Characterization of Inositol 1,4,5-Trisphosphate Receptors and Calcium Mobilization in a Hepatic Plasma Membrane Fraction*

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The distribution of hepatic binding sites for the calcium-mobilizing second messenger, inositol 1,4,5-trisphosphate (IP3), was analyzed in subcellular fractions of the rat liver by binding studies with [32P]IP3 and compared with the Ca2+ release elicited by IP3 in each fraction. Three major subcellular fractions enriched in plasma membrane, mitochondria, and endoplasmic reticulum were characterized for their 5'-nucleotidase, glucose-6-phosphatase, succinate reductase, and angiotensin II binding activities. The fraction enriched in plasma membrane showed 7- and 20-fold increases in IP3 binding capacity over those enriched in endoplasmic reticulum and mitochondria, respectively, and contained a single class of high-affinity binding sites with \( K_d \) of 1.7 ± 1.0 nM and concentration of 239 ± 91 fmol/mg protein. IP3 binding reached equilibrium in 30 min at 0 °C, and the half-time of dissociation was about 15 min. The specificity of the IP3 binding sites was indicated by their markedly lower affinities for inositol 1-phosphate, phytic acid, fructose 1,6-bisphosphate, 2,3-bisphosphoglycerate, and inositol 1,3,4,5-tetrakisphosphate. The Ca2+-releasing activity of IP3 in the subcellular fractions was monitored with the fluorescent indicator, Fura-2. All three fractions showed ATP-dependent Ca2+ uptake and rapidly released Ca2+ in response to IP3. The fraction enriched in plasma membrane was the most active in this regard, releasing 174 ± 67 pmol Ca2+/mg of protein compared to 45 ± 10 and 48 ± 7 pmol/mg protein for the fractions enriched in endoplasmic reticulum and mitochondria, respectively. These data suggest that the [32P]IP3 binding sites represent specific intracellular receptors through which IP3 mobilizes Ca2+ from a storage site (or co-purifying) with the plasma membrane of the rat liver. It is likely that a specialized vesicular system (to which IP3 can bind and trigger the release of Ca2+) is located in close proximity with the plasma membrane and is thus adjacent to the site at which IP3 is produced during stimulation of the hepatocyte by Ca2+-mobilizing hormones.

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The abbreviations used are: IP3, inositol 1,4,5-trisphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylenebis(oxyethylenenitriilo)tetracetic acid; PEG, polyethylene glycol 6000; Gpp(NH)p, guanyl-5'-yl imidodiphosphate.

**For review, see Ref. 14. IP3 acts through specific intracellular receptors that have been demonstrated in the adrenal cortex (15, 16), macrophages (17), liver (18), neutrophils (19), brain (20) and anterior pituitary gland (21) by direct ligand-binding studies. An important question about the mechanism of action of IP3 is the nature and location of the pool from which Ca2+ is mobilized. Currently, the endoplasmic reticulum is believed to be the major source for release of calcium from intracellular stores. However, IP3 may also promote calcium mobilization and/or calcium influx at the level of the plasma membrane during its action on the redistribution of intracellular calcium. We have recently observed that IP3 binding sites are relatively abundant in a plasma membrane-enriched preparation, consistent with an action of IP3 at this location (16). Since liver is readily fractionated into enriched organelles, and has been widely used for the study of calcium-mobilizing hormones (22-26; for review, see Ref. 27), it was employed for a more detailed analysis of the subcellular distribution of IP3 binding sites. In this study, the fraction enriched in plasma membrane was found to be highly enriched in IP3 receptors with binding properties similar to those recently described in adrenal cortex (16) and anterior pituitary gland (21). Significantly, the abundance of IP3 receptors in the plasma membrane fraction was correlated with high IP3-induced Ca2+ release in this fraction. These results indicate that the vesicular system to which IP3 binds and releases Ca2+ is closely associated with the plasma membrane and co-purifies with it during subcellular fractionation procedures.

**EXPERIMENTAL PROCEDURES**

**Materials**—Inositol 1,4,5-trisphosphate and inositol 1-phosphate were obtained from Amersham Corp. [32P]IP3, (20–200 Ci/mmol) and [3H]IP3 (3.6 Ci/mmol) were obtained from New England Nuclear. Fura-2 (pentasodium salt) was from Behring Diagnostics, Monodiiodinated 111I-angiotensin II was prepared by a modified IODO-GEN (Pierce Chemical Co.) method (28) (Meloy Laboratories, Springfield, VA) and had a specific radioactivity of 1600 Ci/mmol. All other reagents were from Sigma or Boehringer Mannheim.

