Characterization of Membrane Fractions and Isolation of Purified Plasma Membranes from Rat Myometrium*

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Plasma membranes from rat myometrium preincubated in Krebs-Ringer solution and homogenized in a Polytron were isolated by differential and sucrose density gradient centrifugation. The enzyme activities of isolated plasma membranes were analyzed to determine the contamination by mitochondrial inner and outer membranes, endoplasmic reticulum, and lysosomes. The activities of succinate-cytochrome c reductase and rotenone-sensitive NADH-cytochrome c reductase, markers for mitochondrial inner membrane, were enriched in the mitochondrial fraction and decreased to a very low level in the plasma membrane fraction. Activities of monoamine oxidase and rotenone-insensitive NADH-cytochrome c reductase, supposed markers for mitochondrial outer membrane, although enriched in the mitochondrial fraction, were also present in the plasma membrane fraction. RNA, protein synthesis, and NADPH-cytochrome c reductase activity, markers for endoplasmic reticulum, were found in small amounts in plasma membrane but in increased amounts in heavier fractions in the sucrose density gradient. The activities of lysosomal enzymes, β-glucuronidase and β-galactosidase, were very low in this tissue and present predominantly in mitochondrial and soluble fractions. The plasma membrane fraction was highly enriched with 5'-nucleotidase activity and with lectin and specific oxytocin binding sites. Electron microscopy of the plasma membrane fraction revealed sealed vesicles with little contamination with other membranes. On the basis of protein concentration and marker enzyme activities, it was calculated that this fraction contained 70 to 80% plasma membrane. Calcium uptake by the plasma membrane fraction was the highest among all fractions both in the absence and in the presence of ATP, supporting its strong involvement in calcium exchange during excitation-contraction coupling and relaxation in intact muscle.

We have reported a standard method for the isolation of subcellular membranes from myometrium and other smooth muscle (1, 2). The fraction markedly enriched in plasma membrane was capable of binding and transporting calcium in the presence of ATP under conditions suggesting that this membrane was involved in the regulation of intracellular levels of this ion (2). However, the studies did not establish markers for different smooth muscle organelles and therefore were incomplete with respect to yield, distribution, and purity of different subcellular fractions. In particular, the degree of contamination of the plasma membrane fractions by endoplasmic reticulum was not determined. In this paper, we report a detailed study of isolation of plasma membrane from rat myometrium, having introduced additional markers for plasma membrane and studied markers for endoplasmic reticulum and mitochondrial inner and outer membranes which were not established in smooth muscle previously. We have also developed a mathematical approach to estimate the purity of different fractions. Our analysis indicates that the plasma membrane fraction is the purest thus far obtained from smooth muscle and supports the contention that this membrane transports calcium and may regulate the intracellular concentration of this ion.

**MATERIALS AND METHODS**

Portions of this paper (including tables and figures) are presented in detail in a miniprint at the end of this paper. In general, tissues were homogenized using a Polytron PT 20, centrifuged to remove nuclei and unbroken cells, fractionated by differential centrifugation into a mitochondrial and a microsomal fraction, and the latter was subdivided on either a continuous or a discontinuous sucrose gradient.

The distribution of a variety of enzyme activities and of specific binding sites was followed during the fractionation. 3'-Nucleotidase activity, wheat germ [H]fagglutinin binding, and [H]oxytocin binding were used as markers for plasma membranes; NADPH-cytochrome c reductase activity was used for smooth endoplasmic reticulum; rotenone-insensitive NADH-cytochrome c reductase for outer mitochondrial membrane, and succinate-cytochrome c reductase and rotenone-sensitive NADH-cytochrome c reductase for inner mitochondrial membrane.

The distribution of Mg²⁺-ATPase, monoamine oxidase, NADPH-peroxidase, β-glucuronidase, β-galactosidase, and kynurenine hydroxylase were also examined, as well as the ability to bind and transport Ca²⁺ in the presence and absence of ATP.

**RESULTS**

**Differential Centrifugation of the Postnuclear Supernatant**—The distribution of various markers and protein in mitochondrial, microsomal, and soluble fractions obtained after differential centrifugation is shown in Table I.

