Triads and Transverse Tubules Isolated from Skeletal Muscle Contain High Levels of Inositol 1,4,5-Trisphosphate*

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We measured the content of inositol 1,4,5-trisphosphate in sarcoplasmic reticulum, transverse tubules, and triads isolated from frog skeletal muscle, as well as in triads isolated from rabbit skeletal muscle. We found that acid extracts of both transverse tubules and triads contained significant amounts of inositol 1,4,5-trisphosphate, in the range of 300-400 pmol/mg of protein as determined by a radioreceptor assay, whereas no detectable amounts were found in sarcoplasmic reticulum vesicles. The identity of inositol 1,4,5-trisphosphate in the extracts was confirmed by comigration with \([3H] \text{inositol 1,4,5-trisphosphate on polyethyleneimine-cellulose plates, and by phosphorylation to inositol 1,3,4,5-tetrakisphosphate using the inositol 1,4,5-trisphosphate 3-kinase present in muscle extracts. These findings may have important physiological implications. First, the results indicate that the muscle plasma membrane regions differentiated as transverse tubules contain high amounts of inositol 1,4,5-trisphosphate, suggesting that they might possess a high density of binding sites for this compound. Second, since inositol 1,4,5-trisphosphate has been proposed as a chemical transmitter in excitation-contraction coupling in skeletal muscle, our finding that this second messenger is present in high density at the site of coupling may contribute to the understanding of its role in this process.}

The inositol 1,4,5-trisphosphate (IP₃) content in amphibian and mammalian skeletal muscle has been measured by different methods (Vergara et al., 1985; Foster et al., 1989; Mayr and Thieleczek, 1991; Scholz et al., 1991). Values for the total IP₃ content of resting muscle in the range of 1.2-2.5 \( \mu \text{M} \) were recently reported using mass measurement determinations (Mayr and Thieleczek, 1991). Although we obtained similar overall values in frog skeletal muscle using a radioreceptor assay, we found that a proportion of the total IP₃ was attached to particulate fractions and was not free in the cytoplasm. To investigate this in greater detail, we measured the IP₃ content of triads, transverse tubules, and sarcoplasmic reticulum (SR) membrane fractions isolated from frog skeletal muscle, and of triads isolated from rabbit skeletal muscle. We found that transverse tubules and triads contained significant amounts of IP₃, in the range of 300-400 pmol/mg protein, whereas SR vesicles did not contain detectable amounts of IP₃. The potential physiological implications of these findings are discussed.

EXPERIMENTAL PROCEDURES

Membrane Preparations—Transverse tubule and SR membranes were isolated from frog muscle as described (Hidalgo et al., 1986a; Bull et al., 1989). Triads were isolated from frog skeletal muscle as follows. Briefly, finely minced muscle was homogenized using a Warner Blender (two bursts of 30 and 20 s at low setting) in four volumes of ice-cold homogenization solution: 0.15 M KCl, 5 mM MgSO₄, 20 mM MOPS/Tris, pH 6.8, and the following protease inhibitors: leupeptin (1 \( \mu \text{g/ml} \)), pepstatin (1 \( \mu \text{g/ml} \)), benzamidine (0.4 mM), and phenylmethylsulfonyl fluoride (1 mM). The resulting suspension was sedimented at 1,500 \( \times \) g, the pellet was resuspended in 15 mM MOPS/Tris, pH 6.8, and the following protease inhibitors: leupeptin (1 \( \mu \text{g/ml} \)), pepstatin (1 \( \mu \text{g/ml} \)), benzamidine (0.4 mM), and phenylmethylsulfonyl fluoride (1 mM). The resulting suspension was incubated at 17,000 \( \times \) g for up to 1 month.

