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Stereospecific inositol 1,4,5-[^{32}P]trisphosphate binding to isolated rat liver nuclei: Evidence for inositol trisphosphate receptor-mediated calcium release from the nucleus

(calcium/nuclear signaling/inositol receptors)

A. N. MALVIYA*, P. ROGUE, AND G. VINCENDON

Centre de Neurochimie du Centre National de la Recherche Scientifique, 5 rue Blaise Pascal, 67084 Strasbourg Cedex, France

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ABSTRACT **It is well known that inositol 1,4,5-trisphosphate binding and release of calcium are mediated by the same protein. Several reports have indicated the location of the inositol 1,4,5-trisphosphate receptor in organelles other than endoplasmic reticulum. Immunocytochemical studies on the subcellular localization of 1,4,5-trisphosphate receptor in the Purkinje cells from two laboratories have given contradictory results regarding the nuclear location of this receptor. In this paper, a high-affinity inositol 1,4,5-[^{32}P]trisphosphate binding site (K_d = 0.11 nM) on nuclei isolated from rat liver and devoid of any microsomal, mitochondrial, or plasma membrane constituents is documented. Furthermore, we present data demonstrating that inositol 1,4,5-trisphosphate is capable of releasing $^{45}Ca^{2+}$ from the intact isolated liver nuclei. A rapid and transient release of calcium that was taken up by nuclei in the presence of ATP is observed. The role of inositol 1,4,5-trisphosphate in the coupling between cytoplasmic second messengers and nuclear events activated during signal transduction is postulated.**

Since the report (1) that inositol 1,4,5-trisphosphate (InsP_3) releases calcium from nonmitochondrial intracellular stores, its role as a second messenger (2) for a multiplicity of neurotransmitters, hormones, and growth factors (3–5) has been documented. Specific InsP_3 binding has been found in a variety of tissues, and the InsP_3 receptor has been purified from rat (6) and mouse (7) cerebellum and cloned (8). Ferris *et al.* (9) have provided conclusive evidence, through reconstitution experiments, that InsP_3 binding and the release of calcium are mediated by the same protein. InsP_3 was initially thought to bind to its specific receptor located on the endoplasmic reticulum. Later studies revealed InsP_3 high-affinity binding to liver (10, 11) and T-lymphocyte plasma membranes (12) and to a vesicular organelle (13) in the adrenal cortex. Immunocytochemical studies have presented conflicting reports regarding the location of the InsP_3 receptor on the nuclear membrane of Purkinje cells. The observation by Ross *et al.* (14) on the nuclear location of the InsP_3 receptor has been contradicted in a recent report (7). It is with this background that we document that a specific high-affinity [^{32}P]InsP_3 binding site is located on isolated rat liver nuclei. We also present data demonstrating that ATP-stimulated calcium uptake (15) by isolated nuclei can be released by InsP_3. This shows that rat liver nuclei possess functional InsP_3 receptors.

MATERIALS AND METHODS

Preparation of Rat Liver Nuclei. Rat liver nuclei were prepared as described by Masmoudi *et al.* (16). Briefly, small pieces of liver were homogenized in 8 vol of a medium containing 1.3 M sucrose, 1.0 mM $MgCl_2$, and 10 mM potassium phosphate buffer (pH 6.8). The homogenate, after filtration through four layers of cheesecloth, was centrifuged for 15 min at 1000 × *g* and the resulting pellet was suspended in a minimum volume of homogenization medium. This suspension was mixed with a medium containing 2.4 M sucrose, 1.0 mM $MgCl_2$, and 10 mM potassium phosphate buffer (pH 6.8) to give a final 2.2 M sucrose concentration, which was checked with a refractometer. In another set of experiments, nuclei were prepared from homogenate supplemented with liver microsomes as described below. The microsomal preparation was adjusted to a final concentration of 1.3 M sucrose before it was added to the homogenate.

