Regulation of a liver plasma membrane phosphoinositide phosphodiesterase by guanine nucleotides and calcium

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Rat liver plasma membranes are enriched in a Ca^{2+}-dependent phosphodiesterase active on phosphatidylinositol 4,5-P_2 and phosphatidylinositol 4-P, but not phosphatidylinositol. Inositol-P_3 is the first product of the reaction, but is rapidly degraded. Micromolar concentrations of GTP and its nonhydrolyzable analogues stimulate the reaction, whereas GDP, GMP and other nucleoside triphosphates are inactive. GTP and its analogues decrease the requirement of the reaction for Ca^{2+} and also increase its activity at saturating Ca^{2+}. These results support the hypothesis that guanine nucleotides and a guanine nucleotide binding regulatory protein are involved in coupling the receptors for Ca^{2+}-mediated agonists to the breakdown of plasma membrane phosphatidylinositol 4,5-P_2.

Inositol trisphosphate *Phosphatidylinositol bisphosphate* *Hormone* *Guanine nucleotide* *Calcium*

1. INTRODUCTION

Many hormones and neurotransmitters exert their effects by mobilizing Ca^{2+} in their target cells. This mobilization coincides with IP_3 formation from PIP_2 [1,2], and IP_3 releases Ca^{2+} from endoplasmic reticulum when added to permeabilized cells or isolated organelles [1,2].

Various reports have shown hormone effects on phosphoinositide hydrolysis in liver plasma membranes [3–6], but the significance of these observations remains questionable since most have dealt with PI hydrolysis [4–6] whereas hormonal effects on inositol phosphates are primarily due to breakdown of PIP_2 [1,2]. Here, we describe a calcium-sensitive PIP_2 phosphodiesterase activity that is enriched in liver plasma membranes. This activity is stimulated by guanine nucleotides suggesting that it may be regulated by a guanine nucleotide binding protein.

Abbreviations: IP_3, *myo*-inositol trisphosphate; IP_2, *myo*-inositol bisphosphate; IP, *myo*-inositol phosphate; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; GTPγS, guanosine 5′-*O*-(thiotriphosphate); GMPPNP, guanyl-5′-yl imidodiphosphate; GMPPCP, guanyl-5′-yl-(β,γ-methylene)diphosphonate

2. MATERIALS AND METHODS

Rats were injected intraperitoneally with 500 μCi of *myo*-[2-^{3}H]inositol 18–20 h prior to preparation of liver plasma membranes [7] with inclusion of leupeptin and antipain (20 μg/ml each) in the isolation medium. The membrane pellet was resuspended in 10 mM Na^+/Hepes, pH 7.5, 1 mM EGTA and 100 μg/ml each of leupeptin and antipain (Transformation Research). Membranes (approx. 0.4 mg protein) were incubated at 30°C for 5 min with the indicated additions in a total of 0.4 ml. Incubations were stopped with 1.5 ml chloroform:methanol (1:2, v/v) for determination of phosphoinositides or with 0.6 ml of 25% (w/v) trichloroacetic acid for determination of inositol phosphates. Phospholipids were extracted [8], dried and deacylated [9], and the water-soluble

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glycero derivatives separated on 2-ml anion exchange columns [10]. Labeled inositol phosphates were determined from the trichloroacetic acid supernatants according to Berridge [10] for individual inositol phosphates, or by counting the diethyl ether-extracted aqueous phases for total inositol phosphates.

[^{32}P]IP_3 was prepared as described by Downes and Michell [11]. *myo*-[2-^{3}H]Inositol was from American Radiolabeled Chemicals. Guanine nucleotides were from Boehringer Mannheim or Sigma.

3. RESULTS AND DISCUSSION

Fig.1A shows that when liver plasma membranes prepared from rats injected 18–20 h previously with *myo*-[2-^{3}H]inositol were incubated in the presence of Ca^{2+}, there was a time-dependent reduction in PIP_2 radioactivity. PI radioactivity was unchanged in the presence or absence of Ca^{2+}, and labeling of PIP was minimal and showed little change.