Binding Studies—Ouabain and nitrendipine binding were measured as previously described (Hidalgo et al., 1986a; Jaimovich et al., 1986). Ryanodine binding was measured as described by Bull et al. (1989). Briefly, to measure ryanodine binding triads (0.1 mg of protein/ml) were incubated with varying concentrations of \([3H] \text{ryanodine and 0.5 mM AMP-PNP in 0.5 mM KCl, 0.1 mM CaCl}_2, 20 \text{ mM Heps/Tris, pH 7.1 (incubation solution), in a total volume of 0.5 ml. Ryanodine concentrations in the range of 0.25-10 \( \mu \text{M} \) were used; concentrations of ryanodine higher than 10 \( \mu \text{M} \) were avoided since lower affinity sites become apparent} (Bull et al., 1989). After 1 h of incubation at 37 °C, 0.40-ml fractions were filtered through GF/B glass fiber filters. Filters were washed with 3 \( \times \) 5 ml of cold incubation media. Nonspecific binding, determined in the presence of 4 \( \mu \text{M} \) ryanodine, was subtracted from the total binding to produce the specific binding values. To measure binding of \([3H] \text{ouabain, isolated}

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‡ The abbreviations used are: IP₃, inositol 1,4,5-trisphosphate; AMP-PNP, adenylyl-5'-yl imidodiphosphate; DHP, dihydropyridine; E-C, excitation-contraction; HPLC, high performance liquid chromatography; IP₃, inositol 1,4,5-trisphosphate; \([3H] \text{IP₃, d-[inositol-1-} ^{2} \text{H]} \text{inositol 1,4,5-trisphosphate; [H]} \text{IP₃, d-myo-[2-} ^{3} \text{H]} \text{inositol 1,3,4,5-tetrakisphosphate; MESS, 4-morpholineethanesulfonic acid; PEI, polyethyleneimine; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol trisphosphate; GTP, guanosine 5'-triphosphate; SR, sarcoplasmic reticulum; MOPS, 4-morpholinoethanesulfonic acid.}

1 C. Hidalgo, E. Jaimovich, and V. Tapia, manuscript in preparation.
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dties (0.1 mg of protein/ml) were incubated with varying concentrations (5-160 nM) of [3H]ouabain in 120 mM NaCl, 10 mM MgCl2, 10 mM ATP, 0.2 mg/ml saponin, and 40 mM Tris/HCl, pH 7.4, for 1 h at 37°C. Nonspecific binding was determined in the absence of ATP. Binding of [3H]nitrendipine to isolated triads was measured by incubating triads (0.05 mg of protein/ml) under dim light with 0.5-8 nM [3H]nitrendipine in 50 mM Tris/HCl, pH 7.5, for 30 min at 10°C. Nonspecific binding was determined in the presence of 1 μM nifedipine.

Radioceptor Assay—To measure the IP3 contents of membrane preparations, all membrane fractions were extracted either with perchloric acid (6%, w/v) or with concentrated trichloroacetic acid. Perchloric acid extracts were neutralized with KOH/ MES after sedimentation of the denatured protein as described elsewhere (Stephens, 1990). Trichloroacetic acid extracts were washed 4 times with ethyl ether to remove the acid, and the aqueous extracts, treated with N2 to evaporate the remaining ethyl ether, were neutralized with 0.5 M Tris/HCl, pH 8.8. Since both extraction methods gave the same results, the simpler perchloric acid extraction method was used in most experiments. The IP3 content of the extracts was analyzed using the radioreceptor assay as described by Brett et al. (1988). For this purpose, cerebellar membranes (0.2 mg), isolated from Sprague-Dawley white male rats, were incubated for 20 min at 4°C in 0.5 ml of a solution containing 1.6 nM [3H]IP3, (D-[inositol-1-3H]IP3, 17 Ci/mmol, Du Pont-New England Nuclear), 1 mM EDTA, 1 mM β-mercaptoethanol, 50 mM Tris/HCl, pH 8.4, and variable concentrations of IP3, phosphatidylinositol 4,5-bisphosphate (PIP2), ATP, or neutralized membrane extracts. To stop the reaction, membranes were sedimented at 12,000 X g for 10 min, the supernatants were carefully removed and the radioactivity associated with the pellets was determined after transfer into liquid scintillation vials. Nonspecific binding was determined in the presence of 1 μM IP3.