Preparation of Microsomes. The microsomal fraction was prepared by homogenizing small pieces of rat liver in 8 vol of a medium containing 0.3 M sucrose, 1 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, and 20 mM Hepes (pH 7.4). The homogenate was filtered through cheesecloth and centrifuged for 5 min at 2500 rpm in a Sorvall SS 34 rotor. The crude nuclei and cell debris were sedimented and washed twice with homogenization medium; washings were mixed with the supernatant from the previous step. The sedimented material was washed a third time and the resulting supernatant was mixed with the post-plasma membrane fraction. The mixed postnuclear supernatant was centrifuged at 8000 rpm in an SS 34 rotor for 10 min to sediment mitochondria. The sedimented mitochondria were washed in an appropriate vol of homogenization medium by centrifuging at 12,000 rpm (SS 34 rotor) for 15 min. Postmitochondrial supernatant plus subsequent washes were centrifuged at 15,000 rpm for 20 min in a K-50 rotor in a Spinco ultracentrifuge. The resulting pellet was the plasma membrane fraction, which was resuspended in homogenization medium and centrifuged as described above. The supernatant thus obtained was mixed with the post-plasma membrane fraction plus the supernatant obtained from the third washing of the first 2500-rpm pellet (as described above) and was centrifuged at 40,000 rpm in a K-50 rotor for 1 hr. The resulting pellet was the microsomal fraction. This fraction was suspended in an appropriate vol of homogenization medium and recentrifuged. The final pellet was suspended in homogenization medium and served as the microsomal fraction. The entire operation was carried out at 4°C.

Marker Enzyme Activity. NADPH cytochrome *c* reductase and antimycin A-insensitive NADH cytochrome *c* reductase activity were determined as described (17) by monitoring the reduction of cytochrome *c* at 550 nm in a medium containing 50 mM potassium phosphate buffer (pH 7.5), 50 μM cytochrome *c*, 33 μM potassium cyanide, 100 μM NADPH or NADH, and 5 mM antimycin A in the case of antimycin A-insensitive activity. Cytochrome *c* oxidase activity was determined (18) by using freshly prepared ferrocytochrome *c*.

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Abbreviations: InsP_3, inositol 1,4,5-trisphosphate; InsP_4, inositol 1,3,4,5-tetrakisphosphate.

*To whom reprint requests should be addressed.

Activity of plasma membrane marker enzymes was determined as described (16).

[^{32}P]InsP_3 Binding. For [^{32}P]InsP_3 binding, the nuclei were suspended in a medium containing 50 mM Tris·HCl (pH 8.0) plus 2 mM EDTA. The binding was done in an Eppendorf tube at 0°C (on ice) for 30 min in 400 μl, and the protein concentration was maintained at 1 mg/ml per assay. Bound and free [^{32}P]InsP_3 were separated by centrifugation (Beckman Microfuge) at 12,000 rpm for 15 min and the supernatant was removed by aspiration. Any remaining fluid was removed with Kleenex and the pellet was suspended in 1 ml of tissue solubilizer (Soluene 350; Packard) plus 70 μl of acetic acid and transferred to 10 ml of Biofluor liquid scintillator, and the radioactivity was determined. Nonspecific binding was determined in the presence of 10 μM InsP_3.

K_d and B_{max} values were determined by Scatchard analysis of saturation experiments carried out with progressively increasing concentrations of [^{32}P]InsP_3. Displacement experiments were done in the presence of 200 pM [^{32}P]InsP_3 at various concentrations of competing ligand.

