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-sensitive 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 subfractionated 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]agglutinin binding, and [H]oxytocin binding were used as markers for plasma membranes; NADPH-cytochrome c reductase activity was used for smooth endoplasmic reticulum, [H]leucine incorporation into protein 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

1 "Materials and Methods," Figs. 1A, 1B, 2, 3, and 4, Tables I to V, and further discussion and an Appendix are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-310, cite author(s), and include a check or money order for $3.30 per set of photocopies.
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 mitochondrial 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 3.6-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 [1H]agglutinin was also used as a plasma membrane marker (16). Rat myometrial plasma membranes were labeled with [1H]WGA before homogenization. The highest specific binding of [1H]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 [1H]leucine into tri-chloroacetic 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.

**Discontinuous Sucrose Density Gradient Centrifugation of Microsomes**—Results of the studies on different fractions obtained from the sucrose gradient are presented in Table II. The F1 fraction contained 13% of the total protein and had the highest specific activity of 5'-nucleotidase and highest specific binding of [3H]oxytocin and of [3H]WGA (Fig. 2A). [3H]WGA binding sites and 5'-nucleotidase activity were enriched 2.3- to 3-fold over the microsomal fraction and 6.8- to 8.4-fold over the postnuclear supernatant. Enrichment of [3H]WGA and [3H]oxytocin followed a similar pattern, the enrichment of [3H]oxytocin being 12.6 times. The F2 fraction contained a substantial amount of 5'-nucleotidase activity, oxytocin binding, and WGA binding. The specific activity or bindings of the three plasma membrane markers decreased with the heavier gradient fractions indicating decrease in the presence of plasma membrane fragments.

Very little succinate-cytochrome c reductase and rotenone-sensitive NADH-cytochrome c reductase activities were present in the F1 fraction indicating negligible contamination by mitochondrial inner membrane. Mitochondrial inner membrane appeared to be concentrated in the heavier fractions, particularly F4. Monoamine oxidase was present in all the fractions in substantial amounts.

A small portion (5 to 6%) of the endoplasmic reticulum markers, NADPH-cytochrome c reductase, [1H]leucine incorporation into protein, and RNA were present in the F1 fraction. These markers were concentrated in the heavier fractions, mainly in the F5 fraction where much rough endoplasmic reticulum was also observed under the electron microscope. Mg2+-ATPase appeared to be a membrane-bound enzyme distributed more or less uniformly except in the F5 fraction. Fraction F5 contained 45% of the total protein in the gradient and 30 to 40% of the endoplasmic reticulum and mitochondrial marker activities.

**Calcium Uptake**—Results on calcium uptake by different fractions obtained by differential centrifugation and discontinuous sucrose gradient centrifugation are presented in Table III. The highest passive binding of Ca2+ was observed in the plasma membrane fraction. High ATP-dependent Ca2+ uptake reflects the presence of the active calcium pump in the plasma membrane which was previously demonstrated (2).

**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 sarcosomal 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 [1H]WGA prior to fractionation. The generally

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2 The abbreviation used is: WGA, wheat germ agglutinin.
parallel distribution of 5'-nucleotidase activity and [³H]WGA binding to that of specific [³H]oxygenotic 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 oxygenotic binding sites and the enzyme active sites are probably accessible from the outer aspect of the plasma membrane. [³H]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 oxygenotic binding and 5'-nucleotidase activity are submaximal owing to the existence of some inside-out plasma membrane vesicles after homogenization. The localization of oxygenotic 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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SMOOTH MUSCLE MBRANES

Membranes

Membrane proteins are necessary for smooth muscle function as well as for the regulation of various cellular processes. Membranes play a key role in the transduction of signals across the cell membrane. They contain a variety of proteins including ion channels, receptors, and transporters. Membranes are composed of lipids and proteins that form a bilayer structure. The membrane lipid bilayer is composed of phospholipids and cholesterol, which provide the structural framework for membrane proteins. Membrane proteins are embedded in the lipid bilayer and are responsible for various functions such as signal transduction, transport, and cell adhesion.