Phosphorylation—Fractions of 0.1 ml of neutralized triad extracts, containing 8-10 μM IP3 as estimated from the radioreceptor assay, were incubated in a total volume of 0.2 ml with 0.9 mM γ32P]ATP (60 μCi/mmol), 1 mM MgCl2, 0.1 mM CaCl2, 50 mM Tris/HCl, pH 8.0, and 0.3 mg of a 100,000 X g supernatant fraction isolated from frog skeletal muscle that displays IP3-3-kinase activity (Carrasco et al., 1993). These phosphorylation conditions were similar to those described elsewhere (Theibert et al., 1990). After 10 min of incubation at 25°C, the reaction was stopped by addition of 0.015 ml of 70% perchloric acid. The pellet was removed by sedimentation, the supernatant, extracted with activated charcoal to remove excess unreacted [γ32P]ATP (Stephens, 1990), was neutralized as above, and 0.25 ml (containing [3H]IP3 and [3H]inositol 1,3,4,5-tetrakisphosphate, [3H] IP3 (as internal standards) were loaded on a Partisil 10 SAX (Whatman) HPLC column. The column was eluted first with 0.5 mM NaH2PO4, adjusted to pH 3.7 with orthophosphoric acid, and then with an ammonium formate gradient, as modified from Batty et al. (1985).

Other Procedures—Protein was determined by the method described by Hartree (1972) using bovine serum albumin as standard. Electrophoresis in SDS-containing polyacrylamide gels was carried out according to Laemmli (1970).

Materials—IP3 was obtained from Calbiochem Corp. PIP2, PEI-cellulose plates, and aldolase were from Sigma. [γ32P]ATP was purchased from ICN; [3H]IP3, [3H]IP4, and [3-methyl-3H]nitrendipine, were obtained from Du Pont-New England Nuclear; [3H]IP3, was from Amersham Corp. [3H]Ryanodine and ryanodine were a kind gift from Dr. John Sutko. All other reagents used were of analytical grade.

RESULTS

Characterization of Triads Isolated from Frog Skeletal Muscle—The protein composition in SDS-gels of a triad fraction isolated from frog skeletal muscle (Fig. 1) shows a prominent calsequestrin band, a significant proportion of Ca2+-ATPase, and the presence of a high molecular weight doublet identified as the ryanodine receptor by immunodetection in Western blots (not shown). The identity of calsequestrin was confirmed because it stained blue with Stains-All (Campbell et al., 1983). Two isoforms of the ryanodine receptor are present in skeletal muscle of frog and other non-mammalian species as well (Olivares et al., 1991). This protein composition was highly reproducible and was characteristic of the triads obtained by the procedure described in this work. Most of the contractile proteins were eliminated by low speed centrifugation and washing, although some contaminating myosin remained associated with the triads, since extractions with high salt solutions were not carried out. Further purification through density gradient centrifugation was also omitted; it increased considerably the isolation time and, as described below, it did not improve significantly the purity of the preparation.

The isolated triads bound ryanodine with high affinity (Kd = 3.0 nM) and maximal binding values in the range of 7-12 pmol/mg protein (Table I). This density is 2-3-fold higher than that reported previously for the high affinity ryanodine binding sites of heavy SR vesicles from frog skeletal muscle (Bull et al., 1989). The high concentrations of protease inhibitors used in this preparation, and the shorter times involved in isolating the triads as compared to heavy SR, may have contributed to the increased ryanodine binding capacity of the triads. It is also possible that the triads contain a higher proportion of terminal cisternae SR than the heavy SR mem-

![Fig. 1. SDS-polyacrylamide gel electrophoresis of two different triad preparations isolated from frog skeletal muscle.](image-url)

TABLE I

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Bmax (pmol/mg protein)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>8.2 ± 2.0 (6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ouabain plus saponin</td>
<td>20.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Ouabain (control)</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Ryanodine</td>
<td>8.9 ± 3.8 (5)</td>
<td>3.0</td>
</tr>
<tr>
<td>Transverse tubules</td>
<td>124 ± 18 (4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Ouabain plus saponin</td>
<td>215 ± 38 (4)</td>
<td>10.4</td>
</tr>
</tbody>
</table>

*Data taken from Jaimovich et al. (1986). Numbers represent mean ± S.D.; number of determinations is given in parentheses.
branes we isolated previously from frog muscle (Bull et al.,
1989).