InsP_3-Induced $^{45}Ca^{2+}$ Release. Isolated nuclei were suspended in a medium containing 0.3 M sucrose, 2 mM EDTA, 50 mM Tris·HCl (pH 8.0). Nuclei at a concentration of 0.1 mg of DNA per tube were incubated at 37°C for 1 hr in the presence of 4 mM $MgCl_2$, 2 mM EGTA, 2 mM K_2HPO_4, 1 mM ATP, 2.5 mM $CaCl_2$, and a trace amount of $^{45}Ca^{2+}$ (2 μCi/ml; 1 Ci = 37 GBq). The uptake of calcium by nuclei was terminated by chilling on ice for 15 min. The release of $^{45}Ca^{2+}$ was performed at 37°C and was initiated by addition of 50 μl of InsP_3 at a final concentration of 10 μM. For control experiments, 50 μl of medium was added. The final volume for release experiments was 200 μl. In another set of experiments, hexokinase (5 units/ml) plus glucose (10 mM) were added in the medium to study the $^{45}Ca^{2+}$ release under ATP-dissipating conditions. At appropriate times, $^{45}Ca^{2+}$ release was terminated by filtration under vacuum over GF/B glass fiber filters followed by rapid rinsing with 5 ml of ice-cold medium containing 0.3 M sucrose, 2 mM EDTA, 2 mM EGTA, 4 mM $MgCl_2$, 2 mM K_2HPO_4, 50 mM Tris·HCl (pH 8.0). The filters were placed in 10 ml of scintillation fluid (Biofluor) and the amount of $^{45}Ca^{2+}$ trapped was determined. $^{45}Ca^{2+}$ release, after a given time, was defined as the radioactivity loaded (time 0) minus the radioactivity trapped on the filters.

Protein was determined according to Bradford (19) with bovine serum albumin used as a standard.

[^{32}P]InsP_3 was obtained from Amersham International or from NEN. $^{45}Ca^{2+}$ was from Amersham International; InsP_3 was from Boehringer; cytochrome *c*, NADPH, NADH, and antimycin A were from Sigma.

RESULTS

Purity of Isolated Nuclei. The nuclei used in this study were devoid of any microsomal, mitochondrial, or plasma membrane contaminants. This was confirmed by measuring the activity of various marker enzymes. NADPH cytochrome *c* reductase activity was only 0.9% of total liver homogenate activity, whereas another microsomal marker, antimycin A-insensitive NADH cytochrome *c* reductase activity was <0.4% of total homogenate activity in the isolated nuclei (Table 1). Yet another line of evidence that [^{32}P]InsP_3 binding observed in isolated nuclei was not due to microsomal adherence is derived from the experiment in which a microsomal preparation (from another batch) was added to the liver homogenate before nucleus purification was initiated. The specific [^{32}P]InsP_3 binding (Table 1) was almost identical in the two sets of nuclear preparations (i.e., one without added microsomes and the one with added microsomes in the liver homogenate). Likewise, activity of the two microsomal marker enzymes did not seem to be altered in the two sets of nuclear preparations. Thus, it may be safely argued that the [^{32}P]InsP_3 binding studied in isolated rat liver nuclei is not due to adherence of the microsomal InsP_3 receptor in the nuclear preparation. Validity of such a statement found further support from the analysis of the final postnuclear material collected at the upper phase of the tubes after centrifugation for 1 hr at 100,000 × *g* in 2.2 M sucrose medium. This

Table 1. [^{32}P]InsP_3 binding and microsomal marker enzyme activity

Fraction derived from rat liver	Protein, mg	[^{32}P]InsP_3 binding: Specific, fmol per mg of protein per min	[^{32}P]InsP_3 binding: Total, pmol	NADPH cytochrome *c* reductase: Specific, μmol per mg of protein per min	NADPH cytochrome *c* reductase: Total, μmol per min	NADH cytochrome *c* reductase (insensitive to antimycin A): Specific, μmol per mg of protein per min	NADH cytochrome *c* reductase (insensitive to antimycin A): Total, μmol per min
Homogenate	4102 (100)	80	328 (100)	0.008	32.8 (100)	0.42	1723 (100)
Nuclei	250 (6.1)	87	22 (6.7)	0.0014	0.34 (1.0)	0.025	6.25 (0.4)
Final postnuclear material	337 (8.2)	104	35 (10.7)	0.012	4.1 (12.4)	0.41	138 (8.0)
Homogenate with added microsomes*	4480 (100)	87	390 (100)	0.012	53.7 (100)	0.50	2240 (100)
Nuclei	296 (6.6)	85	25 (6.4)	0.0017	0.49 (0.9)	0.03	8.8 (0.4)
Final postnuclear material†	324 (7.1)	210	68 (17.4)	0.035	11.3 (21)	0.95	309 (13.8)

Numbers in parentheses represent percentages.

