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Specific Binding Sites for Inositolhexakisphosphate in Brain and Anterior Pituitary

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SUMMARY

[^{3}H]Inositolhexakisphosphate ($InsP_6$) binds to a single population of specific and saturable recognition sites in membranes prepared from cerebral hemispheres, anterior pituitaries, or cultured cerebellar neurons. Binding is temperature and pH dependent, exhibits slow association and dissociation kinetics, and differentiates among various inositolpolyphosphates ($InsP_6$ is much more potent than inositol-1,3,4,5,6-pentakis- and inositol-1,3,4,5-tetrakisphosphate, whereas inositol-1,4,5-trisphosphate is inactive as a displacer). In membranes from cerebral hemispheres, saturation analysis reveals a K_D value of 33 ± 4 nM and a maximal density (B_{max}) of 152 ± 23 pmol/mg of protein. Both affinity and density of [^{3}H]$InsP_6$ binding are lower in membranes from anterior pituitaries. In cultured cerebellar neurons, micromolar concentrations of divalent cations enhance both [^{3}H]$InsP_6$ binding and $InsP_6$-stimulated $^{45}Ca^{2+}$ uptake, suggesting that activation of specific receptors may be involved in the stimulation of $^{45}Ca^{2+}$ uptake produced by $InsP_6$. These data support the recent view that $InsP_6$, as other inositolpolyphosphates, may act as a signal molecule in excitable cells.

Although $InsP_6$ occurs in large amounts in mammalian cells (1), its function is still unclear. Vallejo and co-workers (2, 3) have raised the possibility that $InsP_6$ acts as an extracellular signal stimulating specific groups of neurons in the central nervous system.

We have recently reported that addition of $InsP_6$ or $InsP_5$ enhances $^{45}Ca^{2+}$ uptake in primary cultures of cerebellar granule cells (4) or anterior pituicytes (5). In cerebellar neurons, this effect requires a certain degree of cell maturation and is accompanied by an increase in D-[^{3}H]aspartate release (4). Stimulation of $^{45}Ca^{2+}$ uptake by $InsP_6$ is not consequent to activation of the Na^+/Ca^{2+} antiporter and involves neither dihydropyridine-sensitive voltage-dependent Ca^{2+} channels nor large conductance ion channels linked to excitatory amino acid receptors (4, 5).

To explore the dynamics of interaction between $InsP_6$ and target cells, we have studied [^{3}H]$InsP_6$ binding in membranes prepared from rat cerebral hemispheres, cultured neurons, and anterior pituitaries.

Materials and Methods

Binding assay. [^{3}H]$InsP_6$ binding was studied in membranes prepared from cerebral hemispheres or anterior pituitaries of male adult Sprague Dawley rats (body weight, 200 ± 30 g). Tissue was homogenized in 10 volumes of 0.32 M sucrose and centrifuged at 500 × *g* for 20 min. The pellet was discarded and the supernatant was centrifuged at 10,000 × *g* for 30 min to obtain the crude mitochondrial pellet (P_2 fraction). The pellet was washed once in 10 mM Tris·HCl (pH 7.4) and suspended to a protein concentration of 0.02–0.15 mg in 0.05 ml for binding assays. In some experiments, the crude mitochondrial pellet was processed further to prepare subcellular fractions, as described by Gray and Whittaker (6). In brief, the pellet was washed once in water and layered on the top of a discontinuous sucrose gradient (0.8, 1.0, 1.2, and 1.4 M sucrose solutions). After centrifugation at 75,000 × *g* for 2 hr, the mitochondrial fraction was obtained at the bottom of the tube. The synaptosomal fraction was collected between 1.0 and 1.2 M sucrose, diluted 1:5 with Tris·HCl (10 mM, pH 7.4), and centrifuged at 50,000 × *g* for 1 hr. The whole synaptosomes were lysed by prolonged sonication (60 sec), followed by freezing and thawing. All the subcellular fractions were suspended in 10 mM Tris·HCl (pH 7.4) and used for binding assays. The binding assay was performed in a total volume of 0.5 ml of Tris buffer, containing 3 nM [^{3}H]$InsP_6$, 0.05 ml of membrane suspension, and various concentrations of unlabeled ligand. In saturation experiments, [^{3}H]$InsP_6$ concentrations ranged from 1 nM to 1 μM. In standard experiments, incubations were performed for 20 min at 37° and terminated by centrifugation (2 min in an Eppendorf microfuge at maximal speed). Pellets were rapidly washed with ice-cold buffer and dissolved in 0.5 N NaOH, and the radioactivity was measured by scintillation counting. Nonspecific binding was determined in the presence of 50 μM $InsP_6$.