To determine the transverse tubule content of the isolated
triads, we measured their density of ouabain and nitrendipine
binding sites (Table I). Triads bound 20 pmol of ouabain/mg
protein in the presence of saponin to unmask latent sites,
with a $K_d$ of 13.6 nM. In the absence of detergent, the specific
ouabain binding decreased to 4.6 pmol/mg protein (Table I),
indicating that over 77% of the transverse tubules forming
part of the triad vesicles were sealed.

Nitrendipine bound to the triads with high affinity ($K_d =
0.8$ nM) and with maximal binding values of 9.5 pmol/mg
protein, giving a ratio of ouabain to nitrendipine bound close
to 2 (Table I). The same ratio was reported previously for
transverse tubules isolated from frog muscle (Jaimovich et
al., 1986). From the density of ouabain and nitrendipine binding
sites, we estimate that about 10% of the total protein present
in the triads corresponds to transverse tubule membranes.

That these transverse tubules were truly associated in triads
with terminal cisternae vesicles was established as follows.
After sedimentation of 5 mg of triads through a 25–50% (w/
v) sucrose density gradient, a single band at a density of 47–
47.6% sucrose was found; no protein was detected in the
interface of 10%-25% sucrose where free transverse tubule
membranes band at equilibrium (Hidalgo et al., 1986a). In
agreement with this observation, we found that their dihydro-
pyridine (DHP) receptor content remained unchanged after
density gradient sedimentation; the initial content was 10.3
pmol/mg protein and 10.0 pmol/mg in the triad band collected
from the gradient. These results establish the integrity of the
triads used in this study and indicate that further separation
of the isolated triads through sucrose gradients is pointless,
since the purity of the preparation did not increase with this
procedure.

Radioceptor Assay—The radioceptor assay method fol-
lowed in this work to measure intracellular IP$_3$ concentra-
tion is highly specific for IP$_3$ (Bredt et al., 1989). We found that
PIP$_2$ and ATP also displaced [H]IP$_3$ bound to cerebellar
membranes but with considerably lower affinities than IP$_3$
(Fig. 2). $K_d$ values were $16.7 \pm 1.31$ nM for IP$_3$, $527 \pm 55$ nM
for PIP$_2$, and $447 \pm 31$ $\mu$M for ATP. During the course of
these determinations, we noticed that a large fraction (36%)
of the PIP$_2$ added to the radioassay partitioned into the
cerebellar membranes (Fig. 3A), as determined by measuring
the membrane-bound fraction of [$^{3}$H]phosphatidylinositol-4,5-
 bisphosphate ([H]PIP$_2$). Therefore, the actual $K_d$
value for PIP$_2$ might be significantly different from the value
given in Fig. 2, which was calculated utilizing its total concen-
tration, since we do not know whether PIP$_2$ displaces IP$_3$
from the aqueous solution or from the membrane. Isolated
triads incubated with varying concentrations of [H]PIP$_2$
showed significant partition (32%) of this phospholipid into
the membranes as well (Fig. 3B).

Inositol 1,4,5-Triphosphate Content of Membrane Fractions—We found that extracts of isolated triads and trans-
verse tubules contained considerable amounts of IP$_3$(300–400
pmol/mg protein), while extracts from SR vesicles lacked
measurable levels of IP$_3$ (Table II). Triads or transverse
tubules incubated with saponin at the same concentrations
that unmask their latent ouabain binding activity (Table I)
(Jaimovich et al., 1986) had the same IP$_3$ contents as un-
treated control membranes. These results indicate that IP$_3$
is not simply trapped inside the vesicular lumen. Furthermore,
the results presented in Table I indicate that the triad fraction
isolated from frog skeletal muscle contains about 10% trans-
verse tubules. If we assume that all the IP$_3$ measured in triads
originates from their transverse tubular components, and
correct the results shown in Table II accordingly, triad-at-
tached transverse tubules would contain 3–4 nmol of IP$_3$/mg

