The Isolation and Characterization of Inositol Polyphosphate 4-
Phosphatase*

Vinay S. Bansal, Kevin K. Caldwell, and Philip W. Majerus‡

From the Division of Hematology-Oncology, Departments of Internal Medicine and Biological Chemistry, Washington University
School of Medicine, St. Louis, Missouri 63110

A widely distributed response of cells to hormones, growth
factors, and other extracellular agonists is a rapid receptor-
mediated hydrolysis of inositol phospholipids to produce sev-
reral messenger molecules (1-4). The metabolism of these
noncyclic inositol phosphates (4,5).

We previously identified an alternative pathway for the
metabolism of inositol 1,3,4-trisphosphate (Ins(1,3,4)P3) in calf brain. The enzyme responsible for the degradation of Ins(1,3,4)P3 was designated as
inositol polyphosphate 4-phosphatase (Bansal, V. S.,
Inhorn, R. C., and Majerus, P. W. (1987) J. Biol. Chem.
262, 9644-9647). We have now purified this enzyme
3390-fold from calf brain-soluble fraction. The iso-
lated enzyme has an apparent molecular mass of 110
kDa as determined by gel filtration. On sodium dodecyl
sulfate-polyacrylamide gel electrophoresis, the enzyme
migrates as a protein of 105 kDa, suggesting that it is
monomeric. Among various 4-phosphate-containing
inositol polyphosphates, the enzyme hydrolyzes only
Ins(1,3,4)P3 and inositol 3,4-bisphosphate (Ins(3,4)P2),
yielding inositol 1,3-bisphosphate and inositol 3-phos-
apate as products. The inositol polyphosphate 4-phos-
phatase has apparent K_m values of 40 and 25 μM for
Ins(1,3,4)P3 and Ins(3,4)P2, respectively. The maxi-
mum velocities for these two substrates are 15–20 nmol
of product/min/mg protein. Ins(1,3,4)P3 is a competi-
tive inhibitor of Ins(3,4)P2 hydrolysis with an apparent
K_i of 27 μM implying that the same active site is in-
volved in hydrolysis of both substrates. The final en-
zeyme preparation retained a small inositol polyphos-
phate 3-phosphatase activity (<2% of rate of inositol
polyphosphate 4-phosphatase activity) which most
likely reflects a contaminant. The enzyme displays
maximum activity between pH 6.5 and 7.5. It is not
inhibited by Li^+, Ca^{2+}, or Mg^{2+} except at 10 mM diva-
thent ions. Mn^{2+} inhibits enzyme at high concentrations
IC_50 = 1.5 mM.

EXPERIMENTAL PROCEDURES

Materials

\[^{[3]H}]\text{Ins}(1,3,4,5)P_4 (5 \text{ Ci/mmol}), [^{[3]H}]\text{Ins}(1,4,5)P_3 (3.6 \text{ Ci/mmol}) ,
\[^{[3]H}]\text{Ins}(1,3,4)P_3 (17 \text{ Ci/mmol}), [^{[3]H}]\text{Ins}(1,4)P_2 (15 \text{ Ci/mmol}) \text{ were obtained from Du Pont-New England Nuclear. Unlabeled }
\text{Ins}(1,3,4,5)P_4, \text{ Ins}(1,4,5)P_3, \text{ and } \text{Ins}(1,3,4)P_3 \text{ were purchased from Calbiochem. Ins}(1,4)P_2, \text{ phosphocellulose, and DEAE-Sephrose CL-}
6B were from Sigma. The Mono S (10/10) FPLC column was from Pharmacia LKB Biotechnology Inc. Bio-Gel HTP-hydroxyapatite,
Dowex AG 1- X8 (formate form), polyacrylamide electrophoresis mater-
ials, Bio-Gel TSK HPLC gel filtration columns, Bio-Gel TSK