*Total protein in the homogenate was 4100 mg. Microsomes were prepared as described. Total protein in the starting homogenate was 3891 mg. Total microsomal protein was 380 mg. [^{32}P]InsP_3 specific and total binding were 158 fmol per mg of protein per min and 60 pmol, respectively; NADPH cytochrome *c* reductase specific and total activity were 0.06 μmol per mg of protein per min and 22.8 μmol per min, respectively; antimycin A-insensitive NADH cytochrome *c* reductase specific and total activity were 1.45 μmol per mg of protein per min and 551 μmol per min, respectively.

†Livers were homogenized in 1.3 M sucrose medium and the homogenate was centrifuged at 1000 × *g*. The resulting pellet was suspended in the same medium and was adjusted to 2.2 M sucrose by adding 2.4 M sucrose medium, followed by centrifugation at 100,000 × *g* for 1 hr. Nuclei were sedimented, whereas other cytoplasmic material, including contaminating microsomes, stayed above sucrose in the tube (this is termed final postnuclear material). The final concentration of [^{32}P]InsP_3 was 0.5 nmol in the binding assay. The radioligand was displaced by 10 μM InsP_3. The reduction of cytochrome *c* was monitored at 550 nm and the reductase activity was determined by using an extinction coefficient of 21.1 $mM^{-1} \cdot cm^{-1}$. Antimycin A concentration was 5 mM. These data are means of two independent preparations with replicates that varied by <5%.

material, which is a final washing step in the purification procedure, seems to contain a part of the microsomes as well as some cytoplasmic constituents. This is supported by the presence of microsomal marker enzyme activity, which is not found in the purified nuclei (Table 1). Furthermore, specific [^{32}P]InsP_3 binding in similar material, derived after separation of nuclei without supplementing liver homogenate with exogenous microsomes was 104 fmol per mg of protein per min and the total binding was 186 pmol. In the postnuclear material obtained from the nuclear preparation derived from the homogenate supplemented with exogenous microsomes, the specific binding and total binding were 210 fmol per mg of protein per min and 340 pmol respectively (Table 1).

[^{32}P]InsP_3 Binding to Nuclei. Purified rat liver nuclei showed very high-affinity [^{32}P]InsP_3 specific binding. The total binding was ≈20% of [^{32}P]InsP_3 added in the binding assay medium. Nonspecific binding, defined by including 10 μM InsP_3 was <10% of total bound at 200 pM [^{32}P]InsP_3. The specificity of the InsP_3 receptor located on the nuclei was analyzed by competitive displacement of [^{32}P]InsP_3 by various ligands. InsP_3 at 15 nM inhibited [^{32}P]InsP_3 binding 50% and was the most potent inhibitor of binding (Fig. 1). Binding was also inhibited, although to a lesser extent, by inositol 2,4,5-trisphosphate (2,4,5-InsP_3) or inositol 1,3,4,5-tetrakisphosphate (InsP_4), as well as by 2 mM ATP. Heparin at 100 μg/ml completely abolished [^{32}P]InsP_3 binding, whereas at 1 μg/ml it inhibited 51% of the specific binding. Such a rank order of inhibition of specific [^{32}P]InsP_3 binding is characteristic of the InsP_3 receptor. The [^{32}P]InsP_3 binding to isolated nuclei was sensitive to pH and was clearly enhanced when pH was raised from 7.5 to 8.5 (Fig. 2). The specific binding also increased linearly with the amount of nuclei (in terms of concentration of protein) added in the binding assay (data not shown). Scatchard plots (Fig. 3) of saturation isotherms showed a single class of binding sites with a high affinity (K_d = 0.11 nM) and a B_{max} of 8.9 fmol per mg of protein.