To exclude the possibility that [^{3}H]$InsP_6$ could be metabolized during the binding assay, we have studied its elution profile from anion

ABBREVIATIONS: $InsP_6$, inositolhexakisphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; $InsP_5$, inositol-1,3,4,5,6-pentakisphosphate; $InsP_4$, inositol-1,3,4,5-tetrakisphosphate; $InsP_3$, inositol-1,4,5-trisphosphate; GABA, γ-aminobutyric acid.

exchange columns. Membranes from cerebral hemispheres were incubated in the presence of 3 nM [^{3}H]InsP$_6$ for 20 min at 37°. Incubations were terminated by the addition of 1.8 ml of chloroform/methanol/water (1:1:1). After phase separation, the aqueous phase was applied to Dowex 1-X-8 columns (formate form, 100–200 mesh) and eluted with ammonium formate buffer of increasing ionic strength (0.2–2 M), containing 0.1 M formic acid. [^{3}H]InsP$_6$ standard was added to an equal volume of chloroform/methanol/water and run in parallel. Six-milliliter fractions were collected and the radioactivity was counted after addition of scintillation fluid. [^{3}H]InsP$_6$ standard was eluted as a broad peak between 1.4 and 2 M ammonium formate. No radioactivity was collected with lower concentrations of ammonium formate, which are expected to elute less polar inositolphosphates. The elution profile of [^{3}H]InsP$_6$ was identical after incubation with membranes from cerebral hemispheres, suggesting that dephosphorylation products of [^{3}H]InsP$_6$ are not produced in significant amounts during the binding assay.

Cell culture studies. Primary cultures of cerebellar granule cells were prepared from 8-day-old rats, as described previously (7), and grown in 35- or 100-mm Nunc Petri dishes for the study of $^{45}Ca^{2+}$ uptake and [^{3}H]InsP$_6$ binding, respectively. After 7–9 days of maturation *in vitro*, these cultures contain more than 90% granule cells, 4–6% GABAergic interneurons, and a small amount (2–3%) of glial and endothelial cells as contaminants (7). Stimulation of $^{45}Ca^{2+}$ uptake was measured as described by Wroblewski *et al.* (8). Cultures at 9 days *in vitro* were washed twice with a salt-balanced buffer solution containing 154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 5.6 mM glucose, and 10 mM HEPES (pH 7.4) and were preincubated for 10 min at 37°. Then, 1 μCi of $^{45}CaCl_2$ (specific activity, 10 Ci/g; New England Nuclear) was added, and 10 min were allowed for the equilibration of the radioactive tracer into the various compartments. At the end of this preincubation, InsP$_6$ and/or divalent cations were added, and the incubation was continued for another 10 min. Uptake was terminated by rapid washing of the cultures with a solution containing 154 mM choline chloride, 2 mM EGTA, and 10 mM Tris·HCl (pH 7.35). The cells were dried and dissolved in 0.5 M NaOH, and aliquots were used for measurements of incorporated radioactivity and protein concentration. Values were always calculated without subtracting the amount of radioactivity accumulated during the preincubation and were expressed as nmol of $^{45}Ca^{2+}$ uptake/mg of protein.

In [^{3}H]InsP$_6$ binding studies, cultured cells at 9 days *in vitro* were washed twice with 0.32 M sucrose (10 ml/dish), scraped, and homogenized. Binding was assayed in the P_2 fraction, as described above, with 0.10 mg of membrane proteins incubated for 20 min at 37° in the presence of 3 nM [^{3}H]InsP$_6$.