The abbreviations used are: \text{Ins}(1,4,5)P_3, \text{ inositol 1,4,5-trisphos-
phate}; \text{Ins}(1,3,4)P_3, \text{ inositol 1,3,4-trisphosphate}; \text{Ins}(1,3,4,6)P_4, \text{ inositol 1,3,4,6-tetra-
kisphosphate}; \text{Ins}(1,4,5)P_3, \text{ inositol 1,4,5-bisphosphate}; \text{Ins}(3,4)P_2, \text{ ino-
sitol 3,4-bisphosphate}; \text{Ins}(1,3)P_2, \text{ inositol 1,3-bisphosphate}; \text{Ins}(4)P, \text{ inositol 4 }
phosphate; \text{Ins}(3)P, \text{ inositol 3 phosphate}; \text{Ins}(1)P, \text{ inositol 1-phosphate}; \text{HPLC, high perform-
ance liquid chromatography}; \text{MES}, \text{ 2(N-morpholino)ethanesulfonic acid}; \text{EGTA, [ethylenebis(oxy-
ethylenenitrilo)tetraacetic acid}; ETDMA, ethylenediaminetetraacetic
acid; FPLC, fast protein liquid chromatography; HEPES, 4-(2-hy-
droxyethyl)piperazineethanesulfonic acid.

* This research was supported by Grants 14147 (Specialized Center
for Research in Thrombosis), 16634, and Training &ant 07088 from
the National Heart, Lung, and Blood Institute. The costs of publi-

† To whom correspondence should be addressed.

1806
Inositol Polyphosphate 4-Phosphatase

DEAE 5PW preparative columns, and protein molecular weight standards were from Bio-Rad. Calf brains were obtained from Pel Freez. All other materials were from Sigma or Fisher.

**Preparation of Radiolabeled Inositol Phosphates**

\[ [\text{H}]\text{Ins}(3,4)P_2 (4000 \text{ cpm/nmol}) \text{ and } [\text{H}]\text{Ins}(3,4)P_3 (17 \times 10^3 \text{ cpm/nmol or relatively unlabeled 36 kDa/nmol}] \] were prepared by incubating \([\text{H}]\text{Ins}(1,4)P_2\) and \([\text{H}]\text{Ins}(1,3,4)P_3\) with purified inositol 4-phosphatase and inositol polyphosphate 3-phosphatase as described previously (23). \([\text{H}]\text{Ins}(1,3)P_2(17 \times 10^3 \text{ cpm/nmol}) \text{ and } \text{Ins}(3,4)P_2(40 \text{ kDa/nmol}) \] were prepared from \(\text{Ins}(1,4)P_2\) by using purified inositol 4-phosphatase and inositol polyphosphate 3-phosphatase from calf brain as described previously (20). The inositol polyphosphate 4-phosphatase was heated for 10 min at 50°C to inactivate contaminating inositol 3-phosphatase before use. In each case, substrates were completely converted to inositol bisphosphates (i.e. \(\text{Ins}(1,3)P_2\) or \(\text{Ins}(3,4)P_2\)). The purity of the products was confirmed by Dowex formate chromatography and by HPLC using a Whatman 10 Partisil SAX analytical column (19, 20).

**Determination of Inositol Polyphosphate 4-Phosphatase and Inositol Polyphosphate 3-Phosphatase Activities**

**Assay 1**—The rate of hydrolysis of \(\text{Ins}(1,3,4)P_3\) was determined by incubating \([\text{H}]\text{Ins}(1,3,4)P_3\) (10-250 \(\mu\)M, 500-1000 cpm/nmol) in 25 \(\mu\)l containing 50 mM MES (pH 6.5), 5 mM EDTA, and an amount of enzyme that gave less than 40% (<10% in kinetic experiments) hydrolysis of substrate in a 10-min reaction at 37°C. Reactions were stopped by dilution to 1 ml with cold water and poured onto a 0.4-ml Dowex formate column. The product, \([\text{H}]\text{Ins}(3,4)P_2\), was eluted with 8 ml of 0.4 M NaH_2CO_3, 0.1 M HCOOH, and radioactivity was measured by liquid scintillation spectrometry.

