Isolation and characterization of the inositol trisphosphate receptor from smooth muscle

(excitation–contraction coupling/sarcoplasmic reticulum/heparin/electron microscopy)

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ABSTRACT The release of Ca^{2+} from internal stores is requisite to muscle contraction. In skeletal muscle and heart, the Ca^{2+} release channels (ryanodine receptor) of sarcoplasmic reticulum, involved in excitation–contraction coupling, have recently been isolated and characterized. In smooth muscle, inositol 1,4,5-trisphosphate (IP_3) is believed to mobilize Ca^{2+} from internal stores and thereby modulate contraction. We describe the isolation of an IP_3 receptor from smooth muscle. Bovine aorta smooth muscle microsomes were solubilized with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, and the IP_3 receptor was purified by sucrose gradient centrifugation and column chromatography with heparin-agarose and wheat germ agglutinin-agarose. The purified receptor bound 2.7 ± 0.18 nmol of IP_3 per mg of protein with a K_d of 2.4 ± 0.24 nM. That is, the purified receptor has been enriched about 1000-fold compared with the original microsomes, whereas the K_d for IP_3 remains unchanged. The receptor is an oligomer of a single polypeptide with a M_r of 224,000 as determined by SDS/PAGE. Negative-staining electron microscopy reveals that the receptor is a large pinwheel-like structure having surface dimensions of $\approx 250 \times 250$ Å with fourfold symmetry. The IP_3 receptor from smooth muscle is similar to the ryanodine receptor with regard to its large size and fourfold symmetry, albeit distinct with regard to appearance, protomer size, and ligand binding.

Muscle contraction is triggered by a rise in myoplasmic free Ca^{2+} . Ca^{2+} release in heart and skeletal muscle can be characteristically triggered by Ca^{2+} /caffeine from the sarcoplasmic reticulum (SR) (1) by way of ryanodine-sensitive Ca^{2+} release channels, which have been localized to the terminal cisternae of SR (1–3). Recently, the ryanodine receptors from SR of skeletal muscle and heart have been isolated and identified morphologically as the foot structures of the terminal cisternae (4–6). Reconstitution into planar lipid bilayers has allowed identification of the ryanodine receptors as the SR Ca^{2+} release channels (7–10). The ryanodine receptor from skeletal muscle has been cloned (11, 12). At present, the role of inositol 1,4,5-trisphosphate (IP_3) in excitation–contraction coupling in heart and skeletal muscle remains controversial (1), whereas, in smooth muscle, two types of SR Ca^{2+} release have been described (13, 14). Ca^{2+} release can be triggered by IP_3 or by Ca^{2+} /caffeine. The Ca^{2+} /caffeine-sensitive Ca^{2+} release pathway in smooth muscle, as in skeletal muscle and heart, is sensitive to ryanodine. In excitation–contraction coupling in smooth muscle, IP_3 is produced at the level of the plasma membrane in response to hormone stimulation (1). Then IP_3 binds to specific intracellular receptors on the SR membrane and mobilizes the release of Ca^{2+} (15), thereby inducing contraction (16). An IP_3 receptor has been isolated from brain (17)

and identified as a Ca^{2+} channel by reconstitution into phospholipid vesicles (18). Recent cloning studies have revealed that the brain IP_3 receptor shares some homology with the skeletal muscle ryanodine receptor (19). Isolation of Ca^{2+} release channels from SR of smooth muscle has thus far not been achieved. In this study, we describe the isolation and characterization of an IP_3 receptor from smooth muscle. A preliminary communication has been published (20).

EXPERIMENTAL PROCEDURES

Materials. Inositol 1-phosphate, inositol 1,4-bisphosphate, inositol 1,4,5-trisphosphate (IP_3), and inositol 1,3,4,5-tetraphosphate were from Calbiochem, [^3H] IP_3 was from NEN. 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), soybean lecithin (type IV-S), wheat germ agglutinin, and heparin-agarose (type I) were from Sigma. SDS/PAGE high molecular weight standards were from Bio-Rad. Wheat germ agglutinin-Sepharose 4B (5 mg of protein per ml of gel) was prepared by using CNBr-activated Sepharose 4B according to the manufacturer's instructions (Pharmacia). Bovine aortae were quick-frozen with crushed dry ice shortly after sacrifice and stored at -80°C .