Materials. InsP$_4$ (tetrapotassium salt) and InsP$_5$ (barium salt) were purchased from Calbiochem (San Diego, CA). InsP$_3$ was a generous gift from Dr. J. T. Wroblewski, Fidia Georgetown Institute for the Neurosciences, Georgetown University (Washington, DC). InsP$_6$ (Ca^{2+} salt; 2 Ca^{2+} and 1 H_2O/mol) and all other chemicals were purchased from Sigma (St. Louis, MO). InsP$_6$, InsP$_4$, and InsP$_3$ were soluble in water after agitation. InsP$_5$ (barium salt) was soluble in 0.01 M HCl at the initial concentration of 10 mM. All the inositolpolyphosphates were soluble in buffer at concentrations lower than 0.1 mM. [^{3}H]InsP$_6$ (specific activity 12 Ci/mmol) was a generous gift from NEN-Du Pont.

Results

[^{3}H]InsP$_6$ bound to specific and saturable recognition sites in membranes prepared from cerebral hemispheres, anterior pituitaries, and cultured cerebellar neurons. Specific binding sites for [^{3}H]InsP$_6$ were present in high density in all subcellular fractions, including synaptosomes, mitochondria, and myelin (Table 1). Crude mitochondrial membranes from cerebral hemispheres were used for binding characterization. In a typical binding assay (3 nM [^{3}H]InsP$_6$; 20 min of incubation at 37°), total binding varied from 10 to 60% of the total radioactivity, as a function of protein concentration (from 0.02 to 0.150 mg of membrane proteins). Nonspecific binding was always lower than 5%. Binding was not saturable at protein concentrations higher than 0.2 mg/0.5 ml. [^{3}H]InsP$_6$ binding was maximal at pH 6 to 7.4 and decreased dramatically with pH variations between 7.4 and 8 (Fig. 1). In addition, binding did not change when incubations were performed at 18° but was reduced by more than 80% at 4° (Table 2). Specific binding was no longer detectable when membrane proteins were denaturated (10 min at 100°) before the assay (specific binding = 2400 ± 330 and 120 ± 70 cpm in control and heat-treated membranes, respectively; three experiments).

TABLE 1

[^{3}H]InsP$_6$ binding in subcellular fractions from cerebral hemispheres

Values are means ± standard errors of six determinations from two separate experiments performed in triplicate. [^{3}H]InsP$_6$ concentration = 1 nM. Nonspecific binding was determined in the presence of 50 μM InsP$_6$. Numbers in parentheses, percentage of total binding.

	[^{3}H]InsP$_6$ binding		
	Total	Nonspecific	Specific
	(cpm/mg of protein) × 10^{-2}		
Crude mitochondrial pellet	250 ± 1	9 ± 1.4	241 (96%)
Mitochondrial fraction	900 ± 33	60 ± 4.4	840 (93%)
Myelin fraction	570 ± 60	64 ± 10	506 (90%)
Synaptosomal fraction	560 ± 15	190 ± 22	370 (66%)
Lysed synaptosomes	350 ± 4.2	130 ± 45	220 (63%)

The association of [^{3}H]InsP$_6$ to its binding sites was relatively slow. Binding reached plateau values after 20 min, although half-maximal binding was present after less than 2 min (Fig. 2B). To study the dissociation rate, membranes were incubated with [^{3}H]InsP$_6$ for 20 min, and dissociation was initiated by the addition of 50 μM unlabeled InsP$_6$. About 50% of [^{3}H]InsP$_6$ binding was dissociated after 10 min and more than 80% after 20 min (Fig. 2B).

The [^{3}H]InsP$_6$ binding site differentiated among inositolpolyphosphates and was selective for InsP$_6$. InsP$_4$ and InsP$_5$ were weak competitors of [^{3}H]InsP$_6$ binding ($IC_{50} > 5$ μM), whereas InsP$_3$, ATP, fructose-1,6-bisphosphate, and glutamate were virtually inactive as displacers (Fig. 3).