The mitochondrial fraction (10,000 × g, pellet) was enriched

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about 5-fold with succinate-cytochrome c reductase and rotenone-sensitive NADH-cytochrome c reductase activities, markers for mitochondrial inner membrane (15). This fraction contained 60 to 70% of the total activity of these two enzymes. The microsomal fraction contained 22 to 25% of the activities of both the enzymes without a significant increase in specific activity above that of the postnuclear supernatant. Monoamine oxidase, a possible marker enzyme for mitochondrial outer membrane (22), was enriched only about 2-fold in the mitochondrial fraction which contained 30% of the total activity. However, it was also increased (1.3 times) in the microsomal fraction, which contained 23% of the total activity. These results are consistent with a recent report that this enzyme is also a constituent of endoplasmic reticulum and nuclear membranes and, in some tissues, of plasma membrane (23). No kynurenine hydroxylase activity, another reported mitochondrial outer membrane marker (15), was detected in any fraction. The soluble fraction (113,000 × g supernatant) only showed to contain some (7%) monoamine oxidase activity.

The distribution of plasma membrane markers is also shown in Table I. 5'-Nucleotidase activity successfully used previously as a marker for plasma membrane in rat myometrium (1, 2) was enriched 2.5-fold in the microsomal fraction which contained 43% of the total activity. The mitochondrial fraction contained very little 5'-nucleotidase activity (12%). The soluble supernatant contained 43% of the total 5'-nucleotidase activity. However, its specific activity was very low. This activity was not diminished even after centrifugation at higher speed and for a prolonged period of time and may represent some soluble form of the enzyme.

The distribution of specific binding sites for oxytocin was also investigated as the receptor for this hormone seems to be localized in the plasma membrane of target tissues (4, 5). The microsomal fraction was enriched 3.5-fold in [3H]oxytocin binding with respect to the postnuclear supernatant and contained 60% of the total binding sites. Although some enrichment in the mitochondrial fraction was observed, this fraction contained only 20% of the total binding sites.

Wheat germ [3H]agglutinin was also used as a plasma membrane marker (16). Rat myometrial plasma membranes were labeled with [3H]WGA before homogenization. The highest specific binding of [3H]WGA (2.9 times that of the postnuclear supernatant) was found to be in the microsomal fraction which contained 50% of the total binding sites. The total and specific binding of WGA were low in the mitochondrial and soluble fractions. The pattern of distribution of activities associated with plasma membrane (5'-nucleotidase, oxytocin, and WGA bindings) were found to be similar.

No marker enzyme for endoplasmic reticulum has been yet established for smooth muscle. NADPH cytochrome c reductase, a marker established for endoplasmic reticulum of liver cells (8), was employed in the present study and found to be enriched slightly more in the microsomal fraction (2.3 times) than in the mitochondrial fraction (1.9 times). The activity present in the mitochondrial fraction is probably due to the numerous vesicles of rough and smooth endoplasmic reticulum seen in this fraction by electron microscopy. Protein synthesis measured by incorporation of [3H]leucine into trichloroacetic acid-precipitable proteins, and RNA content as well as Mg2+-ATPase and rotenone insensitive NADH cytochrome c reductase, which are localized both in mitochondrial outer membrane and endoplasmic reticulum of liver cells (24) were similarly distributed. NADPH-peroxidase, which has been demonstrated to be present in rat liver microsomes (12), was present in rat myometrium but the specific activity of this enzyme was about two times higher in mitochondria than in microsomes. The soluble supernatant contained 73% of the total enzyme activity in an unusual total recovery of 126% (Table I).

Most of the activities of lysosomal enzymes, β-glucuronidase, and β-galactosidase were found in the soluble supernatant (Table I), but a small portion (14%) was in the mitochondrial fraction.

**DISCUSSION**

The aim of this work was the development of a reproducible preparation of a purified plasma membrane fraction from rat myometrium and to provide positive identification of this membrane constituent as well as of contamination by other organelle membranes. Previous reports from our laboratories did not extensively examine the markers for endoplasmic reticulum, mitochondrial outer, and inner membrane or the markers for lysosomes.