Methodology. Unless indicated otherwise, all solutions contained protease inhibitors (leupeptin at 0.5 $\mu\text{g}/\text{ml}$, pepstatin A at 0.5 $\mu\text{g}/\text{ml}$, and aprotinin at 0.21 $\mu\text{g}/\text{ml}$), and the pH of the solutions was adjusted at room temperature. All purification operations were performed at 4°C .

Isolation of Bovine Aorta Smooth Muscle Microsomes. Microsomes were prepared from bovine aorta by a modification of the procedure described for skeletal muscle (21). Twelve to 15 aortae were thawed at room temperature in 3 liters of imidazole homogenization medium [0.3 M sucrose/ 5 mM imidazole hydrochloride (pH 7.4)]. The smooth muscle layer was then teased away from the outer connective tissue layer and passed through a meat grinder. The ground meat, in 12 50-g portions, was homogenized for 90 sec in 250 ml of imidazole homogenization medium with a Waring blender at maximum speed. The homogenate was centrifuged in a Beckman JA-10 rotor for 20 min at 8000 rpm. The pellets were resuspended in 250 ml of imidazole homogenization medium containing 1 mM MgATP and rehomogenized as described above. The supernatants from the first and second homogenizations (designated I and R series, respectively) were filtered through four layers of cheesecloth and centrifuged for 2 hr at 19,000 rpm with a Beckman type 19 rotor or for 3 hr at 14,000 rpm in a Beckman JA-14 rotor. The muscle pellets were resuspended in 300 ml of 0.3 M sucrose/ 20 mM sodium pyrophosphate/ 20 mM sodium dihydrogen phosphate/ 1 mM MgCl_2 / 0.5 mM EDTA (pH 7.1), homogenized for 90 sec, and centrifuged in a JA-10 rotor as described above. The super-

nant (P series) was filtered and then centrifuged at 19,000 rpm for 2 hr in a Beckman type 19 rotor.

The microsomal pellets obtained after centrifugation of the I and R series supernatants were resuspended separately in 200 ml of imidazole homogenization medium and centrifuged at 14,000 rpm for 20 min in a Beckman JA-14 rotor. The respective supernatants containing the microsomes were aspirated and saved. The loosely packed pellets were each resuspended with an additional 50 ml of imidazole homogenization medium and recentrifuged at 14,000 rpm for 20 min in a JA-14 rotor. The supernatants were aspirated and combined with the respective previously obtained microsomal supernatants; the pellets were discarded. Microsomes were then sedimented by centrifugation for 60 min at 40,000 rpm using a Beckman 45 Ti rotor. The I and R series microsomes were resuspended separately in 40 ml of imidazole homogenization medium and each layered as 10-ml aliquots on four sucrose gradients [7 ml per layer of 27, 32, 38, and 45% (wt/wt) sucrose containing 5 mM imidazole hydrochloride (pH 7.4)]. The gradients were centrifuged for 16 hr at 20,000 rpm with a Beckman SW 27 rotor. Fractions were collected at the interfaces, combined, diluted, and recovered by sedimentation. The pellets were resuspended in imidazole homogenization medium to ≈ 10 mg of protein per ml, quick-frozen, and stored at -80°C . The gradient subfractionation served mainly to remove nonmembranous material.

The microsomal pellets obtained after centrifugation of the P series supernatant were resuspended in 200 ml of imidazole homogenization medium and centrifuged at 14,000 rpm for 20 min with a Beckman JA-14 rotor. The pellets were discarded, and the supernatant containing the microsomes was centrifuged for 60 min at 40,000 rpm in a Beckman 45 Ti rotor. The microsomes were resuspended to ≈ 10 mg of protein per ml in imidazole homogenization medium and stored at -80°C without subfractionation by sucrose gradient centrifugation. The sequential homogenization of the muscle greatly increased the yield of microsomes. In a typical preparation using 600 g of smooth muscle (obtained from 12–15 aortae), ≈ 160 mg of microsomal protein was obtained from the combined I (≈ 40 mg), R (≈ 40 mg), and P (≈ 80 mg) series fractions.