**Preparation of Subcellular Fractions**—The three subcellular fractions were prepared following a combination of Neville’s (29) and Dawson’s (4) procedures. Livers from 250-400-g male albino rats were minced and homogenized with 10 strokes of a Dounce homogenizer (loose pestle) in a buffer containing: 20 mM HEPES/KOH, pH 7.2, 110 mM KCl, 10 mM NaCl, 2 mM MgCl2, 5 mM KH2PO4, 2 mM EGTA, and 1 mM diethiothreitol. After stirring for 5 min, filtration...
through cheesecloth, and centrifugation at 1500 x g for 20 min, the pellet was resuspended in homogenization buffer and adjusted to 44% in sucrose. From that point, the plasma membrane fraction was purified according to the method of Neville (29) up to step 11. This fraction was washed and resuspended in Buffer A (the homogenization buffer without EGTA) at a concentration of 20–30 mg of protein/ml (30).

The mitochondrial fraction was prepared by centrifugation of the 1,500 x g supernatant at 8,000 x g for 10 min. The pellet was then resuspended in Buffer A, sedimented at 8,000 x g for 10 min and taken up at a high protein concentration (~30 mg/ml) in Buffer A. The microsomal fraction was prepared by centrifugation of the 8,000 x g supernatant at 35,000 x g for 20 min. This pellet was resuspended in Buffer A, recentrifuged at 35,000 x g for 20 min and taken up in Buffer A. The subcellular fractions were used freshly for Ca²⁺-mobilization studies or were frozen in small aliquots for binding or enzymatic analysis.

Characterization of Subcellular Fractions—Glucose-6-phosphatase was measured at 37°C by the method of Salomon et al. (31), 5'-nucleotidase by the method of Bramley and Ryan (32), and succinate-reductase according to Pennington (33). [3H]-Angiotensin II binding was performed for 30 min at room temperature in a buffer containing: 20 mM Tris/HCl, pH 7.4, 100 mM NaCl, 5 mM MgCl₂, 0.2% bovine serum albumin, and 0.5 mM dithiothreitol. The bound and free ligand were separated by vacuum filtration through glass-fiber (GF/C) membranes.

IP₃ Binding Studies—Rat liver fractions containing 200–600 μg of protein were incubated in a medium of the following composition: 25 mM Na₃HPO₄, pH 7.4, 100 mM KCl, 20 mM NaCl, 0.1% bovine serum albumin, and 1 mM EDTA. Incubations were performed, unless otherwise specified, for 30 min at 0°C in a final volume of 500 μl with [32P]IP₃ (~20,000 cpm 100 fmol ~0.3 nM). Non-specific binding was determined in the presence of 1 μM IP₃. Incubations were terminated by filtration through pre-soaked glass-fiber filters (Whatman GF/B) and rapid washing with 2.5 ml of ice-cold incubation medium. The receptor-bound radioactivity was analyzed by liquid scintillation spectrometry.

5'-Nucleotidase activity and IP₃ binding capacities of the three fractions for IP₃ were obtained by enzyme assays.

The three subcellular fractions were analyzed for their IP₃ binding properties. As shown in Fig. 1 (see also Table I), the plasma membrane fraction showed a much higher binding capacity for IP₃ (6 and 20 times higher than the fractions enriched in endoplasmic reticulum and mitochondria, respectively). The upper left panel shows typical displacement curves obtained during inhibition of tracer [32P]IP₃ binding by increasing concentrations of unlabeled IP₃. In this experiment, the IP₃ binding capacity of the plasma membrane fraction was 6.3 and 13 times higher than those of the fractions enriched in endoplasmic reticulum and mitochondria, respectively. In the upper right panel, the data from the same experiment are normalized to show the similar binding affinities of the three fractions for IP₃. Scatchard analyses of the same data (lower panel) also indicate similar binding affinities (0.8, 0.9, and 0.8 nM) and the marked difference between the binding capacities (146, 23, and 11 fmol/mg protein) of the plasma membrane, endoplasmic reticulum, and mitochondria-enriched fractions, respectively.

