependent Ca\(^{2+}\) uptake are sensitive to calmodulin. The modulator shifts the enzyme to a higher Ca\(^{2+}\) affinity state (\(K_m\) shift from about 300 to about 50 nM) and enhances its \(V_{max}\) by about 80%. The present work has also shown that skeletal muscle sarcolemma is practically free of endogenous calmodulin. As isolated in this work, the sarcolemmal membranes are substantially phosphorylated and contain both (an) endogenous Mg\(^{2+}\)-stimulated and F\(^{-}\)-sensitive phosphatase(s) and an endogenous protein kinase system of the cAMP-dependent type. The presence of the cAMP-dependent protein kinase in skeletal muscle sarcolemma has been already reported (2, 33). The system enhances the Ca\(^{2+}\)-ATPase markedly and reversibly. By contrast, the phosphorylation of the sarcolemmal membrane with either Ca\(^{2+}\) or
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Ca²⁺ plus calmodulin fails to influence the Ca²⁺-ATPase. Thus, the Ca²⁺-ATPase in skeletal muscle sarcolemma is regulated directly by calmodulin and indirectly by a cAMP-dependent phosphorylation system. Based on the similar Ca²⁺ dependence of the pumping reaction in the phospho- and dephosphorylated states it is suggested that the phosphorylation-dephosphorylation-controlled process affects the turnover rate of the enzyme but not its apparent affinity for Ca²⁺. The phosphorylation process has three main protein substrates in the sarcolemmal membrane. Whether all three are involved in the regulation of the ATPase is at the moment an open question.

As is the case for other plasma membrane Ca²⁺-ATPases, the solubilized sarcolemmal Ca²⁺-ATPase could also be purified by calmodulin affinity chromatography. The sarcolemmal Ca²⁺-ATPase resembles closely the analogous enzymes so far described in other plasma membranes (8) and is clearly different from that of sarcoplasmic reticulum. Among the similarities to plasma membrane Ca²⁺-ATPases, the molecular weight (140,000 Da), the high affinity for Ca²⁺, the sensitivity to vanadate, and the direct stimulation by calmodulin are of obvious significance (8).

The Ca²⁺-ATPase is very low in the T-tubule membrane (11). Contrary to sarcolemmal membranes, the T-tubule enzyme is not calmodulin and vanadate sensitive and is not affected by the phosphorylation and dephosphorylation of the membrane. These differences point to the usefulness of the Ca²⁺-ATPase as a marker to distinguish the sarcolemmal and T-tubule membranes. The results obtained here have shown that the T-tubule membrane contains endogenous phosphatase(s) and a cAMP-dependent protein kinase, which phosphorylates a protein of M₉ of about 30,000. The significance of this membrane phosphorylation has not been established, but it is possible that it is involved in regulating membrane permeability or a specific transport system.

The significance of the Ca²⁺/calmodulin-dependent phosphorylation observed in both the sarcolemmal and T-tubule membranes remains obscure. The M₉ of the phosphoproteins formed by this process (39,000 and 23,000 Da in sarcolemma, 70,000 and 20,000 Da in the T-tubules) correlates with those described for the calmodulin-dependent phosphorylation or sarcoplasmic reticulum (34, 35). This may reflect contamination of the membrane preparations used here by sarcoplasmic reticulum. On the other hand, the skeletal muscle plasma membrane may contain specific substrates for a calmodulin-dependent phosphorylation. These substrates could be present in other membranes as well.

The role of the Ca²⁺-ATPase in skeletal muscle sarcolemma and especially its dual-regulation system are open to speculation. Unlike cardiac muscle, excitation-contraction coupling in skeletal muscle does not depend on external Ca²⁺. It is, therefore, unlikely that the Ca²⁺-ATPase has a specific role in the process of excitation-contraction coupling. The Ca²⁺-ATPase, operating in parallel with the Na⁺/Ca²⁺ exchange system, will remove Ca²⁺ that has entered the sarcoplasm in direct response to an action potential or as a leak current (36). Given its very high affinity for Ca²⁺, the ATPase is optimally suited to keep the intracellular level of free Ca²⁺ around 0.1 μM.

The regulation of the sarcolemmal Ca²⁺-ATPase by a cAMP-dependent membrane phosphorylation suggests the involvement of the hormonal factors in the eflux of Ca²⁺ from skeletal muscle cells. Skeletal muscle plasma membranes have been shown to contain adrenergic and serotoninergic receptors linked to the adenyl cyclase system (37-39). Seroto-
nergic and adrenergic action on skeletal muscle influences (among other processes) the metabolism of glycogen and amino acids. It thus appears that serotonin, catecholamines, and dopamine may mediate, through a cAMP-dependent mechanism, changes in Ca\(^{2+}\) efflux to the extracellular space. The cAMP-dependent stimulation of sarcolemmal proteins phosphorylation may influence the fluxes of Ca\(^{2+}\) in sarcolemma in other ways as well. The regulation of the opening of Ca\(^{2+}\) channels, documented in heart sarcolemma, is but a prominent example of this (5).

Acknowledgments—Thanks are due to Drs. M. Chiesi and J. Krebs for providing the purified sarcoplasmic reticulum and bovine brain calmodulin, respectively. The assistance of L. Soldati in measuring the Na\(^+\)/Ca\(^{2+}\) exchange activity is gratefully acknowledged.

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