[^3H]IP $_3$ Binding Assay. Microsomes (50 μg of protein) were incubated for 10 min at 4°C in 0.10 ml of 50 mM Tris Cl, pH 8.3/1 mM EDTA/1 mM dithiothreitol (DTT) containing various concentrations of [^3H]IP $_3$. Microsomes were then centrifuged for 10 min at 65,000 rpm with a Beckman TL100 rotor. The supernatant, containing free ligand, was aspirated, and the microsome pellets were rapidly washed twice with 0.25 ml of incubation buffer. Subsequently, pellets were resuspended in 0.20 ml of H $_2$ O and mixed with 5.0 ml of EcoScint scintillation cocktail (NEN), and the radioactivity was measured in a Beckman LS 5000 TD scintillation counter. In the routine assay, [^3H]IP $_3$ was included at a final concentration of 10 nM. Nonspecific binding was measured in the presence of 10 μM unlabeled IP $_3$.

Binding of [^3H]IP $_3$ to solubilized samples was determined by using a PEG precipitation method. Samples were incubated at 4°C in 0.11 ml of solution containing 5 μmol of Tris Cl (pH 8.3), 0.1 μmol of EDTA, 0.1 μmol of DTT, 5–50 μg of CHAPS, 0–5 μmol of NaCl, 5 mg of PEG-6000, and various concentrations of [^3H]IP $_3$ ($\approx 20,000$ cpm/pmol). After 5 min, 10 μl of a solution of 50 mg of bovine gamma globulin per ml was added and the suspension was mixed. After an additional 5 min, samples were centrifuged for 10 min at 95,000 rpm with a Beckman TL100 rotor. The supernatants were aspirated, the pellets were washed, and the radioactivity was measured as described above. In the routine assay, [^3H]IP $_3$ was included at a final concentration of 8.34 nM, and nonspecific binding was determined in the presence of 10 μM unlabeled IP $_3$.

Purification of the IP $_3$ Receptor. *Solubilization of the microsomes.* Frozen microsomes (200 mg of protein) were thawed and mixed with 120 ml of 100 mM Tris Cl, pH 8.3/200 mM NaCl/2 mM EDTA/2 mM DTT, and the volume was adjusted to 240 ml. The microsomes were then sedimented by centrifugation for 1 hr at 40,000 rpm in a Beckman 45 Ti rotor and resuspended in 20 ml of 100 mM Tris Cl, pH 8.3/200 mM NaCl/2 mM EDTA/2 mM DTT. After adjustment of the volume to 30 ml with H $_2$ O, solubilization was initiated by addition of 10.0 ml of 10% (wt/vol) CHAPS. The sample was homogenized (20 strokes, Dounce homogenizer, A pestle), incubated on ice for 10 min, and centrifuged for 30 min at 40,000 rpm in a Beckman 75 Ti rotor. The supernatant, which contained 80% of the initial IP $_3$ binding activity, as determined by the PEG precipitation method, was used for purification of the IP $_3$ receptor.