To define better the subcellular location of the binding sites for IP₃, the plasma membrane fraction was further purified by application to a 13–37% sucrose gradient overlaying a 20% sucrose cushion. After centrifugation for 60 min at 550 x g, the material at the cushion interface was collected and washed in Buffer A. The 5′-nucleotidase activity and IP₃ binding capacity of this more purified membrane fraction were further

| TABLE I | Characterization of subcellular fractions prepared from rat liver |
|---------|--------------------------|
|         | Plasma membrane fraction | Microsomal fraction | Mitochondrial fraction |
| Angiotensin II binding (cpm/25 μg protein) | 6954 ± 416 | 1933 ± 116 | 1882 ± 50 |
| 5′-Nucleotidase (μmol P/mg protein/20 min) | 2.1 ± 0.1 | 0.9 ± 0.1 | 1.3 ± 0.2 |
| Glucose-6-phosphatase (μmol P/mg protein/30 min) | 3.3 ± 0.1 | 9.5 ± 0.2 | 5.9 ± 0.1 |
| Succinate reductase (nmol Formazan/mg/15 min) | 1.2 ± 0.1 | 0.1 ± 0.05 | 1.8 ± 0.2 |
| IP₃ binding (fmol/mg protein) | 209 | 56 | 8 |
enriched by comparison with the original plasma membrane fraction and the homogenate. In a typical preparation, the 5'-nucleotidase enzyme activity was 7.5 and 10.5 times higher in the original membrane fraction and the enriched plasma membrane fraction, respectively, as compared to the homogenate. On the other hand, glucose-6-phosphatase activity was only 1.8 and 1.3 times as active in the same fractions as compared to the homogenate. The IP₃ binding capacity of the enriched plasma membrane fraction was 757 fmol/mg protein, almost 3 times that of the original membrane fraction (264 fmol/mg protein) and about 30-fold higher than that of the homogenate (25 fmol/mg). Similar results were obtained in three different preparations, confirming the co-purification of IP₃ binding sites with plasma membrane components.

Characterization of IP₃ Binding Sites—Since the plasma membrane fraction was highly enriched in IP₃ binding sites, subsequent studies to characterize the sites were performed with this subcellular fraction. Kinetic studies exemplified in Fig. 2 revealed that specific binding of IP₃ at 0 °C was half-maximal in about 5 min and reached a plateau within 30 min.

Binding was reversible, and addition of 10⁻⁶ M unlabeled IP₃ was followed by rapid dissociation of the bound ligand with a half-time of about 15 min, according to a single exponential function as shown in the lower panel of Fig. 2. The association rate data fitted a second-order equation as shown in Fig. 3, upper panel. The association rate constant (kₐ), calculated from the slope of the curve, was 2.1 × 10⁵ M⁻¹·min⁻¹. The dissociation rate constant (kₐ) was 0.02 min⁻¹. The Kᵣ calculated from the ratio of the rate constants for dissociation and association was 0.95 nM, in agreement with the value obtained from steady state binding experiments.

In saturation studies (Fig. 4), tracer binding was reduced by about 10% in the presence of 0.2 nM unlabeled ligand and was progressively inhibited by increasing concentrations up to 100 nM IP₃ (upper panel). Scatchard analysis of the binding data was consistent with a single set of high-affinity sites (upper panel, inset) with Kᵣ of 1.7 ± 1.0 nM and maximal binding capacity of 239 ± 91 fmol/mg protein (n = 12). Since two classes of binding sites for IP₃ have been observed in liver microsomes (18), and to complement the data obtained by competition analysis (in which low-affinity binding of [³²P]IP₃ may be obscured as the measured ligand binding decreases toward the nonspecific value), we also performed saturation analysis with increasing concentrations of the low specific radioactivity radioligand [³H]IP₃ (3.6 Ci/mmol). This method provides a more accurate determination of the receptor binding profile at high ligand concentrations, where low-affinity sites would be detected. As shown in Fig. 4 (lower panel),

![Fig. 1. IP₃ binding capacities of liver subcellular fractions.](image)

