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Regulation of Phosphatidylinositol 4-Kinase Activity in Rat Pancreatic Acini

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SUMMARY

Previous data showed that carbachol (CCh) elicits a concentration-dependent increase in phosphatidylinositol (PtdIns) 4-kinase activity in homogenates derived from agonist-stimulated pancreatic acini. In the present study CCh elicited a time-dependent increase in PtdIns 4-kinase activity, which was blocked by *n*-methylscopolamine and mimicked by muscarine. A membrane-associated PtdIns 4-kinase activity was identified that displayed maximal activity in the pH range of 5.5–8.5. The zymogen granule fraction possessed relatively high specific enzyme activity, which was sensitive to CCh stimulation. The enzyme had apparent K_m values for PtdIns and ATP of 4 and 60 μM, respectively. CCh caused no discernible change in the K_m for either PtdIns or ATP but increased V_{max}. Dioctanoylglycerol and oleoyl acetylglycerol augmented PtdIns 4-kinase activity, which was blocked by staurosporine, thus suggesting a possible role for protein kinase C in the regulation of PtdIns 4-kinase. These collective findings demonstrate a muscarinic receptor-mediated regulation of PtdIns 4-kinase activity in exocrine pancreas, involving a protein kinase C-dependent process.

Receptor-mediated secretagogues for exocrine pancreas, such as CCh, exert their primary action on the surface of the acinar cell to promote the breakdown of phosphoinositides. CCh stimulates the phospholipase C-mediated hydrolysis of PtdIns(4,5)P_2 to yield inositol trisphosphate and 1,2-diacylglycerol, which mediate an increase in Ca^{2+} availability and activation of PKC, respectively (1).

Although phosphoinositides constitute a minor fraction of total cell phospholipids, they undergo continuous phosphorylation and dephosphorylation. The synthesis of PtdIns(4,5)P_2 from PtdIns is mediated by two distinct ATP-dependent reactions. PtdIns is converted to PtdIns(4)P by PtdIns 4-kinase, whereas PtdIns(4)P is converted to PtdIns(4,5)P_2 by PtdIns 4-phosphate-kinase. PtdIns 4-kinase is a particulate enzyme that is primarily, although not exclusively, associated with the plasma membrane (2–4). Membranes associated with secretory granules are also an important source of the enzyme in chromaffin cells (5–7).

As in other cells, when pancreatic acinar cells are incubated with [^{32}P]P_i, PtdIns(4)P and PtdIns(4,5)P_2 become rapidly labeled, indicating a rapid interconversion of the phosphoinositides (8). However, the mechanisms involved in the regulation of polyphosphoinositide synthesis have not been adequately defined. The regulatory process may involve an action on PtdIns 4-kinase exerted by PKC or cAMP-dependent protein kinases (9–12). However, convincing evidence favoring a PKC- or cAMP-mediated activation of PtdIns 4-kinase is still fragmentary and even controversial (13). Substrate availability must also be considered as a possible mechanism for regulating inositol phospholipid resynthesis (14).

Our previous study demonstrated that CCh is capable of eliciting a concentration-dependent increase in PtdIns 4-kinase activity in homogenates prepared from intact pancreatic acini stimulated with CCh or epidermal growth factor (15). The present report describes the cellular distribution of PtdIns 4-kinase in pancreatic acini, as well as the factors that modify its activity, including the kinetic and regulatory properties of the enzyme. The direct regulation of enzyme activity appears to involve a PKC-dependent pathway.

Materials and Methods

Collagenase (0.48 units/mg) derived from *Clostridium histolyticum* was obtained from Boehringer-Mannheim (Indianapolis, IN). [γ-^{32}P] ATP (6000 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Phospholipase C was purchased from American Radiolabeled Chemicals (St. Louis, MO). PtdIns(4,5)P_2 and staurosporine were from

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ABBREVIATIONS: CCh, carbachol; PtdIns, phosphatidylinositol; PtdIns(4)P, phosphatidylinositol-4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; OAG, 1-oleoyl-2-acetylglycerol; diC$_8$, dioctanoylglycerol; PKC, protein kinase C; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid.