Saturation analysis revealed a single population of high affinity binding sites (K_D = 33 ± 4 nM) with high capacity (B_{max} = 152 ± 23 pmol/mg of protein; three experiments) (see Fig. 2A). Binding was still saturable in the presence of the Ca^{2+}-chelator EGTA (1 mM). EGTA increased total binding by about 2-fold but slightly reduced the affinity of the binding site for InsP$_6$ as a displacer (Fig. 4).

In membranes from anterior pituitaries, [^{3}H]InsP$_6$ binding sites exhibited lower affinity and density (K_D = 91 ± 6 nM; B_{max} = 40 ± 9 pmol/mg of protein; three experiments), as compared with membranes from cerebral hemispheres.

In all tissue preparations, [^{3}H]InsP$_6$ binding was insensitive to Na^+ ions (154 mM as NaCl) but was no longer saturable in the presence of 1 mM $CaCl_2$. Under these conditions, the amount of bound [^{3}H]InsP$_6$ was even greater in the presence of an excess of unlabeled InsP$_6$ (Table 3). Micromolar concentrations of various divalent cations (including Mg^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+}) increased specific [^{3}H]InsP$_6$ binding in membranes prepared from cerebral hemispheres or cultured cerebellar neurons. Interestingly, this effect exhibited a high degree of correlation with the ability of divalent cations to potentiate InsP$_6$-stimulated $^{45}Ca^{2+}$ uptake in cultured cerebellar neurons (Fig. 5).

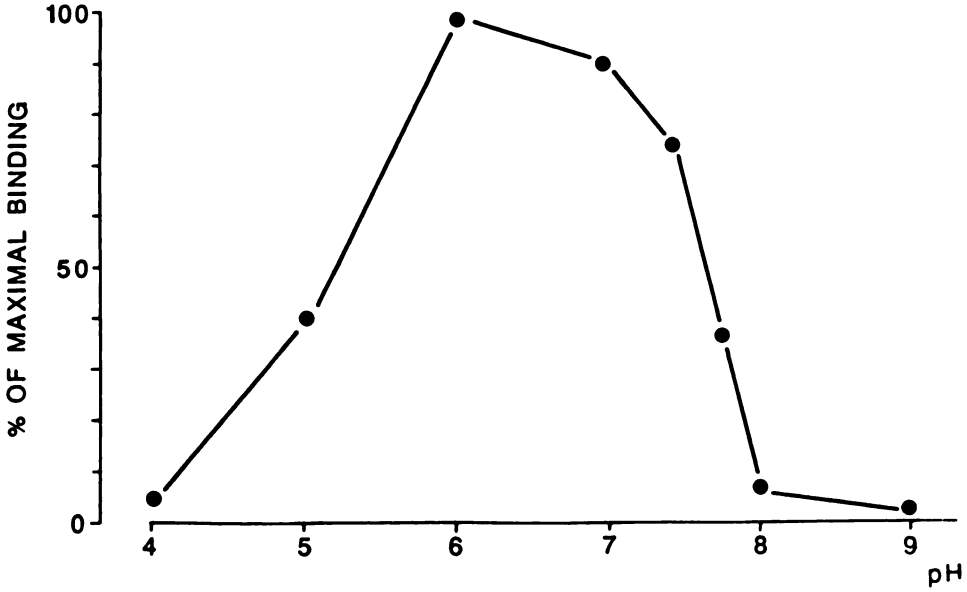

Fig. 1. pH dependency of [^{3}H]InsP$_6$ binding in membranes from cerebral hemispheres. Maximal binding (at pH 6) was 32,000 ± 4,500 cpm/mg of protein. Nonspecific binding was 2,800 ± 550 at pH 6 and did not change significantly at different pH values.

TABLE 2

Temperature dependence of [^{3}H]InsP$_6$ binding

Values are means ± standard errors of six determinations from two separate experiments performed in triplicate.