![Fig. 2. Association and dissociation of [³²P]IP₃ with liver binding sites as a function of time. Binding was initiated at 0 °C by addition of the membrane suspension (300 µg of protein) to assay buffer containing the radioligand (28,000 cpm), and dissociation was initiated by adding 10⁻⁶ M (final concentration) unlabeled IP₃ (arrow). At the indicated times, aliquots were removed and filtered as described under "Experimental Procedures." The dissociation data expressed on a logarithmic scale are shown in the lower panel. This representative experiment performed in duplicate was repeated with three different membrane preparations.](image)
The specificity of the IP₃ binding sites was analyzed in competitive binding experiments performed with inositol phosphates and related compounds. As shown in Fig. 5, phytic acid and inositol 1-phosphate showed extremely low affinities to the binding site (at least 10,000-fold less than the homologous ligand). Other compounds bearing phosphate groups on vicinal carbon atoms, such as fructose 1,6-bisphosphate and 2,3-bisphosphoglycerate (an inhibitor of the enzyme IP₃ 5'-phosphatase) (36, 37), showed extremely low binding affinities. The most closely similar compound, inositol 1,3,4,5-tetrakisphosphate, showed relatively low affinity (about 5% compared with IP₃) for the binding site. The shapes of the displacement curves and their parallelism with the IP₃ binding-inhibition curve were consistent with competitive interaction of these compounds with the IP₃ binding site.

Ca²⁺-releasing Activity of IP₃—Ca²⁺ uptake and release in response to IP₃ were determined in each of the subcellular fractions. The plasma membrane fraction showed ATP-dependent Ca²⁺ sequestering activity (which was inhibited by vanadate), and decreased the ambient Ca²⁺ concentration to about 200 nM (Fig. 6, panel A). This relatively high value should not be considered as the “set point” but was probably due to the limited capacity of the vesicular system in comparison to the large amount of Ca²⁺ in the medium. When the ambient calcium concentration was reduced by adding a small amount of EGTA (~10 μM) or if the plasma membrane preparation was added in higher concentration (10 mg of protein), the ATP-dependent Ca²⁺ uptake process lowered the Ca²⁺ level to below 200 nM (data not shown).

Addition of IP₃ (2.5 μM) caused immediate release of 0.4 nmol of Ca²⁺ followed by slow re-uptake. Subsequent stimulation with IP₃ evoked a comparable response, although with repeated additions the response diminished in magnitude. Addition of 1 μM ionomycin immediately released the accumulated Ca²⁺, indicating the vesicular nature of the Ca²⁺ sequestering process. The fractions enriched in endoplasmic reticulum (Fig. 6, panel B) and mitochondria (Fig. 6, panel C) also showed high ATP-dependent Ca²⁺ sequestering activities, decreasing the ambient Ca²⁺ concentration to about 200 nM. However, these preparations showed smaller responses to IP₃ (2.5 μM) stimulation, releasing 0.13 and 0.07 nmol of Ca²⁺,


Fig. 5. Competitive binding of IP₃ and related compounds in liver plasma membrane fraction. Liver particles (500 μg of protein) were incubated at 0 °C in medium containing [³²P]IP₃ (14,000 cpm) and increasing concentrations of unlabeled compounds. After 30 min the incubations were stopped and analyzed as indicated under “Experimental Procedures.” The data are expressed as values relative to the total binding observed without unlabeled ligand (2,482 cpm) and corrected for nonspecific binding (192 cpm). Liver particles added were 500 pg of protein.

Fig. 6. IP₃-induced Ca²⁺ release in liver subcellular fractions. Liver particles (3 mg of protein) were incubated at 37 °C and their Ca²⁺ uptake and release activities were monitored with Fura-2 (free acid) as described under “Experimental Procedures.” Panels A, B, and C show typical traces derived with plasma membrane, endoplasmic reticulum, and mitochondria-enriched fractions, respectively. C, 0.2 nmol of Ca²⁺; I, 2.5 μM IP₃; Io, 1 μM ionomycin. These traces are representative of several such observations in at least three different liver preparations.

Fig. 7. Dose-dependent effects of IP₃ on release of Ca²⁺ from the liver plasma membrane fraction. The experiment was performed as described in the legend of Fig. 5. Ordinate, amount of Ca²⁺ released expressed as a ratio of the maximal response (290 pmol/mg protein). Abscissa, concentration of IP₃ added. Similar results were obtained with four different liver preparations.
pool is a specialized part of the endoplasmic reticulum which chondria. Attempts to purify a microsomal fraction caused plasma membrane fraction was not examined. The present characterized in several different tissues. The intracellular quent binding studies by Spat et al. and GTP exert their actions through distinct mechanisms, as also indicated by other recent studies (11, 40, 41).