Calbiochem (San Diego, CA). OAG was obtained from Serdary Research Laboratories (London, Ontario, Canada). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Tissue preparations. Dispersed acini were prepared from a single rat by collagenase digestion, as described previously (16). To prepare acinar homogenates, aliquots of suspensions of acini were removed and microfuged for 5 sec. The supernatant was discarded and the cell pellet was washed with ice-cold medium containing 0.25% bovine serum albumin. The suspension was microfuged again and the pellet was resuspended in ice-cold reaction buffer containing 20 mM $MgCl_2$, 1 mM EGTA, 30 mM HEPES, pH 7.0, 0.1 mg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, and 300 μM phenylmethylsulfonyl fluoride. For experiments designed to elucidate the subcellular distribution of PtdIns kinase activity, a reaction buffer was utilized that was identical to the HEPES buffer except that 140 mM KCl and 10 mM NaCl, pH 7.0, were included to mimic the intracellular milieu (17). Cell suspensions in the various reaction buffers (0.2–0.5 mg of protein/ml) were homogenized on ice for 6 sec, using a Brinkmann Polytron. The homogenate was then centrifuged at 120 × *g* for 10 min at 4°. The resulting pellet contained whole cells, intact nuclei, and cell debris. Recovery of PtdIns 4-kinase activity from this first centrifugation averaged 65–85%. The supernatant was centrifuged at 100,000 × *g* for 60 min at 4° to yield the pellet (P_1) and supernatant (S_1) fractions. Purified zymogen granule membranes were prepared as described previously (18, 19), with the following modifications. After isolation from pancreatic fragments using 50% Percoll, the granules were lysed with high pH solution; the lysate was applied to a step gradient containing 10% sucrose plus 600 mM NaCl layered over 20% sucrose, to separate soluble components from pellet. The purity of the granule fraction was verified by the complete absence of mitochondria or plasma membrane (as assessed by membrane markers) and a 6-fold enrichment of amylase in the washed granule fraction, relative to the crude homogenate.

PtdIns 4-kinase assay. PtdIns 4-kinase activity was measured in various fractions by quantitating the transfer of phosphate from [^{32}P]ATP to either endogenous or exogenously added PtdIns. Assays were carried out in homogenization buffer and contained 15–75 μg of protein and 0.5 mM [γ-^{32}P]ATP (4.5 μCi), in a total volume of 75–100 μl. Calcium was omitted from the reaction mixture because it depresses PtdIns 4-kinase activity (20, 21). When homogenate was used as the source of the enzyme, incorporation of ^{32}P into PtdIns(4)P remained linear for only a brief period (15–75 sec). Product formation in the purified zymogen granule membrane fraction remained linear for at least 5–10 min; this was probably a consequence of the removal of degradative enzymes. Therefore, reactions were carried out at 30° for either 45 sec or 5 min, depending upon the preparation used. Reactions were initiated by the addition of [γ-^{32}P]ATP and were terminated by removal of aliquots (50–85 μl) at appropriate intervals. [^{32}P]PtdIns(4)P was extracted with chloroform/methanol (1:2, v/v) containing 0.005 N HCl. The extracted lipids were separated by thin layer chromatography using silica type G plates, identified by autoradiography, and quantitated by scintillation counting as described (8). Enzyme assays were done in duplicate or triplicate. Protein was determined by the method of Bradford (22), using bovine serum albumin as a standard.

To establish that ^{32}P incorporation into PtdIns(4)P was an accurate reflection of synthesis and was not significantly affected by breakdown of [^{32}P]PtdIns(4)P within the enzyme assay system, acinar homogenates were prelabeled for 45 sec with 0.5 mM [^{32}P]ATP and breakdown was monitored after the addition of excess unlabeled ATP (50 mM). The rate of hydrolysis of PtdIns(4)P in homogenates derived from CCh-treated acini was, in fact, slightly greater than that in untreated homogenates (7%/min), presumably due to activation of phospholipase C or phosphatases. Thus, inhibition of PtdIns(4)P hydrolysis cannot account for observed changes in PtdIns(4)P levels. Furthermore, the negligible loss of radiolabeled PtdIns(4)P indicates that ^{32}P incorporation into PtdIns(4)P is a reliable estimate of phosphoinositide synthesis elicited by PtdIns 4-kinase activity in exocrine pancreas.

For kinetic experiments, as well as those designed to elucidate the subcellular distribution of PtdIns kinase activity, exogenous PtdIns was used. PtdIns was solubilized in assay buffer containing 1% (v/v) Triton X-100 before sonication on ice for 5 min. This results in the formation of mixed micelles, which creates an environment that is believed to mimic physiological conditions at the membrane surface (23). The enzyme preparation was preincubated with 0.1% Triton X-100/PtdIns (50 μM) for 5 min before the addition of [^{32}P]ATP, to facilitate interaction with the mixed micelles according to the dual-phospholipid kinetic model (24).