Temperature	Specific [^{3}H]InsP$_6$ binding
	cpm/mg of protein
0°	3,500 ± 120
18°	15,000 ± 1,300
37°	17,000 ± 850

Discussion

InsP$_6$ is present in large amounts in mammalian tissues (1) and has been found in a variety of cultured cells, including neuronal cell lines (9). Despite the widespread distribution of InsP$_6$, its source of synthesis and mechanism of action are largely unknown. We now report that InsP$_6$ binds to specific high affinity recognition sites in membranes prepared from cerebral hemispheres, anterior pituitaries, or cultured cerebellar neurons. Because InsP$_6$ may chelate divalent cations, the high affinity binding we have found might reflect the interaction of [^{3}H]InsP$_6$ with the Ca^{2+} present in the membrane preparations. However, this seems unlikely, inasmuch as [^{3}H]InsP$_6$ binding is abolished after heat treatment and is still saturable in the presence of the Ca^{2+}-chelator EGTA. It might be argued that less polar inositolpolyphosphates, present as contaminants or produced by [^{3}H]InsP$_6$ dephosphorylation, bind to the membranes, simulating a real [^{3}H]InsP$_6$ binding. One candidate is InsP$_3$ which is known to interact with specific binding sites in rat brain membrane preparations (10–14). However, [^{3}H]InsP$_6$ and [^{3}H]InsP$_3$ binding differ in a variety of factors, including pH and temperature dependency, association and dissociation kinetics, and displacement specificity. Accordingly, [^{3}H]InsP$_6$ binding exhibits optimum pH range between 6 and 7.4, has slow association and dissociation rates, and is reduced at low temperature; in contrast, [^{3}H]InsP$_3$ binding is enhanced by about 3-fold at basic pH, exhibits rapid association and dissociation kinetics, and is still maximal at 4° (11). In addition, specifically bound [^{3}H]InsP$_6$ is not displaced by InsP$_3$, whereas InsP$_4$ and InsP$_5$ are weak competitors. Although this confers specificity to [^{3}H]InsP$_6$ binding, we cannot exclude the possibility that other positional isomers of InsP$_3$, InsP$_4$, and InsP$_5$ contribute to the specific binding in our study.

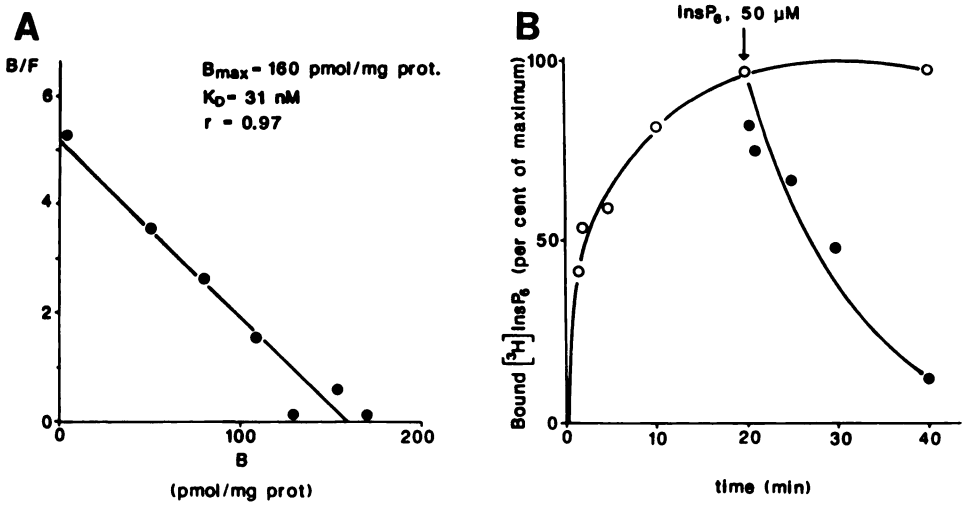

Fig. 2. [^{3}H]InsP$_6$ binding in membranes prepared from cerebral hemispheres. A, Scatchard analysis of the saturation isotherm from a representative experiment. The saturation curve was performed with eight points in a range of concentrations between 1 nM and 3 μM. B, Association (○) and dissociation (●) of [^{3}H]InsP$_6$ binding expressed as a function of time. Dissociation was initiated by addition of 50 μM InsP$_6$ (*arrow*). Values are expressed as percentage of specific binding and were calculated from the mean of three separate experiments.