**Assay 2**—Inositol polyphosphate 4-phosphatase was incubated with \([\text{H}]\text{Ins}(3,4)P_2\) (5-100 \(\mu\)M, 500-1000 cpm/nmol) as described above. The reaction was stopped with 1 ml of cold water and applied to a 0.4-ml Dowex formate column. The product, \([\text{H}]\text{Ins}(3,4)P_2\), was eluted with 6 ml of 0.2 M NaH_2CO_3, 0.1 M HCOOH, and radioactivity was measured as described above. No \(\text{Ins}(3,4)P_2\) was observed under these conditions.

**Assay 3**—Inositol polyphosphate 3-phosphatase activity was measured by incubating calf brain supernatant or purified inositol polyphosphate 4-phosphatase in 25 \(\mu\)l containing \([\text{H}]\text{Ins}(1,3)P_2\) (40 \(\mu\)M, 1700 cpm/nmol) with 50 mM MES (pH 6.5), 5 mM EDTA for 10 min at 37°C. The product, \([\text{H}]\text{Ins}(1,3)P_2\), was separated from \([\text{H}]\text{Ins}(3,4)P_2\) by the procedure described under "Assay 2."

**Preparation of Calf Brain Homogenates**

Frozen calf brains were shredded in a commercial vegetable shredder and then homogenized in 20 mM HEPES (pH 7.5), 10 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.25% sucrose, and 10 mM \(\beta\)-mercaptoethanol at 1:2.5 (w/v) using a Polytron homogenizer. Homogenates were centrifuged at 30,000 \(\times\) g for 30 min, and the crude supernatant fraction was recentrifuged at 49,000 \(\times\) g for 1 h. The pellet was discarded, and the supernatant solution was used for purification as described below.

**Purification of Inositol Polyphosphate 4-Phosphatase**

The purification was carried out at 4°C starting with calf brains.

**DEAE-Sepharose**—A DEAE-Sepharose CL-6B column (10 x 23 cm) was equilibrated with 200 mM HEPES (pH 7.5) and 2 mM EGTA and then re-equilibrated with Buffer A (20 mM HEPES (pH 7.5), 2 mM EGTA, and 10 mM \(\beta\)-mercaptoethanol). The supernatant solution (3.7 liters) was passed onto the column at a flow rate of 5 ml/min. The column was washed with 5 volumes of Buffer A and then eluted with linear gradient of 0-0.4 M NaCl in the same buffer. Enzyme-containing fractions were pooled, solid ammonium sulfate was added to 55% saturation, and the mixture was stirred overnight at 4°C. The suspension was centrifuged for 30 min at 30,000 \(\times\) g. The supernatant was discarded, and the pellet was suspended in a minimal volume of Buffer A and dialyzed against 2 x 4 liters of buffer. After dialysis, a precipitate formed which was removed by centrifugation for 30 min at 40,000 \(\times\) g.

**HPLC-DEAE**—The dialyzed sample was applied to a Bio-Gel TSK DEAE 5-PW (55 x 200 mm) column previously equilibrated with Buffer B (20 mM HEPES (pH 6.0), 2 mM EGTA, 10 mM \(\beta\)-mercaptoethanol), at a flow rate of 10 ml/min. The column was washed with 2 column volumes of Buffer B containing 30 mM NaCl. Enzyme activity was eluted with a linear gradient (4 liters) of 30-175 mM NaCl in Buffer B at a flow rate of 10 ml/min followed by 1 liter each of Buffer B containing 175 mM NaCl and 1 M NaCl, respectively. Enzyme-containing fractions were pooled and concentrated by precipitation in 55% ammonium sulfate as described above. The pellet was suspended in Buffer A and dialyzed against 4 liters of the same buffer overnight. A precipitate was removed by centrifugation for 30 min at 40,000 \(\times\) g, and the clear supernatant solution was applied to phosphocellulose.

**Phosphocellulose Chromatography**—The inositol polyphosphate 4-phosphatase activity was applied at 1 ml/min to a 3 x 5-cm column of phosphocellulose, equilibrated with Buffer A, and subsequently eluted with a 250-ml linear gradient from 0 to 400 mM NaCl in Buffer A. Enzyme-containing fractions were pooled, concentrated by ammonium sulfate precipitation, and dialyzed in Buffer A as described above.