To determine the kinetic parameters of PtdIns 4-kinase with respect to PtdIns, endogenous stores of PtdIns in acinar homogenates (0.5–1.5 mg of protein/ml) were depleted by treatment with exogenous PtdIns-specific phospholipase C (0.9 units/ml) for 15 min at 37°. Endogenous phospholipase C activity was then inactivated by the addition of 1 mM EGTA. The small amount of PtdIns hydrolyzed by exogenous Ca^{2+}-independent PtdIns-specific phospholipase C was subtracted from the mass of PtdIns used to perform kinetic analysis. Treatment with exogenous phospholipase C abrogated basal PtdIns(4)P formation, thereby affirming that this treatment completely depleted endogenous PtdIns stores utilized by PtdIns 4-kinase. To analyze the utilization of PtdIns by PtdIns 4-kinase, 1–50 μM PtdIns and a near-saturating ATP concentration (300 μM) were used. To determine the K_m and V_{max} for ATP, PtdIns(4)P formation was measured in the presence of 30–500 μM ATP and a near-saturating PtdIns concentration (50 μM).

Finally, to demonstrate conclusively that the enzyme studied was in fact a type 2 PtdIns kinase, the reaction product corresponding to PtdIns phosphate on the thin layer plate was deacylated and analyzed by anion exchange chromatography (25). The product was confirmed to be solely PtdIns(4)P.[1]

Statistical analysis. Differences were determined by one- or two-way analysis of variance using Duncan's multiple comparisons test or Student's paired *t* test. Data are represented as means ± standard errors, and a level of $p < 0.05$ was required for significance.

Results

Characterization of PtdIns 4-kinase activity in subcellular fractions. Subcellular fractionation of acini revealed that 97% of the PtdIns 4-kinase activity obtained from the acinar cell extract was associated with the 100,000 × *g* pellet (P_1), thus indicating that the enzyme is predominantly membrane associated. The relative specific activity of the P_1 fraction was 1.6-fold greater than that of the homogenate, indicating an enrichment of PtdIns 4-kinase in the membrane fraction (Table 1). The small amount of total activity present in the soluble fraction (S_1) (19 pmol/min) probably does not constitute a major source of enzyme activity, because its relative specific activity was very low, compared with the membrane-associated enzyme (15 versus 557 pmol/mg/min) (Table 1).

The zymogen granule membrane fraction derived from pan-

TABLE 1

Subcellular distribution of PtdIns 4-kinase in pancreatic acini

Subcellular fractions were prepared and assayed for PtdIns 4-kinase activity as described in Materials and Methods. Values shown are means of three to six independent experiments. Standard errors are within 10–15% of mean values.

Subcellular fraction	Relative specific activity	Total activity
	pmol/mg/min	*pmol/min*
Homogenate (acini)	292	1352
S_1	15	19
P_1	557	590
Homogenate (fragments)	243	3931
Zymogen granule membrane	1466	107

[1] S. P. Soltoff, B. R. Conway, and L. C. Cantley, unpublished observations.

creatic fragments also possessed PtdIns 4-kinase activity. Although this fraction contained only 3% of the total enzyme activity, the relative specific activity was 6-fold higher than that of the homogenate (1466 versus 243 pmol/mg/min) (Table 1).

The pH profile of PtdIns 4-kinase activity of acinar homogenates was very broad, with highest activity in the pH range of 5.5–8.5 (Fig. 1). The broad pH profile of the zymogen granule membrane PtdIns 4-kinase was similar to that of the acinar homogenate enzyme (data not shown).

Mediation of PtdIns 4-kinase activity by muscarinic agents. Our previous study showed that, with a 5-min incubation with the cholinomimetic CCh before homogenization and assay, a discernible increase in PtdIns 4-kinase activity was initially detected with 1 μM CCh (15). The response peaked with 50 μM CCh and then declined with higher CCh concentrations. In the present study, exposure of intact acini to a maximal stimulatory concentration of CCh (50 μM) for various intervals increased PtdIns 4-kinase activity in a time-dependent manner; the maximal response was obtained after a 5-min exposure to CCh (Fig. 2). After 30 min, enzyme activity was still significantly elevated. The time course of CCh stimulation of PtdIns 4-kinase activity is consistent with the findings that the rapid decrease in [^{32}P]PtdIns(4,5)P_2 levels in response to CCh stimulation observed within the first minute of stimulation (15) is followed by a restoration of phosphoinositide levels to near-control values (95 ± 2%) after 30 min.

The stimulatory effect of 10 μM CCh was reduced by 79% by a 20-min pretreatment with 1 nM *n*-methylscopolamine ($p < 0.05$; three experiments). Furthermore, the potent cholinomimetic muscarine produced a concentration-dependent increase in PtdIns 4-kinase activity (Fig. 3). The stimulation of enzyme activity induced by muscarine was observed in a range of concentrations (5–25 μM), which reflects its greater potency, relative to CCh, as a muscarinic receptor agonist (26).