This study provides strong support for our earlier use of 5'-nucleotidase as marker of plasma membrane (1, 2, 4). Wheat germ agglutinin binding sites are localized in sarcolemmal membrane in skeletal muscle (25). This lectin was also found to bind predominantly to the plasma membrane fraction when the outer surface membrane of the rat myometrium was labeled with [3H]WGA prior to fractionation. The generally
parallel distribution of 5'-nucleotidase activity and [\(^{14} \text{H}\)]WGA binding to that of specific [\(^{14} \text{H}\)]oxytocin binding validates their location together in the plasma membrane. This is further supported by the results on the inhibition of the 5'-nucleotidase activity by both WGA and concanavalin A. It should be noted that both lectin and oxytocin binding sites and the enzyme active sites are probably accessible from the outer aspect of the plasma membrane. [\(^{14} \text{H}\)]WGA binding was done in intact cells while the other markers were studied after homogenization. However, all should be enriched in parallel during fractionation even if the observed values for oxytocin binding and 5'-nucleotidase activity are submaximal owing to the existence of some inside-out plasma membrane vesicles after homogenization. The localization of oxytocin receptors in the plasma membrane of rat myometrium supports the validity of using membrane receptors as markers for plasma membrane (26) and provides another useful marker for myometrial plasma membranes, which can be used to investigate the early molecular events in the hormone’s action.

REFERENCES
Smooth Muscle Membranes

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Strength in Motion: A Characterization of Membrane Function and Interaction of Smooth Muscle Membranes with the Extracellular Matrix


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Characteristics

- ATP, Na+, K+, Ca2+, Mg2+, and water
- Membrane potential, ion flux, and energetic processes
- Putative connections between smooth muscle membranes and extracellular matrix

Background

Smooth muscle membranes are part of the complex interplay between cytoskeletal and membrane proteins, which determine the cell's mechanical properties. The membrane plays a critical role in maintaining the cell's integrity and function. Membranes are dynamic structures that undergo constant changes in response to various stimuli. The characteristics of smooth muscle membranes are influenced by the extracellular matrix, which exerts mechanical forces on the cell and regulates the formation of focal adhesions.

Introduction

The aim of this study was to characterize the mechanical properties of smooth muscle membranes in response to various stimuli. The experimental setup involved the measurement of membrane deformation under applied forces. The results showed that the membrane properties were significantly influenced by the extracellular matrix and the cell's mechanical state.

Materials and Methods

- Isolation of intact smooth muscle membranes
- Measurement of membrane deformation using a force-myograph system
- Analysis of membrane properties using拉曼光谱学

Results

- Membrane deformation under applied forces
- Influence of extracellular matrix on membrane properties

Discussion

The results of this study suggest that the mechanical properties of smooth muscle membranes are regulated by the extracellular matrix and the cell's mechanical state. Further research is needed to elucidate the molecular mechanisms underlying these observations.

Conclusion

The study provides insights into the regulation of smooth muscle membrane properties and has implications for understanding the complex interplay between cytoskeletal and membrane proteins.

References


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Smooth Muscle Membranes

The assumption that fractions 7-17 contained Ca-plasma membranes proved to be incorrect. Further investigation revealed that the preparation used for fractionation included plasmalemmae from various tissues. Therefore, additional experiments were performed using mitochrondria or reduced-oxidized plasma membranes as control fractions. In these experiments, the Ca-plasma membranes were obtained from either smooth muscle or reduced-oxidized plasma membranes. These experiments confirmed that the Ca-plasma membranes contained both Ca and Ca-1.3, as well as Ca-plasma and reduced-oxidized plasma membranes.

Fig. 1. Electrophoresis of Ca-plasma membranes.

Fig. 2. Mitochondria and plasma membrane fractions.

Fig. 3. Absorption spectra of mitochondria and plasma membrane fractions.

Fig. 4. Nucleotidease and cytochrome c reductase activities in fractions.

Fig. 5. Sulfite and cytochrome c reductase activities in fractions.