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Displacement of [$^{3}$H]IP$_3$ bound to cerebellar mem-
branes by IP$_3$ (A), PIP$_2$ (B), and ATP (C) using a radioce-
ceptor assay. To determine the apparent $K_d$ values, nonspecific
binding (usually less than 5% of total binding) was subtracted and
the resulting values were adjusted to the function $y = B_{max}/(1 + [x]/K_d)$,
where $B_{max}$ represents maximal binding and $[x]$ the concentration
of IP$_3$, PIP$_2$ or ATP added. The solid lines drawn represent the theo-
retical fit to the data with the $K_d$ values illustrated above. Different
symbols represent different experiments.}
\end{figure}
of tubular protein, 10 times more than the isolated transverse tubules. Triad preparations require significantly shorter isolation times than the procedure followed to isolate transverse tubules. Thus a possible cause for the lower IP₃ content of transverse tubules might be a progressive loss during membrane isolation. Alternatively, the triad structure might stabilize IP₃ binding to its putative sites.

To ascertain the identity of IP₂₃ in the extracts, we analyzed a neutralized triad extract by TLC on polyethyleneimine (PEI) cellulose plates (Spencer et al., 1990). We found that the primary phosphate-containing spot comigrated with [³H]IP₂₃ added as tracer (Fig. 4A), indicating that the extracts contained significant amounts of IP₂₃. To further establish the identity of this compound as 1,4,5-IP₃, we investigated whether it could be enzymatically converted to IP₄ since it is known that the IP₃-kinase is highly specific for the 1,4,5-IP₃ isomer. For this purpose, we incubated a triad extract with [γ-³²P]ATP and a soluble fraction from frog skeletal muscle containing IP₃-3-kinase activity (Carrasco et al., 1993). After separation of the phosphorylated extract by HPLC (Batty et al., 1985), we found that a significant fraction, 150 pmol of the 340 pmol of IP₃ initially present in the triad extract, had been converted to [³²P]IP₄ (Fig. 4B). The identity of the [³²P]IP₄ formed was confirmed by coelution from the HPLC column with a true [³²P]IP₄ standard. Control experiments were carried out by phosphorylating commercial IP₃. The amount of [³²P]IP₄ formed was proportional to the concentration of IP₃ present in the assay; in the absence of IP₃ there was no radioactive peak at the IP₄ retention time. In the experiment conditions used to measure phosphorylation of IP₃ to IP₄, some radioactivity eluted at longer retention times (Fig. 4B), suggesting formation of phosphoinositides containing even more phosphate groups than IP₃ by the possible presence of other kinases in the 100,000 X g extract. A characterization of these compounds was outside the range of the present study and was not pursued in greater detail.

Less than 10% of the IP₃ added to the assay was hydrolyzed to inositol 1,4-bisphosphate, as measured in control experiments by conversion of [³H]inositol 1,4,5-trisphosphate to [³H]inositol 1,4-bisphosphate (not shown).

Since both ATP and IP₂₃ displace [³H]IP₃ in the radioreceptor assay (Fig. 2), it was important to investigate possible interferences of these compounds in the measurements of membrane associated IP₃ concentrations. In the case of ATP, concentrations of the order of 50 μM or higher are needed to obtain measurable displacement (Fig. 2C); at these concentrations ATP displays significant absorbance at 259 nm. Since all membrane extracts had no detectable absorbance at 259 nm, ATP contributions to the displacement assay were ruled out. In the case of IP₂₃, it has been reported that this phospholipid is present in isolated triads and transverse tubules membranes but not in SR vesicles (Hidalgo et al., 1986b; Varsanyi et al., 1986). It can be argued that membrane denaturation with perchloric or trichloroacetic acid might cause hydrolysis of a fraction of this phospholipid, producing IP₃. Alternatively, these acids might release IP₂₃ to the soluble fraction, leading to [³H]IP₃ displacement in the radioreceptor assay by IP₂₃ rather than by IP₃. To investigate these possibilities, 170 pmol (20,000 cpm) of [³H]IP₂₃ were added to 0.1 mg of isolated triads prior to extraction with perchloric acid in the same conditions followed to extract IP₃. After sedimen-
**IP₃ Copurifies with Skeletal Muscle Triads**