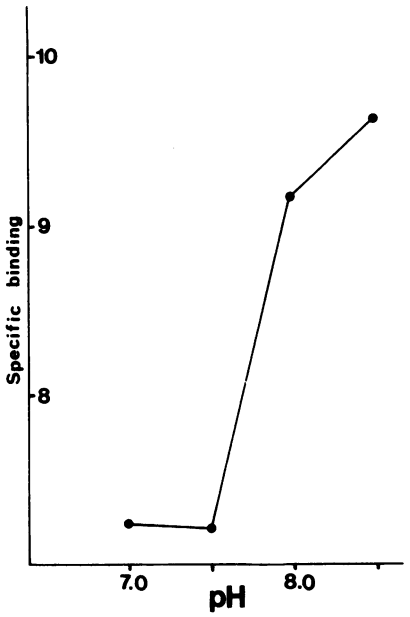

FIG. 2. pH dependence of [^{32}P]InsP_3 specific binding (cpm × 10^{-3}) to nuclei. Tris·HCl buffer (50 mM) was used at the indicated pH plus 2 mM EDTA. The binding was as described in the legend to Fig. 1.

$^{45}Ca^{2+}$ Uptake and Release by Nuclei. To assess the functional relevance of the observed specific InsP_3 binding site on the isolated rat liver nuclei, the nuclei were loaded with $^{45}Ca^{2+}$ and the influence of InsP_3 on calcium release was examined. No calcium uptake was observed in the absence of ATP and no calcium leakage was seen by keeping calcium-loaded nuclei on ice for a prolonged period (data not shown). InsP_3 (10 μM) caused robust release of nearly one-half of the $^{45}Ca^{2+}$ loaded (Fig. 4). The calcium release was time dependent, being rapid and transient with a maximum ≈1 min after