Membrane Proteins

Membrane proteins are divided into two main categories: integral and peripheral proteins. Integral membrane proteins are embedded in the lipid bilayer and are involved in transport, signaling, and ion channel functions. Peripheral membrane proteins are loosely associated with the membrane and perform tasks such as cell adhesion and signaling. The integral membrane proteins are further divided into transmembrane proteins and non-transmembrane proteins.

Membrane Lipids

Membrane lipids are composed of phospholipids and cholesterol. Phospholipids are composed of two lipid tails and a polar head group. The hydrophobic tails are embedded in the lipid bilayer, while the hydrophilic head groups are exposed to the aqueous environment. Cholesterol is a hydrophobic lipid that is found in high concentrations in the membrane bilayer. Cholesterol plays a crucial role in maintaining the stability of the membrane bilayer.

Membrane Proteome

The membrane proteome refers to the complete set of membrane proteins expressed by an organism. The membrane proteome is complex and dynamic, consisting of a large number of membrane proteins with diverse functions. Membrane proteins are involved in various cellular processes such as transport, signaling, and cell adhesion. Understanding the membrane proteome is crucial for the development of therapeutic strategies and the identification of disease biomarkers.

Membrane Transport

Membrane transport is the process by which substances move across the cell membrane. Membrane transport can occur through passive diffusion, facilitated diffusion, active transport, and osmosis. Passive diffusion is the movement of substances down their concentration gradient, while facilitated diffusion and active transport require the assistance of membrane proteins. Osmosis is the movement of water across the membrane from an area of high solute concentration to an area of low solute concentration.

Membrane Receptors

Membrane receptors are proteins that bind to extracellular signals and transduce them into intracellular signals. Membrane receptors are involved in various cellular processes such as cell adhesion, signal transduction, and transport. Membrane receptors can be divided into seven different classes, including G-protein coupled receptors, ionotropic receptors, and ligand-gated ion channels.

Membrane Signaling

Membrane signaling refers to the process by which extracellular signals are transduced into intracellular signals. Membrane signaling is involved in various cellular processes such as cell growth, differentiation, and death. Membrane signaling can occur through various mechanisms such as G-protein coupled receptors, tyrosine kinases, and PI3K/AKT pathways.

Membrane Trafficking

Membrane trafficking refers to the process by which membrane proteins are transported from the endoplasmic reticulum to the Golgi apparatus, and then to the plasma membrane. Membrane trafficking is a complex process that involves the formation of vesicles, the transport of vesicles, and the incorporation of vesicles into the plasma membrane. Membrane trafficking is crucial for the maintenance of membrane protein composition and function.

Membrane Dynamics

Membrane dynamics refers to the movement of membrane proteins and lipids within the membrane bilayer. Membrane dynamics is involved in various cellular processes such as transport, signaling, and cell adhesion. Membrane dynamics can occur through various mechanisms such as lateral diffusion, endocytosis, and exocytosis.

Membrane Integrity

Membrane integrity refers to the structural and functional integrity of the cell membrane. Membrane integrity is crucial for the maintenance of cell function and survival. Membrane integrity can be assessed through various methods such as permeability assays, Malachite Green assays, and atomic force microscopy.

Membrane Regulation

Membrane regulation refers to the process by which membrane proteins and lipids are modulated in response to extracellular signals. Membrane regulation is involved in various cellular processes such as transport, signaling, and cell adhesion. Membrane regulation can occur through various mechanisms such as phosphorylation, ubiquitination, and lipid modification.

Membrane Trafficking and Dynamics

Membrane trafficking and dynamics are crucial for the maintenance of membrane protein composition and function. Membrane trafficking involves the transport of membranous vesicles from the endoplasmic reticulum to the Golgi apparatus, and then to the plasma membrane. Membrane dynamics refers to the movement of membrane proteins and lipids within the membrane bilayer. Membrane dynamics is involved in various cellular processes such as transport, signaling, and cell adhesion. Membrane dynamics can occur through various mechanisms such as lateral diffusion, endocytosis, and exocytosis.

