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MODULATION OF PHOSPHOLIPASE A_2 ACTIVITY IN ZYMOGEN GRANULE MEMBRANES BY GTP[S]; EVIDENCE FOR GTP-BINDING PROTEIN REGULATION

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Summary: In membranes associated with purified pancreatic zymogen granules, GTP[S] elicited a concentration-dependent activation of phospholipase A_2 (PLA_2), which was converted to inhibition in the presence of added Ca^{2+}. The GTP-binding protein inhibitor GDP[S] blocked both the stimulatory and inhibitory actions of GTP[S]. We conclude that in zymogen granule membranes GTP-binding proteins exert a dual regulation of PLA_2 activity.

Recently, members of the GTP-binding protein (G protein) family have been identified in membranes derived from secretory vesicles of chromaffin cells, neutrophils, and exocrine pancreas (1-4). Although the specific cellular function of these G proteins is unknown, an outcome of recent work on intact and permeable cells is the recognition that phospholipase A_2 (PLA_2), like phospholipase C, is coupled to receptors by G proteins (5).

Cell signaling in pancreatic acinar cells during receptor activation is associated with a rise in cytosolic Ca^{2+} and the release of arachidonic acid from the sn-2-position of phospholipids (6,7). The products of PLA_2 activation, arachidonic acid and lysophospholipids, have long been considered potential mediators of cell fusion reactions, including exocytosis (8). Insight into the cellular localization and possible functional role(s) of PLA_2 in cell

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Abbreviations: PLA_2, phospholipase A_2; GTP[S], guanosine 5'-[γ-thio]triphosphate; GDP[S], guanosine 5'[β-thio]diphosphate; GTP, guanosine triphosphate.

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signaling in exocrine pancreas was attained in a previous study, in which we identified and partially characterized a Ca^{2+} activated PLA_2 in membranes associated with purified pancreatic zymogen granules (9). This PLA_2 was activated by Ca^{2+} concentrations which correspond to those observed in intact acinar cells after exposure to physiological stimuli. To ascertain whether this membranous PLA_2 might be subject to G protein regulation, the present investigation explores the effects of the non-hydrolyzable guanine nucleotide GTP[S] on Ca^{2+}-independent and -dependent PLA_2 activity. We provide evidence for the dual regulation of PLA_2 activity of the granule membrane fraction by stimulatory and inhibitory G proteins.

Materials and Methods

Materials: 1-Stearoyl-2-[1-^{14}C]arachidonoyl phosphatidylcholine (54 mCi/mmol) was obtained from Amersham Research Products (Arlington Hts., IL). GTP[S], GDP[S], and GTP were purchased from Sigma Chemical Co, (St. Louis, MO).

Methods: Zymogen granule membranes were prepared as previously described (9). The assay for PLA_2 was carried out using 1-stearoyl-2-[1-^{14}C]arachidonoyl phosphatidylcholine as the exogenous substrate (9). The standard reaction mixture included substrate (0.2 µCi/ml), 0.2mM $MgCl_2$, 0.11M sucrose, 0.2M Tris-HCl (pH 7.5) and 25-50 µg protein in a final volume of 250µl. The reaction was carried out at 37°C for 15 min. The experiments which utilized added Ca^{2+} were conducted over a range of Ca/EGTA ratios to give appropriate pCa values, which were calculated using the program generously provided by Dr. Alexandre Fabiato of the Medical College of Virginia (10).

Statistical analysis: Differences between percent hydrolysis were determined using Student's t-test.

Results

To determine possible G protein regulation of PLA_2 activity in membranes associated with purified zymogen granules, the effect of the non-hydrolyzable GTP analogue GTP[S] was investigated. GTP[S] (0.1-10µM) stimulated PLA_2 activity from basal levels (2.0 $\pm$ 0.5% hydrolysis) in a concentration-dependent manner (Fig. 1), with half-maximal stimulation estimated to be 0.2µM. In the next series of experiments, although mean PLA_2 activity was somewhat lower (0.5 $\pm$ 0.2% hydrolysis), 10µM GTP[S] increased PLA_2 activity to 40% above basal levels ($P < 0.05$)(Fig. 2). The putative G protein inhibitor GDP[S] antagonized the stimulatory action of GTP[S] and had a variable but non-significant effect on basal PLA_2

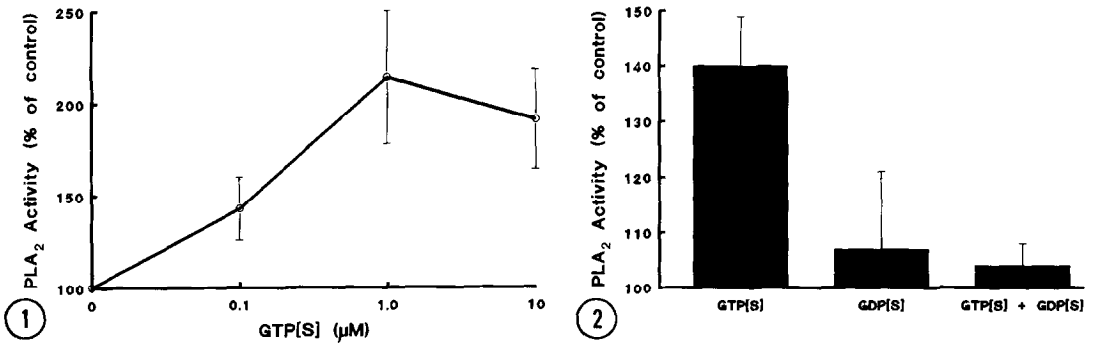

Fig. 1. Stimulatory effect of GTP[S] concentration on PLA_2 activity in membranes associated with purified zymogen granules. Results are expressed as a mean percentage of basal PLA_2 activity ($\pm$ SE) for 3-4 different experiments.