**Mono S Chromatography**—The enzyme activity from phosphocellulose was applied at a flow rate of 4 ml/min to a Pharmacia Mono S 10/10 FPLC column equilibrated with Buffer A. The column was washed with 40 ml of Buffer A and eluted with a 140-ml linear 0-43 mM NaCl gradient in the same buffer. The fractions containing enzyme activity were pooled, concentrated by ammonium sulfate precipitation, and dialyzed as described above.

**Gel Filtration Chromatography**—The pooled concentrated enzyme from Mono S was applied to three Bio-Gel TSK 250 HPLC gel filtration columns (60 x 7.5 mm) connected in series and equilibrated with Buffer C (20 mM HEPES (pH 7.5), 2 mM EGTA, and 0.15 M NaCl). The columns were eluted with the same buffer at a flow rate of 0.5 ml/min. Enzyme-containing fractions were pooled and concentrated to 400 \(\mu\)l in a Micro-Pro DiCon concentrator using Buffer C containing 10% glycerol and stored at -80°C.

**Hydroxylapatite Chromatography**—The purification scheme for 4-phosphatase was modified in some cases by introducing hydroxylapatite chromatography after the Mono S step in order to remove contaminating inositol phosphatase 3-phosphatase activity. The hydroxylapatite column (1 x 5 cm) was equilibrated in Buffer A containing 2 mM sodium phosphate buffer (pH 7.5) (Buffer D). The sample was applied at a flow rate of 0.2 ml/min, then the column was washed with 2 column volumes of the same buffer, and the inositol polyphosphate 4-phosphatase activity was eluted at a 25-linear gradient 2-900 mM phosphate (pH 7.5) in Buffer A. The enzyme-containing fractions were pooled and concentrated to a min in a Micro-Pro DiCon concentrator using Buffer C containing 10% glycerol.

**Miscellaneous Methods**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (24). Protein concentrations were determined using the Bio-Rad protein assay with bovine serum albumin as standard.

**RESULTS**

We purified inositol polyphosphate 4-phosphatase from the soluble fraction of calf brain which contains 60-75% of the total 4-phosphatase activity in this tissue. After the initial DEAE-Sepharose chromatography the enzyme was chromatographed on a DEAE-HPLC column as shown in Fig. 1. The active fractions from this column were pooled, further purified by ammonium sulfate precipitation, chromatographed on phosphocellulose, and then on Mono S as shown in Fig. 2. We obtained a sharp peak of inositol polyphosphate 4-phosphatase activity; however, this peak did not correspond to the protein peak. The inositol polyphosphate 4-phosphatase was further purified by gel filtration as shown in Fig. 3. The gel filtration profile shows a major peak of contaminating protein eluting just before inositol polyphosphate 4-phosphatase activity, but there was a small peak of protein that did correspond to the enzyme-containing fractions. The inositol polyphosphate 4-phosphatase had an apparent molecular mass of 110 kDa. A sodium dodecyl sulfate-polyacrylamide gel of fractions containing enzyme activity is shown in Fig. 4A. Numerous proteins are present, but there is good correlation between the staining intensity of a protein migrating at ap-
FIG. 1. DEAE-HPLC anion exchange chromatography of inositol polyphosphate 4-phosphatase. 78 ml of partially purified inositol polyphosphate 4-phosphatase from calf brain supernatant was applied to a Bio-Gel TSK DEAE 5-PW (55 x 200 mm) column and eluted with a 4-liter linear gradient (0-0.175 M NaCl). The flow rate was 10 ml/min, and 20-ml fractions were collected. Enzyme activity was estimated using Ins(1,3,4)P₃ (40 μM) as described under "Assay 1." %B (- - -) refers to proportion of eluant containing 1 M NaCl.

proximately 105 kDa and the profile of enzyme activity in each fraction. Another protein migrating at 79 kDa partially correlated with enzyme activity but seemed unlikely to be the inositol polyphosphate 4-phosphatase, since it appeared to be absent in fractions 82 and 83. In order to establish further that the protein migrating at approximately 105 kDa represents the enzyme, we repeated the purification and added hydroxylapatite chromatography before the gel filtration step. Although this additional procedure did not yield material of higher specific activity, it did remove the 79-kDa protein as shown in Fig. 4B. In this case, the major contaminant is a 70-kDa protein not present in enzyme-containing fractions of the preparation shown in Fig. 4A. We have carried out this purification five times, and in each case the protein of 105 kDa corresponds to enzyme activity.