Analysis of the water-soluble products showed a rapid accumulation of IP_3 and minimal release of *myo*-inositol or other inositol phosphates, when the membranes were incubated in the presence of 5 mM pyrophosphate and the absence of Mg^{2+} (fig.1B). The formation of IP_3 was completely suppressed by the addition of EGTA. When the incubations were carried out in the presence of 10 mM $MgCl_2$ and the absence of pyrophosphate, only transient increases in IP_3 and IP_2 were observed and most of the label was in the inositol fraction at later times (not shown). The effects of Mg^{2+} and pyrophosphate were attributable to stimulation and inhibition, respectively, of IP_3 phosphatase activity as shown by studying the hydrolysis of exogenous [^{32}P]IP_3.

The phosphodiesterase activity exhibited an apparent K_a for free Ca^{2+} of 100 nM. Studies of its subcellular distribution showed that it was enriched 13-fold in the plasma membrane preparation compared with 26-fold for 5′-nucleotidase and 1–3-fold for markers of other organelles. Seyfred and Wells [3] have similarly reported a Ca^{2+}-sensitive polyphosphoinositide phosphodiesterase in rat liver plasma membranes. However, their preparations were highly contaminated with other subcellular organelles and no data were

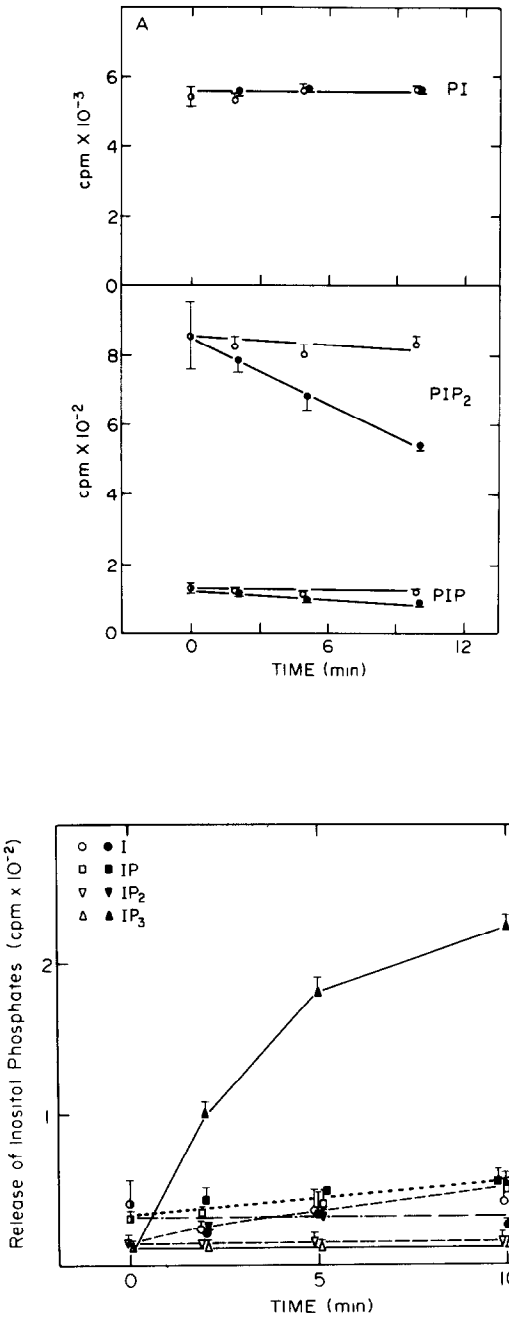

Fig.1. Calcium-dependent hydrolysis of plasma membrane phosphoinositides and release of inositol phosphates. Incubations included 5 mM Na pyrophosphate and either 1.0 mM EGTA (open symbols) or 1.0 mM EGTA plus 1.4 mM $CaCl_2$ (closed symbols). The experiments shown in each panel are representative of at least 3.

presented regarding plasma membrane recovery of the activity.

The release of water-soluble [^{3}H]inositol-containing products from membranes was increased by GTP and its analogs GTPγS, GMPPNP and GMPPCP, but not by GDP, GMP, ATP or CTP (fig.2). Analysis of the products showed the greatest increases in IP_3 and IP_2. The stimulation by guanine nucleotides occurred at micromolar concentrations (fig.3) and was completely dependent upon Ca^{2+}. Stimulation of inositide release by GTPγS was only seen at 1 nM or higher free Ca^{2+}, with the greatest-fold stimulation occurring at submaximal Ca^{2+} concentrations (fig.3). Experiments with GTP and GMPPNP gave similar results. Addition of 0.1 mM GTP to labeled membranes caused a 15% decrease in PIP_2 radioactivity, which was not seen in the presence of EGTA. The stimulation of inositide release by guanine nucleotides was time dependent, requiring 5–10 min for maximal stimulation. Guanine nucleotides acted by decreasing the Ca^{2+} requirement of the phosphodiesterase as well as increasing its activity at maximal Ca^{2+} (fig.3).