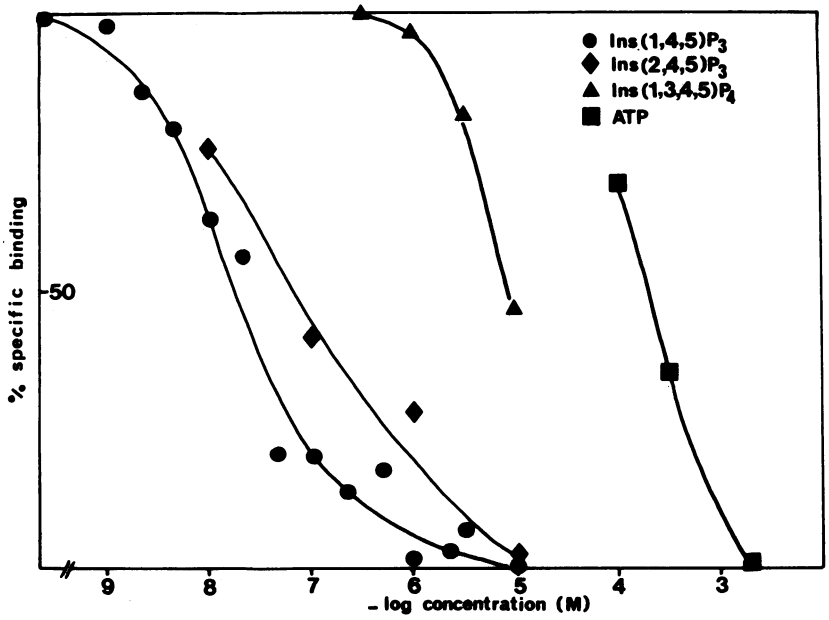

FIG. 1. Characterization of specific [^{32}P]InsP_3 binding to isolated rat liver nuclei. [^{32}P]InsP_3 binding was done in an Eppendorf tube at 0°C (on ice) for 30 min in a medium containing 50 mM Tris·HCl (pH 8.0) and 2 mM EDTA. The protein was 1 mg/ml and the total vol of the assay mixture was 400 μl. The binding was terminated by centrifugation (Beckman Microfuge) at 12,000 rpm for 15 min followed by aspiration of the supernatant. Any remaining fluid was removed with Kleenex and the pellet was suspended in 1 ml of tissue solubilizer (Soluene 350; Packard) plus 70 μl of acetic acid and transferred to 10 ml of Biofluor liquid scintillator, and the radioactivity was determined. Nonspecific binding was determined in the presence of nonradioactive InsP_3. Displacement experiments were carried out with 200 pmol of [^{32}P]InsP_3 at various concentrations of competing ligand. These experiments were performed twice in quadruplicate with replicates varying by <10%.

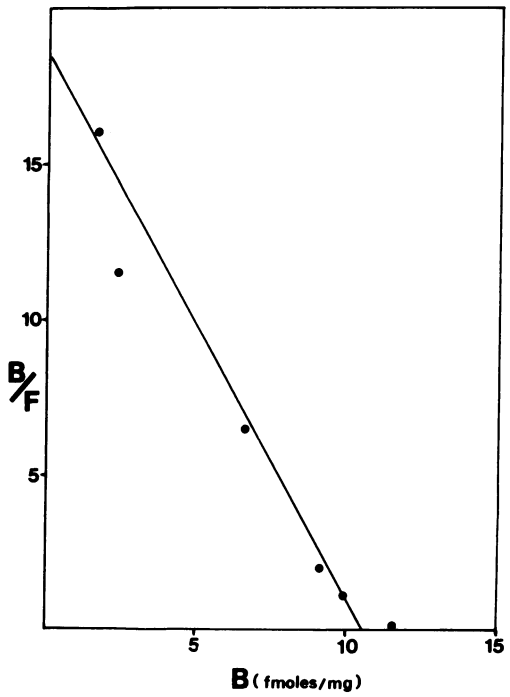

FIG. 3. Scatchard plot of [^{32}P]InsP_3 specific binding. The K_d was determined by Scatchard analysis under classical saturation binding conditions—i.e., by utilizing progressively increasing concentrations of [^{32}P]InsP_3. These experiments were performed on two independent nuclear preparations in triplicate or quadruplicate with replicates varying by <10%. B, bound; F, free.

the addition of InsP_3. The transient nature of the release indicates the occurrence of $^{45}Ca^{2+}$ reuptake, possibly due to the continued presence of ATP. To confirm this process, calcium release was studied in the presence of hexokinase and glucose. Dissipation of ATP abolished reuptake of calcium 5 or 10 min after InsP_3 addition (Fig. 4). Heparin at 100 μg/ml, which alone had no effect, antagonized the ability of InsP_3 to release $^{45}Ca^{2+}$ (Table 2) from isolated nuclei. InsP_4 also had no effect on calcium release (Table 2), whereas the same compound at 10 μM significantly inhibited the binding of [^{32}P]InsP_3 to the nuclei (Fig. 1).

DISCUSSION

Through the use of high molarity sucrose in the homogenization medium (1.3 M) and by maintaining a still higher sucrose concentration (2.2 M) in subsequent centrifugations, it has been possible to obtain a nuclear preparation from rat liver devoid of any microsomal contamination. This has been confirmed by determining NADPH cytochrome *c* reductase activity (<1% of the total homogenate activity) and antimycin A-insensitive NADH cytochrome *c* reductase activity (<0.5% of the total homogenate activity) as shown in Table 1. The fact that even a large amount of exogenous microsomes did not contaminate the nuclei confirmed the efficacy of the purification procedure adopted in this study. Analysis of the final postnuclear material, which in the two sets of preparations contained the associated microsomes washed away, is further proof for this contention. The purified nuclei did not even show a trace of cytochrome *c* oxidase activity, which rules out any mitochondrial contamination. The question of plasma membrane contamination in the nuclear preparation has been dealt with thoroughly in another context (16, 21). Na^+,K^+-ATPase activity (0.24% of total homogenate) and 5'-nucleotidase activity (0.29% of total homogenate) was found in the nuclei as purified for the present study. These factors confirm that the procedure for