DISCUSSION

The messenger action of IP₃ is expressed through its interaction with intracellular receptors that have recently been characterized in several different tissues. The intracellular location of this receptor is an important aspect of the mechanism of action of IP₃. This question was addressed by Dawson and Irvine (4) in the liver by studies which showed that IP₃ releases Ca²⁺ from a vesicular system other than mitochondrial. Attempts to purify a microsomal fraction caused loss of the IP₃ effect, suggesting that the IP₃-sensitive Ca²⁺ pool is a specialized part of the endoplasmic reticulum which co-purifies with heavier cellular organelles. Similar experiments by Joseph et al. (1) led to the same conclusion. Subsequent binding studies by Spat et al. (18) showed enrichment of IP₃ binding sites in the microsomal as compared to the cytosolic and mitochondrial fractions of rat liver, but the plasma membrane fraction was not examined. The present data clearly demonstrate that the plasma membrane fraction is highly enriched in IP₃ receptors with binding properties similar to those present in the adrenal cortex (16) and anterior pituitary gland (21). Our finding that the plasma membrane fraction contains the highest level of IP₃-induced Ca²⁺ release activity also lends further significance to the presence of membrane binding sites for IP₃.

Comparison of the subcellular distributions of IP₃ binding activity and IP₃-induced calcium-release activity required analysis of each of these functions by procedures that differed widely in their requirements for optimal assay conditions. Also, the conditions required for the preparation of optimally responsive microsomes for Ca²⁺ release studies differ considerably from those employed for liver fractionation. The homogenization medium is iso-osmotic, containing Hepes buffer, EGTA, and dithiothreitol, whereas a hypotonic medium containing 1 mM NaHCO₃ is used for plasma membrane purification by the Neville procedure (29). Furthermore, the subcellular preparations are relatively unstable and rapidly lose their Ca²⁺ mobilizing properties. For these reasons, we employed methods that allow rapid fractionation of the liver tissue, and the purity of our routine subcellular fractions was somewhat less than that attainable by more rigorous fractionation schemes. However, when additional purification of the plasma membrane fraction was performed by sucrose density gradient centrifugation, concomitant enrichment of 5'-nucleotidase activity and IP₃ binding capacity was again observed.

A current question of major interest is whether IP₃ serves to control Ca²⁺ influx directly across the plasma membrane, and/or its release from a calcium-containing structure associated with the plasma membrane, as well as from the endoplasmic reticulum. IP₃ was recently found to activate Ca²⁺ channels in the plasma membrane of T-lymphocytes (42), consistent with Michell’s (43) original proposal that phospholipid breakdown could regulate the influx of external Ca²⁺ through transmembrane channels. In addition to its possible entry through IP₃-regulated calcium channels, our data show that Ca²⁺ is released by IP₃ from vesicular structures after uptake by an ATP-dependent process (which is inhibited by vanadate and can be completely reversed upon addition of a calcium ionophore). Although such structures could represent inside-out vesicles originating from the plasma membrane during homogenization, this would not account for the Ca²⁺-releasing activity of IP₃ in a wide variety of permeabilized cells (8, 44-46) including hepatocytes (1-3, 47) which do not contain inside-out vesicles. The most likely explanation is that IP₃ binds to and triggers the release of Ca²⁺ from a vesicular system that is closely associated with the plasma membrane, and which purifies with the membrane when subcellular fractions are prepared.

Several recent reports have provided evidence for a mechanism whereby direct entry of extracellular calcium into an IP₃-sensitive pool contributes to receptor-operated calcium influx and is responsible for sustained calcium entry in agonist-stimulated cells (45-52). The IP₃-sensitive pool, with its associated IP₃ receptors and calcium releasing mechanism, would thus be expected to be adjacent to the plasma membrane. At least three current models of receptor-activated calcium entry involve a close apposition between IP₃-sensitive regions of endoplasmic reticulum and the plasma membrane. In Putney’s (52) capacitative scheme, agonist-induced entry of calcium from a component of the endoplasmic reticulum (the receptor-regulated calcium pool) serves as a signal for calcium entry by promoting its transport from the subplasmalemmal space into the endoplasmic reticulum. A more complex model developed by Gill and colleagues (53) to explain the role of GTP in calcium movements involves the formation of junctional processes between adjacent membranes, including connections between the plasma membrane.
and the endoplasmic reticulum. Likewise, the model proposed by Irvine and Moor (54) for the conjoint actions of IP$_3$ and IP$_3$ on calcium entry also includes functional coupling between plasma membrane and endoplasmic reticulum, in this case promoted by Ins-1,3,4,5-P$_4$, to permit direct entry of external calcium into the endoplasmic reticulum.