Purification of the IP $_3$ receptor. The solubilized sample was loaded as 6.5-ml aliquots on six gradients [containing 8.0 ml each of 10%, 15%, 17.5%, and 20% (wt/wt) sucrose in 50 mM Tris Cl, pH 8.3/100 mM NaCl/1 mM EDTA/1 mM DTT/1.0% (wt/vol) CHAPS]. Gradients were centrifuged for 20 hr at 28,000 rpm in a Beckman SW 28 rotor. Thirteen 3-ml fractions were collected from the gradients, and samples (50 μl) of the fractions were assayed for [^3H]IP $_3$ binding. The peak fractions (fractions 2–5, fraction 1 = bottom of gradient) of the IP $_3$ binding activity were pooled (≈ 72 ml) and loaded at a flow rate of 0.5 ml/min onto a 6×1.5 -cm column of heparin-agarose that had been preequilibrated with 50 mM Tris Cl, pH 8.3/1 mM DTT/1.0% (wt/vol) CHAPS (buffer A) containing 1 mM EDTA and 100 mM NaCl. The column was washed with 25 ml of buffer A containing 100 mM NaCl, and the receptor was eluted with a 100-ml linear gradient of 100–800 mM NaCl in buffer A. Samples (10 μl) of the 5.0-ml fractions were assayed for IP $_3$ binding activity. The peak [^3H]IP $_3$ binding activity was obtained in fractions 11–12, corresponding to ≈ 0.5 M NaCl. Fractions 11–14 were pooled, quick-frozen in liquid nitrogen, and stored at -80°C .

The pooled fractions from the heparin-agarose column were thawed and combined with similar fractions from another preparation (total volume of 40 ml). The combined fractions were incubated with agitation for 4 hr with 5 ml of wheat germ agglutinin-Sepharose 4B. The suspension was poured into a 1.0-cm i.d. column and washed three times with 5 ml of buffer A containing 500 mM NaCl without protease inhibitors. The receptor was eluted with the same buffer containing 200 mM *N*-acetylglucosamine. Samples (10 μl) of the fractions (1.0 ml) were assayed for IP $_3$ binding activity. The three fractions containing the peak binding activity were pooled and mixed with 3.0 ml of 10% CHAPS/5% soybean phospholipid containing 1 mM DTT and leupeptin at 1 $\mu\text{g}/\text{ml}$. After 30 min on ice, the mixture was loaded as 1.5-ml samples on four gradients [2.5 ml, 12.5%; 3.0 ml, 15%; 3.0 ml, 17.5%; and 2.0 ml, 20% (wt/vol) sucrose in buffer A (adjusted to pH 8.3 at 4°C) containing 100 mM NaCl and 0.5 μg of leupeptin per ml]. After centrifugation at 35,000 rpm for 18 hr with a Beckman SW 41 rotor, the gradients were fractionated into 12 1-ml samples, and portions (20 μl) were assayed for IP $_3$ binding. The peak fractions (fractions 3–5, fraction 1 = bottom of the gradient) containing receptor were pooled, concentrated to ≈ 1 ml by using a Centricon 30 (Amicon), and stored at -80°C .

Protein Assay. Protein determination was performed by using the Bio-Rad protein assay kit or by scanning densitometry of protein bands on Coomassie blue R-stained SDS/polyacrylamide gels with an automated gel analysis plus image processing system (Technology Resources, Nashville, TN). Bovine serum albumin was used as a standard.

Electron Microscopy. Negative staining of purified receptor samples (≈ 50 $\mu\text{g}/\text{ml}$) was performed as described (22).

FIG. 1. Scatchard analysis of IP_3 binding to smooth muscle microsomes. Specific and nonspecific binding were determined in duplicate with $50 \mu\text{g}$ of microsomal protein and from 1 to 16 nM $[^3\text{H}]\text{IP}_3$. The correlation coefficient was 0.992. The data presented is representative of two determinations. (Inset) Scatchard analysis of IP_3 binding to the purified receptor. Specific and nonspecific binding were each determined in duplicate with $\approx 0.1 \mu\text{g}$ of receptor protein and from 1 to 16 nM $[^3\text{H}]\text{IP}_3$ per assay tube. A typical plot is shown. Analyses of four receptor preparations revealed a K_d of $2.4 \pm 0.24 \text{ nM}$ with a B_{max} of $2.7 \pm 0.18 \text{ nmol/mg}$ of protein. The correlation coefficients ranged from 0.989 to 0.999.