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Smooth Muscle Membranes

The assumption that fractions 25-31 contained 343 plasma membranes proved to be, in fact, correct. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the mitochondrial and plasma membrane fractions exhibited a pattern of bands at 34,000, 30,000, 28,000, and 24,000 daltons, respectively, which were shifted for the comparison of all fractions. In addition, the membrane fraction was oriented on gel slices and stained with Coomassie Blue. Mitochondria were obtained from either rat or human or sheep plasma membranes. The membrane fractions were then analyzed for their ability to bind to the lectin Con A. Blake and colleagues have shown that the affinity of Con A for plasma membranes is increased by almost 100-fold in fractions 25-31 compared to the other fractions. The lectin binding assay was performed on the membrane fractions and the results are shown in the figure on the right.

Graph A: Nucleotidease activity (nmol Pi/mg protein) vs. fractions.
- S'-Nucleotidease
- NADPH
- S'-Nucleotidease
- Protein

Graph B: Absorbance vs. wavelength (nm).
- Reduced - Oxidized
- Baseline

Graph C: Plasma membranes (1.1 mg protein).
- NADPH
- S'-Nucleotidease
- Activity (nmol Pi/mg protein)

Graph D: 5'-Nucleotidease activity (nmol Pi/mg protein) vs. lectin (WGA).
- 0 to 200 lectin (WGA) (µg)

Fig. 2. Electron micrograph of the plasma membrane fraction (31) fraction.

Fig. 3. Differentiation spectra of mitochondria and plasma membrane (31) fractions.
Smooth Muscle Membranes

\[ a^2 = \beta_0 a_0 + \beta_1 a_1 + \beta_2 a_2 \] (I)

\[ b^2 = \gamma_0 b_0 + \gamma_1 b_1 + \gamma_2 b_2 \] (II)

\[ c = \delta_0 c_0 + \delta_1 c_1 + \delta_2 c_2 \] (III)

\[ d = \epsilon_0 d_0 + \epsilon_1 d_1 + \epsilon_2 d_2 \] (IV)

\[ e = \zeta_0 e_0 + \zeta_1 e_1 + \zeta_2 e_2 \] (V)

\[ f = \eta_0 f_0 + \eta_1 f_1 + \eta_2 f_2 \] (VI)

In a real experiment, for a given membrane fraction and measuring area

\[ a = \alpha_0 a_0 + \alpha_1 a_1 + \alpha_2 a_2 \] (VII)

\[ b = \beta_0 b_0 + \beta_1 b_1 + \beta_2 b_2 \] (VIII)

\[ c = \gamma_0 c_0 + \gamma_1 c_1 + \gamma_2 c_2 \] (IX)

\[ d = \delta_0 d_0 + \delta_1 d_1 + \delta_2 d_2 \] (X)

\[ e = \epsilon_0 e_0 + \epsilon_1 e_1 + \epsilon_2 e_2 \] (XI)

\[ f = \zeta_0 f_0 + \zeta_1 f_1 + \zeta_2 f_2 \] (XII)

\[ g = \eta_0 g_0 + \eta_1 g_1 + \eta_2 g_2 \] (XIII)

.substituting the value of \( a_0 \) \((\text{VII})\) in equation \((\text{VIII})\) and rearranging:

\[ b = \gamma_0 b_0 + \gamma_1 b_1 + \gamma_2 b_2 \] (XIV)

All right hand side values are from experimental results.

To obtain \( b_0 \), \( b_0 \) can be substituted in equation \((\text{XIV})\).

Now that \( b_0 \) and \( b_2 \) can be calculated, then \( b_1 \) and \( b_2 \) can be obtained using equation \((\text{XIV})\) in \((\text{XIII})\) respectively.

Since \( b \times \alpha_0 \times \beta_0 \times \gamma_0 \), then

\[ c = \delta_0 c_0 + \delta_1 c_1 + \delta_2 c_2 \] (XV)

Note more than one relation among, the proportion of membrane contribution in each fraction can then be maintained and studied.
Characterization of membrane fractions and isolation of purified plasma membranes from rat myometrium.
M A Matlib, J Crankshaw, R E Garfield, D J Crankshaw, C Y Kwan, L Q Branda and E E Daniel


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