The molecular weight determined by gel filtration also agrees with that determined by sodium dodecyl sulfate gel

FIG. 3. Gel filtration chromatography of inositol polyphosphate 4-phosphatase. Sample (2.9 mg) from Mono S was applied onto a gel filtration column. The flow rate was 0.5 ml/min, and the fraction size was 0.5 ml. Enzyme activity was estimated using Assay 1. Elution positions of molecular mass standards are indicated by arrows: 1, bovine thyroglobulin (670 kDa); 2, bovine γ-globulin (158 kDa); 3, chicken ovalbumin (44 kDa); 4, horse myoglobin (17 kDa); 5, cyanocobalamin (1.3 kDa). The inset shows the relative elution positions of standards and inositol polyphosphate 4-phosphatase (indicated by arrow).
Inositol Polyphosphate 4-Phosphatase Activity in Inositol Polyphosphate 4-Phosphatase Preparation—When the final preparation was assayed for a prolonged time for inositol polyphosphate 4-phosphatase activity using Ins(1,3,4)P$_3$ as substrate, we observed both the formation of Ins(1,3)P$_2$ as well as Ins(1)P. This suggests inositol polyphosphate 3-phosphatase activity on the product of inositol polyphosphate 4-phosphatase. When assays were carried out using Ins(1,3)P$_3$ as substrate, we found 0.29 pmol of Ins(1,3)P$_2$ hydrolyzed/min/mg protein. The activity of inositol polyphosphate-3-phosphatase in crude calf brain supernatant is 2 nmol of Ins(1,3)P$_2$ hydrolyzed/min/mg protein. Thus inositol polyphosphate-3-phosphatase is purified about 150-fold. The ratio of inositol polyphosphate 4-phosphatase to inositol polyphosphate 3-phosphatase increases from 1.6 in the crude to 60 in the final preparation implying that inositol polyphosphate 3-phosphatase activity is most likely due to a contaminant protein and not an activity intrinsic to the inositol polyphosphate 4-phosphatase. This conclusion is supported further by heat inactivation experiments, where after 10 min at 50 °C 90% of inositol polyphosphate 3-phosphatase activity is lost while 50% of inositol polyphosphate 4-phosphatase remains.

Properties of Inositol Polyphosphate 4-Phosphatase—We assayed inositol polyphosphate 4-phosphatase for activity with the other 4-phosphate containing substrates as shown in Table II. Only Ins(1,3,4)P$_3$ and Ins(3,4)P$_2$ were hydrolyzed. The enzyme was not inhibited by 2 mM of other phosphate esters as shown in Table II.

The enzyme has optimum activity between pH 6.5 and 7.5. The effect of varying Ins(1,3,4)P$_3$ concentration on inositol polyphosphate 4-phosphatase is shown in Fig. 5. In the experiment shown, the apparent $K_m$ was 40 μM Ins(1,3,4)P$_3$, and the maximum velocity was 18 pmol of Ins(1,3,4)P$_3$ hydrolyzed/min/mg protein as determined by Lineweaver-Burk plot. In other experiments, the apparent $K_m$ for Ins(1,3,4)P$_3$ ranged from 35 to 48 μM. Hydrolysis of varying concentrations of Ins(3,4)P$_2$ showed an apparent $K_m$ of 25 μM Ins(3,4)P$_2$ while the maximum velocity was 14 pmol of Ins(3,4)P$_2$ hydrolyzed/min/mg protein. In separate experiments, the range of $K_m$ values for Ins(3,4)P$_2$ was 20–25 μM. The ability of Ins(1,3,4)P$_3$ to compete with Ins(3,4)P$_2$ as a substrate is shown in Fig. 6. Different concentrations of $[^{3}H]$Ins(3,4)P$_2$ were incubated with inositol polyphosphate 4-phosphatase both in the absence or presence of a fixed amount of unlabeled Ins(1,3,4)P$_3$. Ins(3,4)P$_2$ competitively inhibits Ins(3,4)P$_2$ breakdown with an apparent $K_i$ of 27 μM. From this experiment we concluded that the same active site is involved in the removal of the 4-phosphates from Ins(1,3,4)P$_3$ and Ins(3,4)P$_2$.