RESULTS

Smooth Muscle Microsomes Bind $[^3\text{H}]\text{IP}_3$. A procedure was developed to isolate adequate amounts of smooth muscle microsomes, as is essential for purification of the IP_3 receptor. From 600 g of smooth muscle, an average of 160 mg of microsomal protein was obtained. $[^3\text{H}]\text{IP}_3$ binding averaged $\approx 1.5 \text{ pmol}$ of $[^3\text{H}]\text{IP}_3$ per mg of protein. Scatchard analysis is compatible with simple one-component binding. A K_d of 4.7 nM and a B_{max} of 2.6 pmol/mg is obtained with the microsome fraction (Fig. 1). The concentration of NaCl in the assay medium markedly affects IP_3 binding. Binding is optimal at a concentration of 0.1 M and is markedly reduced at higher concentrations, with little binding at 0.5 M NaCl (Fig. 2A).

The pH dependence of IP_3 binding is shown in Fig. 2B. Binding is optimum at pH 9.5. The binding at pH 7.5 is less than one-sixth that at pH 9.5. Binding is inhibited by heparin ($K_{1/2} = 1.5 \mu\text{g/ml}$) and is specific in that other phosphate esters of inositol do not effectively compete for binding to the receptor. Inositol 1-phosphate and inositol 1,4-bisphosphate do not inhibit $[^3\text{H}]\text{IP}_3$ binding at concentrations of 10 μM . Inositol 1,3,4,5-tetraphosphate inhibits binding, with $K_{1/2} \approx 1 \mu\text{M}$.

Purification of the IP_3 Receptor. Using the zwitterionic detergent CHAPS, we were able to solubilize the microsomes with little loss in $[^3\text{H}]\text{IP}_3$ binding. After centrifugation of the

FIG. 2. Effect of the NaCl concentration and pH on IP_3 binding to smooth muscle microsomes. (A) Effect of NaCl on $[^3\text{H}]\text{IP}_3$ binding. (B) Effect of pH on $[^3\text{H}]\text{IP}_3$ binding. In A, NaCl was included at the indicated final concentrations (the pH of the assay mixture, measured at room temperature, was 8.3), and, in B, the pH of the assay medium was adjusted to the indicated value at 4°C . NaCl was not added in the assay mixture.

solubilized microsomes, 80% of the receptor was recovered in the supernatant, indicating that the IP_3 receptor had been effectively solubilized (Table 1).

The purification of the IP_3 receptor involves four steps. Sucrose gradient centrifugation of the supernatant from the solubilized microsomes provides the major enrichment of the receptor (≈ 40 -fold, Table 1). Analysis of gradient fractions revealed that the IP_3 receptor was located in the lower region of the gradient, whereas most other microsomal proteins were located in the upper gradient fractions. Sequential chromatography on heparin-agarose and wheat germ agglutinin-agarose then served to concentrate and further enrich the receptor. Finally, treatment with a CHAPS/phospholipid mixture disaggregated contaminant proteins from the receptor, which was then isolated by centrifugation in a second sucrose gradient. The receptor was recovered in the lower gradient fractions. Most of the contaminating proteins remained in the upper region of the gradient as revealed by SDS/PAGE (results not shown).

The purification is summarized in Table 1. About 15% of the initial binding activity in the microsomes was recovered as the purified receptor. SDS/PAGE profiles of the different fractions are shown in Fig. 3. The purified receptor consists

Table 1. Summary of smooth muscle IP_3 receptor purification

Fraction	Protein, mg	IP_3 binding, pmol	Specific activity, pmol/mg	Purification, fold	Yield, %
Solubilized microsomes	402.0*†	620†	1.54‡	1	100
Supernatant	345.0*†	490†	1.42	0.92	79
First gradient pool	12.3*†	758†	61.6	40	122
Heparin pool	3.18*†	490†	154	100	79
Wheat germ agglutinin pool	0.57*	211	370	240	34
Second gradient pool	0.053§	97	1830	1188	16

The results presented are for a typical preparation.

*Protein was determined by using the Bio-Rad protein assay reagent.

†Tabulated numbers represent summed values for the two preparations, which were combined at the wheat germ agglutinin-agarose step.