**A**

![TLC analysis of the triad extract on PEI-cellulose plates.](image)

**B**

![HPLC analysis of phosphorylation products obtained following incubation of a triad extract with [γ-³²P]ATP and a 100,000 × g supernatant from frog skeletal muscle containing IP₃ 3-kinase activity.](image)

**FIG. 4.** A, TLC analysis of the triad extract on PEI-cellulose plates. To an extract containing 2 nmol of IP₃, determined by the radioreceptor assay, 3 pmol of [³H]IP₃ were added; this amount of [³H]IP₃ is well below the limit of detection of the phosphate staining technique (Spencer et al., 1990). This mixture was lyophilized, resuspended in a small volume of 0.3 N HCl, 0.2 M KH₂PO₄, and analyzed on PEI-cellulose TLC plates developed using 0.5 N HCl as described (Spencer et al., 1990). After drying and staining for phosphate, the plate shown above was cut in 0.5-cm strips and the radioactivity associated with the strips was determined by liquid scintillation counting. B, HPLC analysis of phosphorylation products obtained following incubation of a triad extract with [γ-³²P]ATP and a 100,000 × g supernatant from frog skeletal muscle containing IP₃ 3-kinase activity. To prepare the triad extract, 0.1 ml of 8% (w/v) perchloric acid were added to 3.7 mg of protein. After discarding the pellet by sedimentation, the supernatant was neutralized with 0.04 ml of KOH/MES buffer (Stephens, 1990). The resulting extract had a displacement activity corresponding to 8.9 pM IP₃. A fraction (0.1 ml) of this extract was incubated as described under "Experimental Procedures" with [γ-³²P]ATP and a soluble fraction from frog skeletal muscle containing IP₃ 3-kinase activity, in a total volume of 0.2 ml. After stopping the reaction, 0.25 ml of the neutralized extract containing tracer amounts of [³H]IP₃ and [³H]IP₄ was loaded in the HPLC column. Fractions of 1 ml were collected at 0.8 min intervals, and their radioactivity was determined by liquid scintillation counting. Only part of the elution pattern, displaying the retention times for ATP, [³H]IP₃, and [³H]IP₄, is shown in the figure. The specific activity of the [γ-³²P]ATP used was 150,000 cpm/nmol, so that the 4,000 cpm present in each of the two peak fractions after background subtraction correspond to 27 pmol of [³²P]IP₄ present in each one. Inset, the elution profile of a time zero control (solid symbols) is compared to the experimental points (open symbols); to compare both experiments the cpm obtained at the ATP peak were scaled to 100%. Only a zone of the elution profile, where [³H]IP₄ is found, is illustrated in the inset.
tation, all the [3H]PIP2 added was found in the perchloric acid pellet and no radioactivity appeared in the soluble fraction, where IP3 is found. Thus, these results indicate that the procedure followed to denature the membranes caused neither PIP2 hydrolysis nor IP3 release to the soluble fraction. Other control experiments, carried out by extracting triad membranes with chloroform, methanol, 1 N HCl (conditions that lead to the complete extraction of PIP2 into the organic phase as controlled using [3H]PIP2), indicated that all the displacement activity derived from the triads remained in the aqueous phase. These combined observations ruled out the possibility that PIP2 contributed to the displacement activity of the membrane extracts, confirming that this was solely due to IP3.