It has not been established whether distinct structural associations between the plasma membrane and endoplasmic reticulum, which could represent the membrane-associated vesicular system implicit in the above models and suggested by the present findings, are present in non-excitable cells. However, the presence of associations between the plasma membrane and sarcoplasmic reticulum in smooth muscle (55) and endoplasmic reticulum in platelets (56), as well as the recent proposal that specialized organelles (calciosomes) are involved in calcium mobilization (57), suggest that discrete perimembrane structures may play a general role in the process of calcium entry and distribution within the cell. Such structures may be present in plasma membrane vesicle fractions from human platelets (56) and rat parotid acini (58), both of which have been shown to release Ca$^{2+}$ in an IP$_3$-sensitive manner.

The IP$_3$ binding sites observed in the plasma membrane fraction of the rat liver possess all the characteristics of a true receptor. IP$_3$ binds to a particulate component and the level of binding is proportional to the amount of membrane protein. The binding is rapidly reversible as expected for a normal ligand-receptor interaction, and this would permit rapid termination of the response. The rapid rates of association and dissociation are consistent with the calcium mobilizing activity of IP$_3$. The binding sites are saturable and binding of the ligand is competitive, being inhibited by increasing concentrations of unlabeled ligand. The binding is also highly specific for IP$_3$; inositol-derived compounds and organic compounds bearing phosphoryl groups on vicinal carbons showed weaker affinities for the binding site. Finally, the important criterion of a functional response is demonstrated by the ability of IP$_3$ to release Ca$^{2+}$ from the same membrane preparation used for the binding experiments.

One question arising from our study is the high affinity of the binding site by comparison with the potency of IP$_3$ for releasing Ca$^{2+}$. Such a discrepancy was also observed in the adrenal cortex (16) and anterior pituitary gland (21), and may be largely attributable to the divergent experimental conditions necessary for the two assays. In particular, the optimal conditions for IP$_3$ binding are very different from those within the cell. Also, the degradation of added IP$_3$ by the plasma membrane fraction at 37 °C is very rapid and could lower the actual IP$_3$ concentration during Ca$^{2+}$ release studies. Furthermore, ATP inhibits the binding of IP$_3$ by a mechanism that is not yet clear, and its presence at millimolar concentration in the Ca$^{2+}$ release assays could substantially lower the potency of IP$_3$. Thus, the discrepancy probably results from the combination of higher apparent affinity in the binding assay, due to choice of optimal binding conditions, and lower apparent affinity in the Ca$^{2+}$ release assays, due to rapid degradation of IP$_3$ at 37 °C in the presence of Mg$^{2+}$ together with the competitive effect of ATP. The difference might also reflect decreased efficiency of the calcium-gating mechanism due to loss or impairment of a putative regulatory component during preparation of the subcellular fractions.

One such component could be a guanine nucleotide regulatory protein associated with the calcium release mechanism. However, the effect of GTP on Ca$^{2+}$ release from the liver plasma membrane fraction was independent of IP$_3$, as previously observed in the adrenal cortex and the anterior pituitary gland (21). In several tissues, GTP seems to act through a mechanism distinct from IP$_3$ and in parotid acini the GTP-sensitive and IP$_3$-sensitive calcium pools appear to reside in different regions of the endoplasmic reticulum (58). Thus, further studies are needed to clarify the importance of this effect of GTP in the regulation of Ca$^{2+}$ mobilization during hormonal stimulation.

It should be noted that two previous studies using subcellular fractions of rat pancreas (59) and rat adipocytes (60) showed that IP$_3$ mobilized Ca$^{2+}$ from endoplasmic reticulum but not from plasma membrane. Whether this is a reflection of differences between specific tissues or among fractionation procedures remains to be clarified. We are attempting to answer this question by analysis of IP$_3$ binding in adrenal cortex and anterior pituitary plasma membranes to establish the intracellular site of action of IP$_3$ in these tissues. However, the presence of IP$_3$ binding and/or IP$_3$-induced calcium mobilization in hepatic, platelet (56), and parotid acinar (58) plasma membrane fractions suggests that the IP$_3$-sensitive calcium pool may be closely related to the plasma membrane in a wide variety of target cells that respond to calcium-mobilizing stimuli.