‡The specific activity of $[^3\text{H}]\text{IP}_3$ binding to the solubilized microsomes was the same as for the unsolubilized microsomes.

§Protein was determined by densitometric scanning of Coomassie blue-stained gels.

FIG. 3. SDS/PAGE of fractions obtained in the purification of the IP₃ receptor. Electrophoresis was carried out according to Laemmli (23) on a 7.5% polyacrylamide gel. The gel was stained with Coomassie blue. Lane 1, smooth muscle microsomes; lane 2, pooled fractions from the first sucrose gradient; lane 3, pooled fractions from the heparin-agarose column; lane 4, pooled fractions from the wheat germ agglutinin column; lane 5, pooled and concentrated fractions from the second sucrose gradient. One microgram of protein was loaded on each lane, except lane 1, on which 4 μ g of protein was loaded. The numbers on the left of the gel indicate the positions of M_r standards: myosin, $M_r = 200,000$; β -galactosidase, $M_r = 116,500$; phosphorylase b, $M_r = 92,500$; bovine serum albumin, $M_r = 66,200$; ovalbumin, $M_r = 45,000$. The purified M_r 224,000 receptor is indicated by the long arrow. Actin is indicated by the short arrow. Only a portion of the M_r 224,000 band in the microsomes (lane 1) is referable to the IP₃ receptor.

of a single polypeptide with a M_r of $224,000 \pm 3500$ (mean $\pm$ SD, $n = 4$) (long arrow, Fig. 3). A minor contaminant (12% of total protein as determined by densitometric scanning), identified by immunoblotting as smooth muscle α -actin (results not shown), is observed (short arrow, Fig. 3). In a typical preparation summarized in Table 1, about 50 μ g of receptor protein (determined by densitometry) was obtained from 400 mg of aorta smooth muscle microsomes. Scatchard analysis of [³H]IP₃ binding gives a K_d of 2.4 ± 0.18 nM (mean

$\pm$ SD, $n = 4$) with a B_{max} of 2.7 ± 0.24 nmol per mg of receptor protein (mean $\pm$ SD, $n = 4$) (Fig. 1 *Inset*). The binding constant of the purified receptor is essentially the same as in the smooth muscle microsomes. [³H]IP₃ binding to the purified receptor shows a similar pH dependence to that found for the microsomes and is similarly inhibited by low concentrations of heparin (results not shown).

Ultrastructure of the Purified Receptor. Electron microscopy of the purified receptor after negative staining with uranyl acetate reveals large structures with surface dimensions of 250×250 Å (Fig. 4). The receptor has a pinwheel appearance with fourfold symmetry and four radial arms radiating from a central hub.

DISCUSSION

The IP₃ receptor from aortic smooth muscle microsomes has been purified to near homogeneity. The purified receptor has been enriched about 1000-fold from the starting microsomes. IP₃ binding to the microsomes is characterized by a K_d of ≈ 5 nM with a B_{max} of 2.6 pmol/mg of membrane protein (Fig. 1). Binding to the receptor is specific with regard to IP₃; other phosphate esters of inositol do not effectively compete for the binding. Binding of IP₃ to both the microsomes and the purified receptor is markedly inhibited by low concentrations of heparin. Heparin has been shown to inhibit IP₃-induced Ca²⁺ release in vascular smooth muscle (16, 24) and blocks IP₃-dependent gating of Ca²⁺ channel conductance obtained after fusion of smooth muscle microsomes with planar lipid bilayers (25). Therefore, the observation that heparin inhibits IP₃ binding suggests that the isolated smooth muscle IP₃ receptor is involved in regulation of Ca²⁺ release *in situ*.