Inositol polyphosphate 4-phosphatase does not require divalent metal ions (e.g. Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$) for its activity. Li$^+$ up to 50 mM has no effect on inositol polyphosphate 4-phosphatase activity. Phosphate and ammonium sulfate strongly inhibit enzyme activity having an IC$_{50}$ = 10 and 2 mM, respectively. Mn$^{2+}$ ions inhibited the enzyme as shown in Fig. 7 although significant inhibition was achieved only at concentrations over 1 mM. In contrast inositol polyphosphate 1-phosphatase is inhibited by lower concentrations of Mn$^{2+}$ with 50% inhibition at 0.01 mM. There was also inhibition of inositol polyphosphate 4-phosphatase at 10 mM Mg$^{2+}$ and Ca$^{2+}$ as seen in Fig. 7. EDTA had no effect on enzyme activity although, when enzyme was diluted into buffers without EDTA, enzyme activity was lost rapidly (80% in 2 h).

**DISCUSSION**

Inositol polyphosphate 1-phosphatase, a Mg$^{2+}$ dependent, lithium ion-inhibited enzyme was isolated previously and...
The rate of Ins(3,4)P₃ hydrolysis was determined using Assay 2 as unlabeled Ins(1,3,4)P₃ with inositol polyphosphate 4-phosphatase. The apparent Kₘ of 40 and 25 for the hydrolysis of Ins(1,3,4)P₃ and Ins(3,4)P₃ with an inhibition by Li⁺ ions. A single active site is responsible for hydrolysis of Ins(1,3,4)P₃ and Ins(3,4)P₂. In contrast to Ca²⁺, A, Mn⁺⁺.

We have now purified and studied the properties of inositol polyphosphate 4-phosphatase containing the indicated additions. For the zero EDTA point, enzyme was diluted without EDTA and assayed immediately. 0, EDTA; 0, 40 mM EDTA and then diluted 1:5 in the final assay buffer containing 0.1 mM EDTA. For the zero EDTA point, enzyme was diluted 1:500 in buffer containing 0.1 mM EDTA and then diluted 1:5 in the final assay buffer containing the indicated additions. For the zero EDTA point, enzyme was diluted without EDTA and assayed immediately. 0, EDTA; 0, 40 mM EDTA.

characterized from calf and rat brain tissues (21–23, 25–27). We have now purified and studied the properties of inositol polyphosphate 4-phosphatase, which specifically removes the 4-phosphate from Ins(1,3,4)P₃ and Ins(3,4)P₂. In contrast to inositol polyphosphate 1-phosphatase, the inositol polyphosphate 4-phosphatase is divalent metal ion-independent and is not inhibited by Li⁺ ions. A single active site is responsible for the hydrolysis of Ins(1,3,4)P₃ and Ins(3,4)P₂ with an apparent Kₘ of 40 and 25 μM, respectively. Recently, Hansen et al. (27) reported a 4-phosphatase activity in rat brain which had a Kₘ of 50 μM for Ins(3,4)P₂. This crude preparation also contained the indicated additions. For the zero EDTA point, enzyme was diluted without EDTA and assayed immediately. 0, EDTA; 0, 40 mM EDTA.