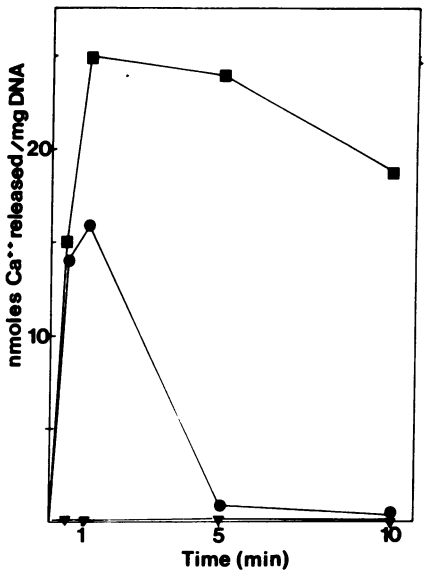

FIG. 4. InsP_3-induced $^{45}Ca^{2+}$ release from nuclei. Isolated nuclei (0.1 mg of DNA per tube) were loaded with $^{45}Ca^{2+}$ by incubating for 1 hr at 37°C in the presence of 1 mM ATP and traces of $^{45}Ca^{2+}$ (2 μCi/ml). The uptake was stopped by placing the nuclei on ice. After a 15-min incubation on ice, the nuclei were preincubated (2 min) at 37°C and the release was initiated by addition of 50 μl of InsP_3 (final concentration, 10 μM) or medium A in controls. The final vol was 200 μl. At appropriate times, the release was terminated by filtration over GF/B glass fiber filters, followed by rapid rinsing with 5 ml of ice-cold medium. The filters were placed in 10 ml of scintillation fluid (Biofluor) for counting trapped $^{45}Ca^{2+}$ radioactivity. $^{45}Ca^{2+}$ release, after a given time, was defined as the radioactivity loaded (time 0) minus the radioactivity trapped on the filter. These experiments were carried out on three independent nuclear preparations in quadruplicate with replicates varying by <10%. ▼, 50 μl of buffer with no InsP_3; ●, InsP_3 in the presence of ATP; ■, InsP_3 plus ATP plus glucose plus hexokinase. The DNA content was determined as described (20).

nuclei isolation did succeed in avoiding any cytoplasmic contamination including microsomes.

Data in this paper clearly show that nuclei are endowed with specific [^{32}P]InsP_3 binding sites. The potency rank order for inhibition of this binding by using other inositol phosphate derivatives (InsP_3, 2,4,5-InsP_3, InsP_4) and ATP, as well as the pH dependency, are characteristic of the InsP_3 receptor. Heparin, a known inhibitor of InsP_3 binding (22), completely abolished nuclear InsP_3 binding at 100 μg/ml, whereas 1 μg/ml inhibited only 51% of the specific binding. Scatchard analysis of saturation isotherms indicated a density of 8.9 fmol per mg of protein and a very high affinity (K_d = 0.11 nM) for these sites. Saturation techniques are more precise than

Table 2. [^{32}P]InsP_3 specific binding to nuclei and InsP_3-stimulated $^{45}Ca^{2+}$ release from isolated rat liver nuclei

Addition	Inhibition of specific [^{32}P]InsP_3 binding, % of maximum bound	$^{45}Ca^{2+}$ release, % of loaded
Medium A	—	0
InsP_3 (10 μM)	100	49
InsP_4 (10 μM)	46	0
InsP_3 (10 μM) + heparin (100 μg/ml)	—	0
Heparin (100 μg/ml)	100	0

[^{32}P]InsP_3 binding was determined as described in the legend of Fig. 1. Calcium release was measured as described in the legend of Fig. 4 and was assayed 1 min after the addition of 10 μM InsP_3. These experiments were performed on three different nuclear preparations in quadruplicate with replicates varying by <10%.

the commonly used isotopic dilution method for the determination of binding parameters. When a kinetic approach is used, very high-affinity specific InsP_3 binding sites have been characterized on adrenal cortex membranes (23).