Other recent evidence has suggested a role for guanine nucleotides in calcium-mediated responses. Introduction of GTP analogues into permeabilized mast cells or platelets stimulates

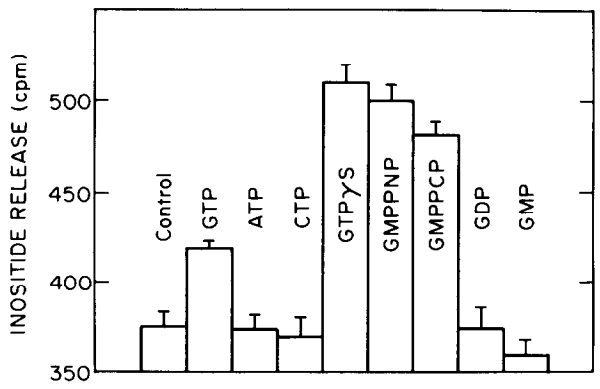

Fig.2. Guanine nucleotide stimulation of phosphoinositide phosphodiesterase activity. Plasma membranes were incubated for 5 min with 140 nM free calcium and 100 μM of the indicated nucleotides. Phosphocreatine (5 mM) and creatine phosphokinase (50 U/ml) were included with GTP. Each nucleotide was tested at least 3 times.

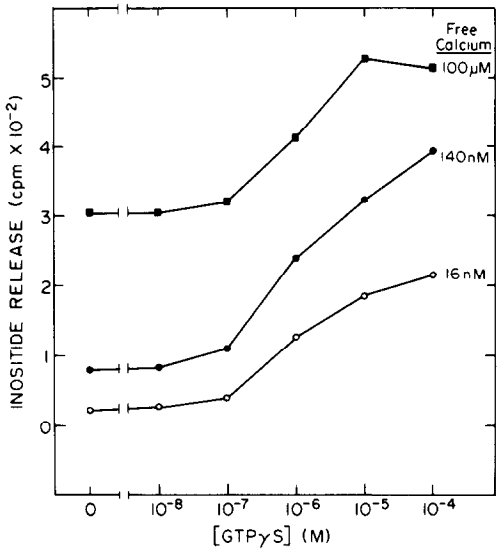

Fig.3. Stimulation of calcium-dependent phosphoinositide hydrolysis by GTPγS. Plasma membranes were incubated with the indicated concentrations of GTPγS for 20 min. $CaCl_2$ was then added to the indicated concentrations and the incubation continued for an additional 5 min. The experiment is representative of 3.

secretion in the presence of calcium [12,13]. Islet-activating protein, which inactivates the guanine nucleotide binding protein involved in inhibition of adenylate cyclase, also suppresses histamine release and PIP_2 breakdown induced by IgE or compound 48/80 in mast cells [14,15] and arachidonic acid release induced by chemotactic peptide in neutrophils [16].

Our data suggest that the stimulation of PIP_2 phosphodiesterase by GTP and its analogues is probably mediated by a guanine nucleotide regulatory protein since the effect is observed with micromolar concentrations of GTP and with non-hydrolysable derivatives of GTP. This gives rise to the further suggestion that the receptors for Ca^{2+}-mobilizing hormones couple to a guanine nucleotide binding protein analogous to the adenylate cyclase system. Other evidence suggesting this comes from experiments showing that GTP and its analogues inhibit agonist binding to these receptors [17–20], and from studies showing that calcium-mediated agonists stimulate guanine nucleotide hydrolysis or exchange in certain membranes [21,22].

Cockcroft and Gomperts [23] utilizing mast cell membranes and Litosch et al. [24] using membranes from blowfly salivary glands have also obtained evidence of a guanine nucleotide effect on PIP_2 phosphodiesterase activity, and Litosch et al. [24] have presented additional evidence for a hormonal stimulation of phosphodiesterase which is potentiated by guanine nucleotides. In studies to be reported elsewhere, we have also observed a hormonal stimulation of the release of inositol phosphates in liver membranes which is potentiated by GTP.

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