Since IP3 remained attached as such to triads and transverse tubule membranes all throughout the isolation procedure but was not present in isolated SR, it is presumably bound to some component of the transverse tubule membranes. The nature of the putative IP3 binding sites present in these membranes remains to be established. Since aldolase binds IP3 (Thieleczek et al., 1989) and is present in the triads (Thieleczek et al., 1988; Brandt et al., 1990), this protein might be a possible source of IP3 binding sites. However, SDS-gel electrophoresis analysis of the protein composition of our triad preparations indicates that they contain little or no aldolase (Fig. 1). Membrane incubation with fructose 1,6-bisphosphate, a procedure that releases aldolase from the membranes, fails to release IP3 from the membranes (Table II); washing and pelleting the vesicles increased somewhat their IP3 content, although the differences are not statistically significant. Experiments are under way to analyze the nature of the IP3 binding sites.

DISCUSSION

In several cell types it has been observed that increases in the cellular IP3 content enhance calcium entry into the cells by a mechanism yet unknown; a role for both IP3 and IP2 in this process is emerging (see Irvine (1992) and Petersen (1992) for recent reviews). Physiological IP3 receptors may be calcium channels (Luckoff and Clapham, 1992), and it has been speculated that they may belong to the DHP receptor family (Irvine, 1992). The IP3 receptor in T cells appears to be responsible for calcium entry (Khan et al., 1992a), and IP3 regulates the activity of voltage-dependent calcium channels present in plasma membranes of cerebellar granule neurons (De Waard et al., 1992). Direct activation by IP3 of two of two types of channels in cell-free patches of plasma membranes from lobster olfactory receptor neurons has been described (Fadool and Ache, 1992), supporting the presence of plasma membrane IP3 receptors in these cells as well. It has been demonstrated that plasma membrane IP3 receptors of T lymphocytes are different from those existing in cerebellar membranes (Khan et al., 1992b), and it is likely that different kinds of IP3 receptors will be found in other cells as well. Our results, which demonstrate the presence of large amounts of IP3 associated with the muscle plasma membrane regions differentiated as transverse tubules, strongly suggest that these membranes possess receptors for IP3. Since IP3 activates the DHP sensitive calcium channels of transverse tubules (Vilven and Coronado, 1988), the putative voltage sensor proteins in excitation-contraction (E-C) coupling in skeletal muscle (Rios and Brum, 1987), the DHP receptors may be a possible source of IP3 binding sites. Experiments are in progress to determine the nature of the sites that maintain IP3 bound to the transverse tubule membranes during isolation. Establishing the nature of these putative sites might help understand how IP3 stimulates calcium influx into cells.

Physiological Relevance in Excitation-Contraction Coupling—A role for IP3 as a chemical transmitter in E-C coupling in skeletal muscle has been postulated (Vergara et al., 1985; Volpe et al., 1985). Several reports either in favor or against this proposal make a role for IP3 in E-C coupling still controversial (Hidalgo and Jaimovich, 1989; Jaimovich, 1991). In particular, identification of the source of IP3 and how it is released from the transverse tubules following depolarization remains a central problem. The present results reveal for the first time that large amounts of IP3 are present in both triads and transverse tubules vesicles isolated from skeletal muscle, presumably bound to some transverse tubule component since IP3 is not released after making the vesicles permeable with saponin. Resting muscle has free IP3 concentrations in the range of 1.2–2.5 μM (Mayr and Thieleczek, 1991). At these concentrations IP3 opens the SR calcium channels incorporated in bilayers (Suárez-Isla et al., 1991) and rapidly releases calcium from SR vesicles (Valdivia et al., 1992) but not from SR vesicles (Hannon et al., 1992). Hence, it is plausible that activation of the SR calcium channels by IP3 is blocked in resting muscle either by the transverse tubular voltage sensor itself or by a secondary protein under the control of the voltage sensor and that depolarization removes this hindrance. That the IP3 effect is under voltage control is supported by the observations that IP3 added to skinned or ruptured muscle fibers is much more effective in releasing calcium or producing a contraction when the transverse tubules are depolarized (Donaldson et al., 1988; Rojas and Jaimovich, 1990; Lopez and Parra, 1991; Hannon et al., 1992).

In summary, we have found large amounts of IP3 in plasma membrane containing preparations isolated from skeletal muscle. How IP3 stays attached to the membranes during isolation, as well as the physiological significance of this finding, remain to be established.

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