Fig. 6. Nucleotidease activity in fractions of Ca-plasma membranes.
Table I: Distribution of Markers in Fractions Obtained after Differential Centrifugation

<table>
<thead>
<tr>
<th>Markers</th>
<th>1000 x g Supernatant</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Solute-Phase</th>
<th>Total Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth Muscle Membranes</td>
<td>852</td>
<td>764</td>
<td>665</td>
<td>764</td>
<td>100%</td>
</tr>
<tr>
<td>Specific Activity</td>
<td>122</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>100%</td>
</tr>
<tr>
<td>% Recovery</td>
<td>80%</td>
<td>90%</td>
<td>85%</td>
<td>85%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table II: Distribution of Markers in Fractions Obtained after Discontinuous Sucrose Density Gradient Centrifugation

<table>
<thead>
<tr>
<th>Markers</th>
<th>Fractons</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth Muscle Membranes</td>
<td>852</td>
<td>100%</td>
</tr>
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</tr>
<tr>
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<td>100%</td>
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Table III: Calculated Values for Unit Membrane Protein

<table>
<thead>
<tr>
<th>Fractons</th>
<th>1000 x g Supernatant</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Solute-Phase</th>
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<td>100%</td>
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</table>

Table IV: Distribution of Membrane Markers in Discontinuous Sucrose Density Gradient Centrifugation

<table>
<thead>
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<tr>
<td>% Recovery</td>
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Table V: Relative Activity of New Fractons

<table>
<thead>
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<tr>
<td>% Recovery</td>
<td>80%</td>
<td>100%</td>
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</table>
\[ a^2 = b^2 + c^2 \]

For another fraction:
\[ a^2 = b^2 + c^2 \]

A true number for \( a \) would have the activities:
\[ a_{\text{true}} = \text{true finite value} \]
\[ a_{\text{meas}} = a_{\text{true}} \]

A true number for \( b \) would have the activities:
\[ b_{\text{true}} = \text{true finite value} \]
\[ b_{\text{meas}} = b_{\text{true}} \]

A true number for \( c \) would have the activities:
\[ c_{\text{true}} = \text{true finite value} \]
\[ c_{\text{meas}} = c_{\text{true}} \]

For further simplification:
\[ a^2 = c^2 + d^2 \]

\[ b^2 = c^2 + e^2 \]

\[ c^2 = f^2 + g^2 \]

\[ d^2 = f^2 + h^2 \]

\[ e^2 = g^2 + h^2 \]

Same as above, with:
\[ a_{\text{true}} = \text{true finite value} \]
\[ a_{\text{meas}} = a_{\text{true}} \]

\[ b_{\text{true}} = \text{true finite value} \]
\[ b_{\text{meas}} = b_{\text{true}} \]

\[ c_{\text{true}} = \text{true finite value} \]
\[ c_{\text{meas}} = c_{\text{true}} \]

\[ d_{\text{true}} = \text{true finite value} \]
\[ d_{\text{meas}} = d_{\text{true}} \]

\[ e_{\text{true}} = \text{true finite value} \]
\[ e_{\text{meas}} = e_{\text{true}} \]

\[ f_{\text{true}} = \text{true finite value} \]
\[ f_{\text{meas}} = f_{\text{true}} \]

\[ g_{\text{true}} = \text{true finite value} \]
\[ g_{\text{meas}} = g_{\text{true}} \]

\[ h_{\text{true}} = \text{true finite value} \]
\[ h_{\text{meas}} = h_{\text{true}} \]

In a real experiment, for a given membrane fraction and measuring true finite values:
\[ a_{\text{true}} = \text{true finite value} \]
\[ b_{\text{true}} = \text{true finite value} \]
\[ c_{\text{true}} = \text{true finite value} \]

\[ d_{\text{true}} = \text{true finite value} \]
\[ e_{\text{true}} = \text{true finite value} \]

\[ f_{\text{true}} = \text{true finite value} \]
\[ g_{\text{true}} = \text{true finite value} \]

\[ h_{\text{true}} = \text{true finite value} \]

Acceptable results for true finite values are:
\[ a_{\text{true}} = \text{true finite value} \]
\[ b_{\text{true}} = \text{true finite value} \]
\[ c_{\text{true}} = \text{true finite value} \]

\[ d_{\text{true}} = \text{true finite value} \]
\[ e_{\text{true}} = \text{true finite value} \]

\[ f_{\text{true}} = \text{true finite value} \]
\[ g_{\text{true}} = \text{true finite value} \]

\[ h_{\text{true}} = \text{true finite value} \]

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