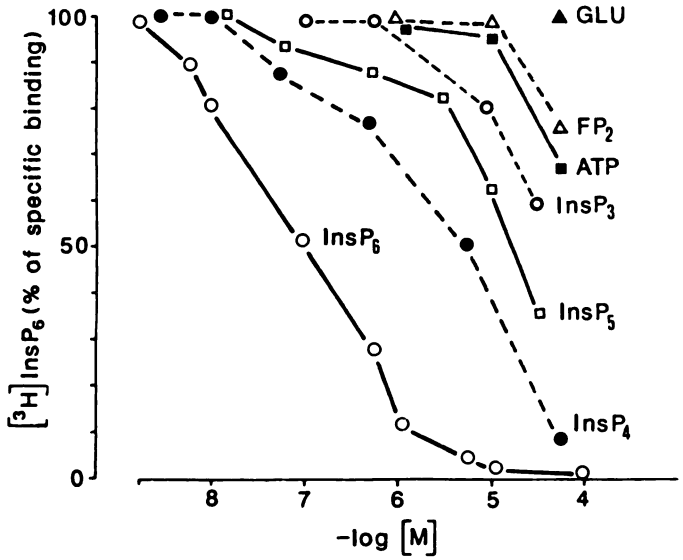

Fig. 3. Concentration-dependent inhibition of [^{3}H]$InsP_6$ binding by $InsP_6$, $InsP_5$, and $InsP_4$ in membranes from cerebral hemispheres. Data were calculated from the mean of three independent experiments performed in triplicate. Binding was assayed for 20 min at 37°, as described in text. The rank order of potency for $InsP_6$, $InsP_5$, and $InsP_4$ in displacing [^{3}H]$InsP_6$ binding was similar in membranes prepared from cultured cerebellar neurons and from anterior pituitaries. *GLU*, glutamate; *FP_2* fructose-1,6-bisphosphate.

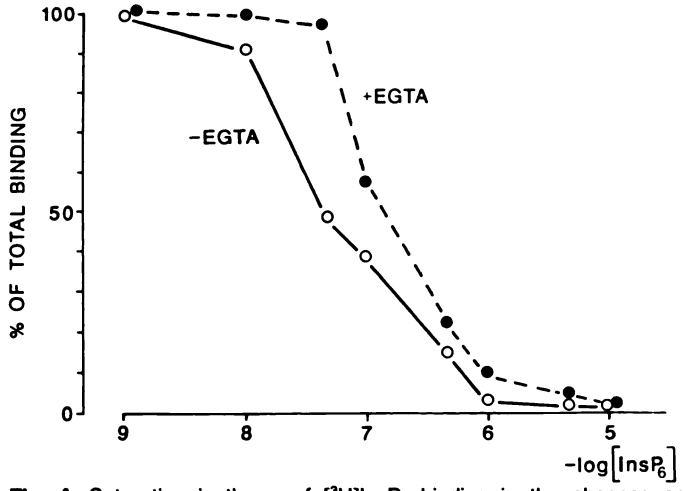

Fig. 4. Saturation isotherm of [^{3}H]$InsP_6$ binding in the absence or presence of 1 mM EGTA. Total binding was 26,000 ± 1,800 in the absence and 60,000 ± 8,500 cpm/mg of protein in the presence of 1 mM EGTA.

TABLE 3

[^{3}H]$InsP_6$ binding in membranes from cerebral hemispheres and anterior pituitaries incubated in the presence of 1 mM Ca^{2+}

Binding was assayed in the crude mitochondrial pellet (P_2 fraction) from cerebral hemispheres and anterior pituitaries. Membranes were incubated at 37° for 20 min, in the presence of 3 nM [^{3}H]$InsP_6$. Values are means ± standard errors of six determinations from two separate experiments performed in triplicate.