The second major thrust of this paper is the function of InsP_3 receptor in nuclear calcium movements. Rat liver nuclei, unlike liver microsomes, are not calcium stores. The resting nuclear calcium level is reported to be only 1% (24) of total liver calcium. There is a definite ATP-stimulated calcium-uptake system in the rat liver nuclei (15). We have studied the effect of InsP_3 on $^{45}Ca^{2+}$ release, which was taken up in an ATP-dependent manner. In this study, we have observed no calcium uptake in the absence of ATP, and no calcium leak from the isolated nuclei was detected in the absence of InsP_3. A rapid and transient release of $^{45}Ca^{2+}$ is seen after the addition of InsP_3 to $^{45}Ca^{2+}$-loaded nuclei (Fig. 4). The release of calcium from isolated nuclei is its own and is not due to mitochondrial contamination, since not even a semblance of cytochrome *c* oxidase activity was seen in isolated nuclei. [^{32}P]InsP_3 specific binding and $^{45}Ca^{2+}$ were antagonized by the different inhibitors used with the same rank order of potency. Both $^{45}Ca^{2+}$ release and specific [^{32}P]InsP_3 binding were inhibited by heparin. Similar heparin effects are reported in the literature (9). Within 5 min of InsP_3 addition, there was a calcium reuptake, which could be circumvented when an ATP-dissipating system (glucose plus hexokinase) was included in the release experiments. Hexokinase (5 units/ml) plus 10 mM glucose was sufficient to dissipate ATP, as has been reported (25). Thus, nuclear InsP_3 receptor mediates partial release from the nucleus of calcium taken up directly into the matrix under the influence of ATP (15). The calcium release observed here followed a similar time scale as that of uptake documented elsewhere (15). It may be mentioned that no calcium uptake into the nuclear envelope has been shown. These data confirm that ATP and InsP_3 are two essential components in the regulation of calcium movement into and out of the nucleus. In this context, it may be worth noting that Ferris *et al.* (26) have postulated that modulation of InsP_3-induced calcium release by ATP could provide a signaling mechanism between Ca^{2+}-ATPase and InsP_3 receptor.

We have documented the presence of functional InsP_3 receptor on rat liver nuclei. This is in accord with the immunocytochemical localization of this receptor (14) on the nuclei of Purkinje cells. This raises the question of the role of this receptor. Intranuclear free calcium concentrations appear to be regulated independently of cytoplasmic calcium (27). Electrical stimulation acting as a signal to nuclear calcium release has been seen in a voltage-clamped vertebrate neuron (28). In adrenal chromaffin cells an agonist-induced calcium increase is localized in the nuclear region (29). One possible role for the nuclear InsP_3 receptor lies in the regulation of nuclear calcium transients. Potassium-selective ion channels have been described in the nuclear envelope (30). They may participate in InsP_3-controlled calcium movement as potassium channel blockers inhibit InsP_3-induced calcium release (31) from brain microsomes.

As to the physiological role of nuclear calcium movements, one may speculate on their involvement in phosphorylation of certain nuclear proteins, including the proteins implicated in gene transcription, by calcium-activated protein kinases, such as protein kinase C isozyme type II (21). Therefore, it is tempting to postulate that InsP_3 receptors may play a key role in the coupling between cytoplasmic second messenger and nuclear third messenger (32) triggered events.

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