REFERENCES

1. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F., and Williamson, J. R. (1984) J. Biol. Chem. 259, 3077-3081
2. Burgess, G. M., Godfrey, P. P., McKinley, J. S., and Putney, J. W. (1984) Nature 310, 63-66
3. Burgess, G. M., Irvine, R. F., Berridge, M. J., McKinley, J. S., and Putney, J. W., Jr. (1984) Biochem. J. 224, 741-746
4. Anderson, A. P., and Irvine, R. P. (1984) Biochem. Biophys. Res. Commun. 120, 855-864
5. Muallem, S., Schoefferd, M., Pandol, S., and Sachs, G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4433-4437
6. Bitar, K. N., Bradford, P. G., Putney, J. W., and Makhlof, G. M. (1986) J. Biol. Chem. 261, 16591-16596
7. Hashimoto, T., Hirata, M., Isho, T., Kamura, Y., and Kuriyama, H. (1986) J. Physiol. 370, 605-618
8. Smith, J. B., Smith, L., and Higgins, B. L. (1985) J. Biol. Chem. 260, 14143-14416
9. Somlyo, A. V., Bond, M., Somlyo, A. P., and Scarpa, A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 5231-5235
10. Chueh, S.-H., and Gill, D. L. (1986) J. Biol. Chem. 261, 13883-13886
11. Jean, T., and Klee, C. B. (1986) J. Biol. Chem. 261, 16414-16420
12. Gershengorn, M. C., Geras, E., Purrello, V. S., and Rebecchi, M. J. (1984) J. Biol. Chem. 259, 10675-10681
13. Biden, T. J., Wellheim, C. B., and Schlegel, W. (1986) J. Biol. Chem. 261, 7223-7229
14. Abdel-Latif, A. A. (1986) Pharmacol. Rev. 38, 227-272
15. Baukal, A. J., Guillemette, G., Rubin, R., Spat, A., and Catt, K. J. (1985) Biochem. Biophys. Res. Commun. 133, 532-538
16. Guillemette, G., Balla, T., Baukal, A. J., Spat, A., and Catt, K. J. (1987) J. Biol. Chem. 262, 1010-1015
17. Hirata, M., Saagauri, T., Hamashi, T., Hashimoto, T., Kukita, M., and Koga, T. (1985) Nature 317, 725-725
18. Spat, A., Fabiato, A., and Rubin, R. P. (1986) Biochem. J. 233, 929-932
19. Spat, A., Bradford, P. G., McKinley, J. S., Rubin, R. P., and Putney, J. W. (1986) Nature 319, 514-516
20. Worley, P. F., Baraban, J. M., Colvin, J. S., and Snyder, S. H. (1987) Nature 325, 159-161
21. Guillemette, G., Balla, T., Baukal, A. J., and Catt, K. J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8195-8199
22. Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michel, R. H., and Kirk, C. J. (1985) Biochem. J. 212, 733-747
23. Thomas, A. P., Marks, J. S., Coll, K. E., and Williamson, J. R. (1983) J. Biol. Chem. 258, 5716-5725
24. Irvine and Moor (54) for the conjoint actions of IP$_3$ and IP$_3$ on calcium entry also includes functional coupling between plasma membrane and endoplasmic reticulum, in this case promoted by Ins-1,3,4,5-P$_4$, to permit direct entry of external calcium into the endoplasmic reticulum.
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24. Litosch, I., Liu, S.-H., and Fain, J. N. (1983) *J. Biol. Chem.* **258**, 13727–13732
25. Rhodes, D., Prpíc, V., Exton, J. H., and Blackmore, P. F. (1983) *J. Biol. Chem.* **258**, 2770–2773
26. Mauger, J. P., Poggioli, J., Guesdon, F., and Claret, M. (1984) *Biochem. J.* **221**, 121–127
27. Williamson, J. R., Cooper, R. H., Joseph, S. K., and Thomas, A. P. (1985) *Am. J. Physiol.* **248**, C203–C216