	[^{3}H]$InsP_6$ binding			
	Cerebral hemispheres		Anterior pituitaries	
	None	1 mM Ca^{2+}	None	1 mM Ca^{2+}
	(cpm/mg of protein) × 10^{-2}			
Total	270 ± 22	380 ± 7.3	50 ± 3.5	65 ± 3.8
$InsP_6$ (50 μM)	18 ± 2.4	730 ± 24	22 ± 1.6	210 ± 45

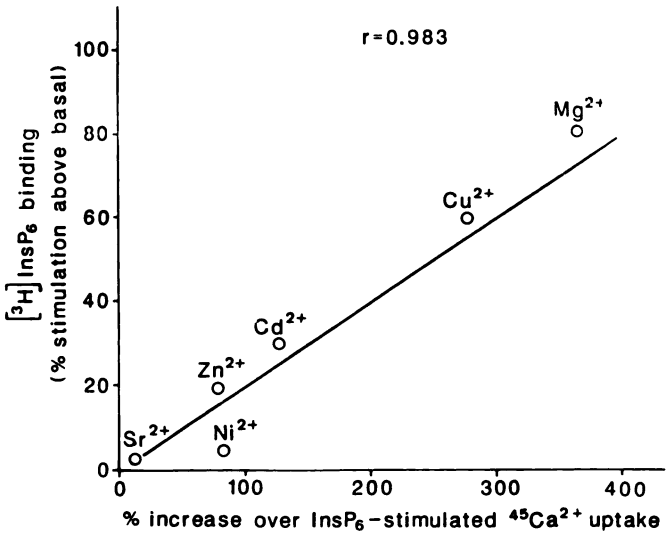

Fig. 5. Micromolar concentrations of divalent cations enhance both [^{3}H]$InsP_6$ binding and $InsP_6$-stimulated $^{45}Ca^{2+}$ influx in cultured cerebellar granule cells. $^{45}Ca^{2+}$ uptake was stimulated by maximal concentrations of $InsP_6$ (50 μM) (for a detailed characterization of $InsP_6$-stimulated $^{45}Ca^{2+}$ uptake, see Ref. 4). Binding assay was performed in granule cell membranes incubated for 2 min at 37° in the presence of 3 nM [^{3}H]$InsP_6$. The final concentration of divalent cations was 25 μM in both experiments. At this concentration, none of the cations modified basal $^{45}Ca^{2+}$ uptake or nonspecific [^{3}H]$InsP_6$ binding. Values are expressed as percentage of $InsP_6$-stimulated uptake and specific [^{3}H]$InsP_6$ binding and were calculated from the mean of 9–15 individual determinations from three to five separate experiments performed in triplicate. The standard deviation was always less than 10% of the mean value. Mean ± SE of basal and $InsP_6$-stimulated $^{45}Ca^{2+}$ uptake was 11 ± 2.4 and 30 ± 1.5 nmol of $^{45}Ca^{2+}$/mg protein, respectively. Total [^{3}H]$InsP_6$ binding was 18,000 ± 1,500 cpm/mg of protein. Nonspecific binding (as determined in the presence of 50 μM $InsP_6$) was 1,400 ± 210 cpm/mg of protein.

$InsP_6$ is dephosphorylated by a specific myoinositolhexakisphosphate phosphohydrolase (EC 3.1.3.8), designated as phytase (15). Although [^{3}H]$InsP_6$ might bind with high affinity to a membrane-bound phytase, this seems unlikely for the following reasons: (i) incubation with membranes under conditions that favor the enzymatic activity (37°) is not followed by the formation of metabolites in appreciable amounts (as assessed by anion exchange chromatography); in addition, binding is not reduced at incubation times as long as 40 min, suggesting that the [^{3}H]$InsP_6$ specifically bound to the membranes is not enzymatically degraded; (ii) the affinity of $InsP_6$ for the binding site characterized here is about 30-fold greater than the half-saturating concentrations (K_m) of $InsP_6$ for the phytase (16); and (iii) the affinity of [^{3}H]$InsP_6$ binding is only slightly reduced by EGTA, whereas phytase activity from different sources is inhibited in the presence of Ca^{2+}-chelating agents (15, 17).