Fig. 2. Inhibitory effect of GDP[S] on GTP[S]-enhanced PLA_2 activity. Membranes were exposed to either 10μM GTP[S] or 10μM GDP[S] or a combination of 10μM GTP[S] and 100μM GDP[S]. Results are means ($\pm$ SE) for 4 different experiments.

activity (Fig. 2). Additionally, GTP (10 μM) failed to elevate PLA_2 activity relative to basal levels (108 $\pm$ 18%)(n=4).

Similar to our previous study (9), Ca^{2+} (5uM) produced a 2.4 ($\pm$0.2) fold increase in PLA_2 activity in zymogen granule membranes. GTP[S] (100nM), which produced a 30% elevation in PLA_2 activity in the absence of added Ca^{2+} (Fig. 1), caused a 22% inhibition of PLA_2 activity stimulated by 5uM Ca^{2+} (Fig. 3)($P < 0.05$). GDP[S] reversed the inhibitory effect of GTP[S]

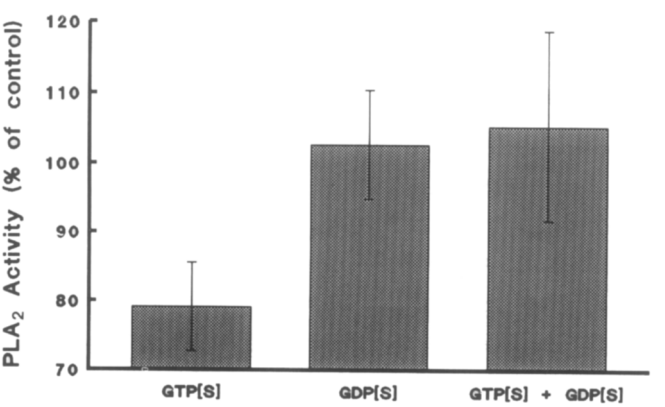

Fig. 3. Inhibitory effect of GTP[S] on PLA_2 activity stimulated by Ca^{2+}. Membranes were exposed to either 100nM GTP[S] or 100nM GDP[S] or a combination of 100nM GTP[S] and 100μM GDP[S]. Results are means ($\pm$ SE) for 4 separate experiments.

but had no discernible effect on basal PLA_2 activity (Fig. 3). GTP (10μM) also failed to alter Ca^{2+}-stimulated PLA_2 activity relative to control levels (97±13%)(n=4).

Discussion

According to this study, PLA_2 associated with zymogen granule membranes is subject to regulation by G-proteins. Thus, PLA_2 was activated in a graded manner by increasing concentrations of GTP[S], and GTP[S]-induced activation of PLA_2 was inhibited by GDP[S]. The stimulatory effect of GTP[S] was not shared by GTP, presumably because of the ability of the hydrolysis-resistant GTP[S] to persistently activate G-proteins. These collective findings suggest that at neutral pH a G-protein may mediate Ca^{2+}-independent PLA_2 activation.

This study also provides evidence for a G protein dependent inhibition of PLA_2. Thus, GTP[S] attenuated Ca^{2+}-stimulated PLA_2 activity, which was reversed by GDP[S]. This implicates a negative modulation by a G-protein. Additional evidence for the dual regulation of PLA_2 by G proteins derives from studies on rod outer segments and macrophages (5,11).

A clue to the identity of the G protein(s) involved in PLA_2 regulation may be inferred from our preliminary experiments which disclose a lack of pertussis toxin sensitivity. Thus, pretreatment of zymogen granule membranes with 40 μg activated pertussis toxin for 30 min failed to modify the inhibition of Ca^{2+}-activated PLA_2 activity produced by 100nM GTP[S]. This latter result fits well with the recent report that substrates for pertussis toxin are not detectable in zymogen granule membranes (3).

On the other hand, low molecular weight G proteins have recently been identified in zymogen granule membranes, including a 25kDa protein which was ADP-ribosylated by botulinum toxin (3,4). It is not yet known what relationship, if any, these low molecular weight G proteins have to the regulation of PLA_2 activity. There is also no specific information available regarding a relationship between PLA_2 activation and amylase release. However, there is evidence that G-proteins may be directly involved in exocytosis in acinar cells (12-14). Furthermore, the ability of GTP[S] to promote interactions between zymogen granules and pancreatic plasma membranes in the absence of Ca^{2+} (15) prompts speculation that G protein regulated PLA_2 activation may be involved in a step

in the secretory process that is distal to the Ca^{2+}-requiring event, perhaps at the level of membrane fusion.

Acknowledgments

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