The relative activities of inositol polyphosphate 1-phosphatase versus inositol polyphosphate 4-phosphatase may determine the levels of various intermediates produced from the metabolism of Ins(1,3,4)P₃. In human umbilical vein endothelial cells cultured in the presence of Li⁺, the levels of Ins(1,3,4)P₃ versus Ins(3,4)P₂ varied considerably in response to different agonists (29). The relative activities of inositol polyphosphate 1-phosphatase and inositol polyphosphate 4-phosphatase toward Ins(1,3,4)P₃ have been measured in crude extracts of various bovine tissues (23). The proportion that was utilized by inositol polyphosphate 4-phosphatase ranged from 5 to 20% in various tissues except in brain where it was ~70%. These observations suggest that Ins(1,3,4)P₃ may play some role in neuronal function. Sherman et al. (30) have directly measured the levels of Ins(1)P and Ins(4)P in rat brain and found the predominant inositol monophosphate in both control and lithium-treated animals. In many other tissues, Ins(4)P is the predominant inositol monophosphate (8). Ins(1,3,4)P₃ is a potential source for the large amount of Ins(1)P in brain.

The physiological role of Ins(1,3,4)P₃ is unclear. Ins(1,3,4)P₃ has been shown to mobilize Ca²⁺ in Xenopus laevis oocytes and permeabilized Swiss mouse 3T3 cells at a potency about 10–30-fold less than Ins(1,4,5)P₃ (31, 32). In certain systems, the concentration of Ins(1,3,4)P₃ rises slowly, but it remains elevated much longer after agonist stimulation (14, 33). Therefore Ins(1,3,4)P₃ may play some role in sustained responses. Higashida and Brown (34) and Tertoolen et al. (35) observed membrane currents caused by Ins(1,3,4)P₃ in NG108-115 and N1E-115 cells, respectively. A messenger role for Ins(1,3,4)P₃ in the nervous system would implicate inositol polyphosphate 4-phosphatase as a signal-terminating enzyme. Isolation of inositol polyphosphate 4-phosphatase will allow further experiments to define the tissue distribution and molecular structure of this enzyme.

Acknowledgments- We thank Dan Lips for valuable discussions; Roger Inhorn, Brian Whiteley, Christine Mitchell, and Theo Ross for help during purification; Theo Ross also for providing inositol polyphosphate 1-phosphatase; Tom Cunningham for HPLC of reaction products; and Ann Delaney for the preparation of the manuscript.

REFERENCES
1. Hokin L. E. (1985) Annu. Rev. Biochem. 54, 205–235
2. Berridge, M. J. (1987) Annu. Rev. Biochem. 56, 159–193
3. Nishizuka, Y. (1986) Science 233, 305–312
4. Majerus, P. W., Connolly, T. M., Deckmyn, H., Ross, T. S., Bross, T. E., Ishii, H., Bansal, V. S., and Wilson, D. B. (1986) Science 234, 1519–1526
5. Rhee, S. G., Suh, P. G., Ryu, S. H., and Lee, S. Y. (1989) Science 244, 546–550
6. Berridge, M. J., and Irvine, R. F. (1984) Nature 312, 315–321
7. Majerus, P. W., Wilson, D. B., Connolly, T. M., Bross, T. E., and Neufeld, E. J. (1985) Trends Biochem. Sci. 10, 168–171
8. Majerus, P. W., Connolly, T. M., Bansal, V. S., Inhorn, R. C., Ross, T. S., and Lips, D. L. (1988) J. Biol. Chem. 263, 3051–3054
9. Rhee, S. G., Letcher, A. J., Heslop, J. P., and Berridge, M. J. (1986) Nature 320, 681–684
10. Johansson, R. A., Hansen, C. A., and Williamson, J. R. (1988) J. Biol. Chem. 263, 7465–7471
11. Connolly, T. M., Bansal, V. S., Bross, T. E., Irvine, R. F., and Majerus, P. W. (1987) J. Biol. Chem. 262, 2146–2149

FIG. 6. Inhibition of inositol polyphosphate 4-phosphatase activity by Ins(3,4)P₂. Enzyme was diluted 1:500 in buffer containing 0.1 mM EDTA and then diluted 1:5 in the final assay buffer containing the indicated additions. For the zero EDTA point, enzyme was diluted without EDTA and assayed immediately. 0, EDTA; 0, Ca²⁺; A, Mg²⁺; A, Mn²⁺.