Interestingly, total [^{3}H]$InsP_6$ binding was increased when 1 mM Ca^{2+} was present in the incubation assay. Under these conditions, the amount of [^{3}H]$InsP_6$ bound to the membranes was even greater in the presence of an excess of unlabeled $InsP_6$. This may suggest that low affinity binding sites with large capacity are unmasked by millimolar concentrations of Ca^{2+}. However, we cannot exclude the possibility that the increased amount of bound radioactivity found in the presence of 1 mM Ca^{2+} and saturating concentrations of $InsP_6$ reflects the formation of $InsP_6$-Ca^{2+} complexes that may precipitate in the membrane due to their low solubility at neutral pH.

Recent evidence suggests that $InsP_6$ acts extracellularly as a potent neural stimulant in the central nervous system. When microinfused into the nucleus tractus solitarius or iontophoretically applied to the dorsal horn of the spinal cord, $InsP_6$ mimics the action of the excitatory transmitter glutamate (2, 3). As an extension of these original contributions, we have shown that addition of $InsP_6$ to cultured cerebellar neurons stimulates $^{45}Ca^{2+}$ uptake and D-[^{3}H]aspartate release (4). To support a functional role for $InsP_6$ binding sites, we have attempted to correlate [^{3}H]$InsP_6$ binding and $InsP_6$-stimulated $^{45}Ca^{2+}$ uptake in cultured cerebellar neurons. Stimulation of $^{45}Ca^{2+}$ uptake by various inositolpolyphosphates correlates roughly with their ability to displace specifically bound [^{3}H]$InsP_6$. Like $InsP_6$, $InsP_5$ stimulates $^{45}Ca^{2+}$ uptake (albeit to a lesser extent), whereas $InsP_4$ is inactive (4). This suggests that, although $InsP_4$ may interact with the $InsP_6$ recognition site, it is devoid of intrinsic activity in stimulating $^{45}Ca^{2+}$ uptake. Both [^{3}H]$InsP_6$ specific binding and $InsP_6$-stimulated $^{45}Ca^{2+}$ uptake are potentiated by micromolar concentrations of divalent cations with a high degree of correlation. Hence, we suggest that stimulation of $^{45}Ca^{2+}$ uptake follows the interaction between $InsP_6$ and specific high affinity recognition sites present in the membrane. However, a major reservation concerns the difference between the K_D of [^{3}H]$InsP_6$ binding (30 nM) and the EC_{50} value of $InsP_6$ relative to the stimulation of $^{45}Ca^{2+}$ uptake (about 30 μM) (4, 5). The low potency of $InsP_6$ in stimulating $^{45}Ca^{2+}$ uptake may be due to the presence of millimolar concentrations of Ca^{2+}, which may uncover a subpopulation of low affinity binding sites, as discussed above. An accurate estimation of the effects of Ca^{2+} on [^{3}H]$InsP_6$ binding is hampered by the low solubility of $InsP_6$ at concentrations higher than 0.1 mM in the presence of Ca^{2+}. Alternatively, stimulation by nanomolar concentrations of $InsP_6$ may not be visible, due to the low sensitivity of $^{45}Ca^{2+}$ uptake as a method to study the influx of extracellular Ca^{2+}. Accordingly, using a fluorescent probe, Law *et al.* (18) found that 1 nM $InsP_6$ causes a rapid rise in intracellular Ca^{2+} and prolactin secretion in perifused anterior pituitary cells.

In conclusion, the discovery of high affinity membrane binding sites supports the view that $InsP_6$ serves as a signal molecule in excitable cells (2, 3, 19, 20). The presence of binding sites in the mitochondrial fraction raises the possibility that $InsP_6$ acts also as an intracellular regulator of Ca^{2+} homeostasis, as reported for $InsP_3$ (reviewed in Ref. 21) and recently also for $InsP_4$ (22–25). It is possible that $InsP_6$, produced inside the cell, is compartmentalized into different pools, each with its own specific functions (such as cell to cell communication, regulation of intracellular Ca^{2+}, etc.). Further clarification of the route of $InsP_6$ synthesis and its possible relation to inositol phospholipid hydrolysis will be helpful in defining the role of $InsP_6$ as a signal molecule in mammalian cells.

Acknowledgments

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