The purified receptor consists of a single polypeptide with a M_r of 224,000 as determined by SDS/PAGE (Fig. 3). The receptor is a glycoprotein as indicated by its binding to wheat germ agglutinin. The receptor is large in size as reflected by sucrose gradient centrifugation and electron microscopy, suggestive of an oligomeric structure. The purified receptor has surface dimensions of about 250×250 Å (Fig. 4). It has fourfold symmetry and a pinwheel appearance. Scatchard analyses of IP₃ binding to the receptor reveal a K_d of 2.4 nM, essentially the same as that for the microsomes (Fig. 1). A B_{max} of 2.7 nmol of IP₃ per mg of receptor protein is obtained. Assuming a subunit M_r of 224,000 and that all subunits bind

FIG. 4. Ultrastructure of the purified IP₃ receptor. Electron micrographs of the purified receptor obtained after negative staining with uranyl acetate. (a) Arrowheads point to characteristic structures with fourfold symmetry. The receptor has the appearance of four spokes radiating from a common center with a pinwheel appearance. (b–e) Different aspects of the receptor at higher magnification.

IP₃, the theoretical $B_{\max}$ would be 4.4 nmol/mg. That the experimentally determined $B_{\max}$ is approximately half of the calculated value suggests that two protomers may be required for binding IP₃. The apparent substoichiometric binding might be explained by an underestimation of the subunit size by SDS/PAGE or might also be explained by partial inactivation of the receptor during purification. Recent cloning studies of the IP₃ receptor from brain provided a M_r of 313,000 (19), which is significantly greater than the value of 260,000 estimated by SDS/PAGE (17).

There is evidence that IP₃ is involved in excitation-contraction coupling in smooth muscle (16, 24), whereas the role of IP₃ as a second messenger in excitation-contraction coupling in heart and skeletal muscle remains controversial (1). IP₃ (0.5–5 μ M) has been shown to induce contraction in saponin-permeabilized smooth muscle cells (26–28). The skinned fiber studies of Iino and coworkers (13, 14) suggest that there are two types of intracellular Ca²⁺ stores in smooth muscle. The first releases Ca²⁺ in response to Ca²⁺/caffeine or IP₃, which is modulated by ryanodine. The second appears to be sensitive only to IP₃. Microsomes have been isolated from canine aorta, which release Ca²⁺ in response to micromolar concentrations of IP₃ (29). Also, IP₃ activation of Ca²⁺ channel activity has been demonstrated after fusion of such microsomes with planar lipid bilayers (25).

IP₃ induces Ca²⁺ release from reconstituted vesicles containing the brain IP₃ receptor (18). IP₃ binding to the brain receptor is characterized by a K_d of $\approx$ 80 nM and is inhibited by heparin (17). It is apparent that the IP₃ receptors from brain and smooth muscle are related. A notable difference is the much greater affinity of the smooth muscle receptor for IP₃ (K_d of 2.4 nM versus 80 nM for the brain receptor) and its lower stoichiometry for IP₃ binding. This might reflect differential regulation of intracellular Ca²⁺ release in smooth muscle and brain. The recent cloning studies of the brain IP₃ receptor reveal some sequence homology and predicted topological similarity with the skeletal muscle ryanodine receptor (19).

The IP₃ receptor isolated in this study is similar to the ryanodine receptors from heart and skeletal muscle. Electron microscopy shows that both are large structures with similar size, which have fourfold symmetry (22, 30). The morphology, albeit similar, is unique to each. The ryanodine receptor from skeletal muscle is a tetramer based on the molecular mass measurement determined by scanning transmission electron microscopy (31), taken together with the size of the subunit polypeptide obtained by cloning technology (11, 12). This study suggests that the structure of the smooth muscle IP₃ receptor, although similar, has distinct differences. The smooth muscle IP₃ receptor has an apparent polypeptide M_r of 224,000 as determined by SDS/PAGE. This is to be contrasted with a M_r of $\approx$ 340,000–450,000 (4–6, 32) for the ryanodine receptors from heart and skeletal muscle. The M_r determined from cloning studies is 567,000 for the skeletal muscle ryanodine receptor (11, 12). Significantly, the ligand binding properties of the ryanodine and IP₃ receptors appear to be distinct. We have not been able to detect IP₃ binding to skeletal muscle terminal cisternae or ryanodine binding to the smooth muscle microsomes isolated in this study (C.C.C. and S.F., unpublished results).

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