28. Markwell, M. A. K., and Fox, C. F. (1979) *Biochemistry* **17**, 4807–4817
29. Neville, D. M., Jr. (1988) *Biochim. Biophys. Acta* **154**, 540–552
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
31. Salomon, Y., Yanovsky, A., Mintz, Y., and Lindner, H. R. (1977) *J. Cyclic Nucleotide Res.* **3**, 163–176
32. Bramley, T. A., and Ryan, R. J. (1978) *Endocrinology* **103**, 778–785
33. Pennington, R. J. (1961) *Biochem. J.* **80**, 649–654
34. Tsien, R. Y., Pozzan, T., and Rins, T. J. (1982) *J. Cell Biol.* **94**, 329–334
35. Fiske, C. H., and Subbarow, Y. (1925) *J. Biol. Chem.* **66**, 375–400
36. Downes, C. P., Mussat, M. C., and Michell, R. H. (1982) *Biochem. J.* **203**, 169–177
37. Seyfred, M. A., Farrell, L. E., and Wells, W. W. (1984) *J. Biol. Chem.* **259**, 13204–13208
38. Guillemette, G., Baukal, A. J., Balls, T., and Catt, K. J. (1987) *Biochem. Biophys. Res. Commun.* **142**, 15–22
39. Dawson, A. P. (1983) *FEBS Lett.* **186**, 147–150
40. Gill, D. L., Ueda, T., Chueh, S. H., and Noel, M. W. (1986) *Nature* **320**, 461–464
41. Hamachi, T., Hirata, M., Kimura, Y., Ikeda, T., Ishimatsu, T., Yamauchi, K., and Koga, T. (1987) *Biochem. J.* **242**, 253–260
42. Kuno, M., and Gardner, P. (1987) *Nature* **326**, 301–304
43. Michell, R. H. (1975) *Biochem. Biophys. Acta* **415**, 81–147
44. Ueda, T., Chueh, S. H., Noel, M. W., and Gill, D. L. (1986) *J. Biol. Chem.* **261**, 3184–3192
45. Irvine, R. F., Letcher, A. J., Lander, D. J., and Berridge, M. J. (1986) *Biochem. J.* **240**, 301–304
46. Pentki, M., Biden, T. J., Janjic, D., Irvine, R. F., Berridge, M. J., and Willhite, C. B. (1984) *Nature* **309**, 562–563
47. Joseph, S. K., and Williamson, J. R. (1986) *J. Biol. Chem.* **261**, 14658–14664
48. Casteels, R., and Droogmans, G. (1981) *J. Physiol. (Lord.)* **317**, 643–658
49. Poggioli, J., Mauger, J. P., Guesdon, F., and Claret, M. (1985) *Biochem. J.* **221**, 121–127
50. Williamson, J. R., Cooper, R. H., Joseph, S. K., and Thomas, A. and Wollheim, C. B. (1984) *Nature* **309**, 562–563
51. Joseph, S. K., and Williamson, J. R. (1986) *J. Biol. Chem.* **261**, 14658–14664
52. Putney, J. W., Jr. (1986) *Cell Calcium* **7**, 1–12
53. Mullaney, J. M., Chueh, S.-H., Ghosh, T. K., and Gill, D. L. (1987) *J. Biol. Chem.* **262**, 13865–13872
54. Irvine, R. F., and Moor, R. M. (1987) *Biochem. Biophys. Res. Commun.* **146**, 284–290
55. Devine, C. E., Somlyo, A. V., and Somlyo, A. P. (1972) *J. Biol. Chem.* **247**, 69–78
56. O'Rourke, F. A., Halenda, S. P., Zavoico, G. B., and Feinstein, M. B. (1985) *J. Biol. Chem.* **260**, 956–962
57. Krause, K.-H., Volpe, P., Zorzato, F., Hashimoto, S., Pozzan, T., Meldolesi, J., and Lew, P. D. (1987) *Cell Calcium Meta.* **(Abstr. 64)
58. Henne, V., Piiper, A., and Soling, H.-D. (1987) *FEBS Lett.* **218**, 153–158
59. Strebl, H., Bayerdorffer, E., Hause, W., Irvine, R., and Schulz, I. (1984) *J. Membr. Biol.* **81**, 241–253
60. Deffert, D. M., Hill, S., Perahdasinhg, H. A., Sberman, W. R., and McDonald, J. M. (1986) *Biochem. J.* **236**, 37–44