FIG. 7. Effect of EDTA and divalent metals on inositol polyphosphate 4-phosphatase. Enzyme was diluted 1:500 in buffer containing 0.1 mM EDTA and then diluted 1:5 in the final assay buffer containing the indicated additions. For the zero EDTA point, enzyme was diluted without EDTA and assayed immediately. 0, EDTA; 0, Ca²⁺; A, Mg²⁺; A, Mn²⁺.
12. Hansen, C. A., Johanson, R. A., Williamson, M. T., and Williamson, J. R. (1987) J. Biol. Chem. 262, 17319–17326
13. Mitchell, C. A., Connolly, T. M., and Majerus, P. W. (1989) J. Biol. Chem. 264, 8873–8877
14. Irvine, R. F., Anggard, E. D., Lotcher, A. J., and Downee, C. P. (1985) Biochem. J. 229, 505–511
15. Tarver, A. P., King, W. G., and Rittenhouse, S. E. (1987) J. Biol. Chem. 262, 17303–17307
16. Shears, S. B., Parry, J. B., Tang, E. K. Y., Irvine, R. F., Michell, R. H., and Kirk, C. J. (1987) Biochem. J. 246, 139–147
17. Balla, T., Hunyady, L., Baukal, A. J., and Catt, K. J. (1989) J. Biol. Chem. 264, 8866–8870
18. Stephens, L. R., Hawkins, P. T., Barker, C. J., and Downes, C. P. (1988) Biochem. J. 253, 121–125
19. Inhorn, R. C., Bansal, V. S., and Majerus, P. W. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2170–2174
20. Bansal, V. S., Inhorn, R. C., and Majerus, P. W. (1987) J. Biol. Chem. 262, 9444–9447
21. Inhorn, R. C., and Majerus, P. W. (1987) J. Biol. Chem. 262, 15946–15952
22. Gee, N. S., Reid, G. G., Jackson, R. G., Barnaby, R. J., and Ragan, C. I. (1988) Biochem. J. 253, 777–782
23. Inhorn, R. C., and Majerus, P. W. (1988) J. Biol. Chem. 263, 14559–14565
24. Laemmli, U. K. (1970) Nature 227, 680–685
25. Neely, M. D., Letcher, A. J., and Nahorski, S. R. (1989) Biochem. J. 258, 23–32
26. Irvine, R. F., Moore, R. M., Pollock, W. K., Smith, P. M., and Wreggett, K. A. (1988) Philos. Trans. R. Soc. Lond. B Biol. Sci. 320, 281–288
27. Hansen, C. A., Inubushi, T., Williamson, M. T., and Williamson, J. R. (1989) Biochim. Biophys. Acta 1001, 194–144
28. Takimoto, K., Motoyama, N., Okada, M., and Nakagawa, H. (1987) Biochim. Biophys. Acta 929, 327–335
29. Pollock, W. K., Wreggett, K. A., and Irvine, R. F. (1989) Biochem. J. 256, 371–376
30. Sherman, W. R., Mansell, L. Y., Gish, B. G., and Honchar, M. P. (1985) J. Neurochem. 44, 786–807
31. Snyder, P. M., Krause, K.-H., and Welsh, M. J. (1988) J. Biol. Chem. 263, 11048–11051
32. Irvine, R. F., Letcher, A. J., Lander, D. J., and Berridge, M. J. (1986) Biochem. J. 240, 301–304
33. Burgess, G. M., McKinney, J. S., Irvine, R. F., and Putney, J. W. (1985) Biochem. J. 223, 237–243
34. Higashida, H., and Brown, D. A. (1986) FEBS Lett. 208, 283–286
35. Tertoolen, L. G. J., Tilly, B. C., Irvine, R. F., and Moolenaar, W. H. (1987) FEBS Lett. 214, 365–369
The isolation and characterization of inositol polyphosphate 4-phosphatase.
V S Bansal, K K Caldwell and P W Majerus

J. Biol. Chem. 1990, 265:1806-1811.

Access the most updated version of this article at http://www.jbc.org/content/265/3/1806

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/3/1806.full.html#ref-list-1