When pancreatic fragments were exposed to 10 μM CCh and the zymogen granule membrane fraction was then isolated, PtdIns 4-kinase activity was maximally increased by 64 ± 26% after a 10-min exposure to CCh ($p < 0.05$; four experiments). Thus, in the granule membrane fraction there is an enduring activation of PtdIns 4-kinase, which retains the state of CCh stimulation achieved in fragments.

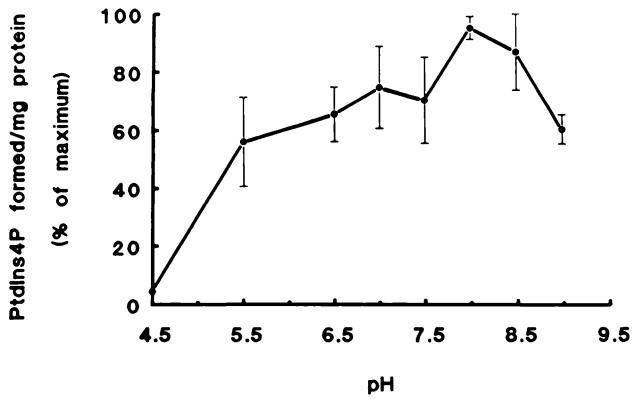

Fig. 1. pH dependency of PtdIns 4-kinase activity. PtdIns kinase activity was determined in isolated acini homogenized in 30 mM sodium acetate/acetic acid (pH 4.5–5.5), MES (pH 6.5), HEPES (pH 7.0), or Tris·HCl (pH 7.5–9.0) buffer. Data are expressed as percentage of maximal activity, which averaged 59 ± 12 pmol of [^{32}P]PtdIns(4)P/mg of protein. Values are means ± standard errors for three independent determinations.

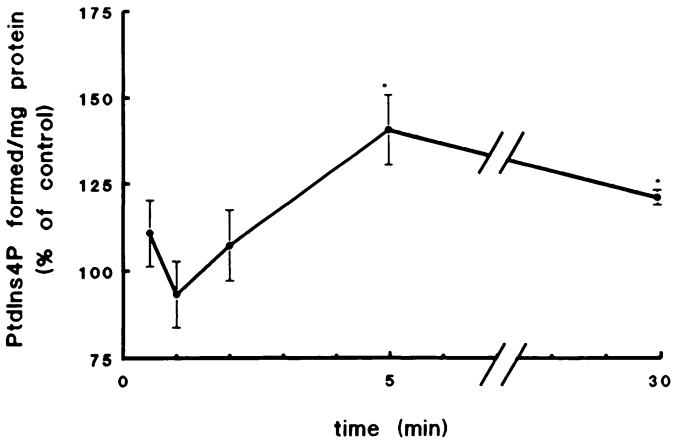

Fig. 2. Time-dependent effect of CCh on PtdIns 4-kinase activity. Intact acini were preincubated with CCh (50 μM) for the times indicated before homogenization and assay of PtdIns activity. PtdIns(4)P formation is expressed as a percentage of paired control values [162 ± 15 pmol of [^{32}P]PtdIns(4)P/mg of protein], which did not change over time. Values are the means ± standard errors of five independent determinations. *, Significantly different from control values, as determined by analysis of variance ($p < 0.05$).

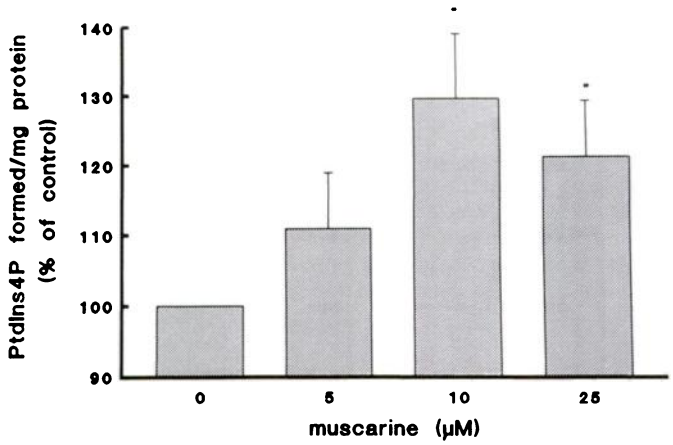

Fig. 3. Concentration-dependent effects of muscarine on PtdIns 4-kinase activity. Intact acini were incubated for 5 min with increasing concentrations of muscarine before enzyme assay. Data are expressed as a percentage of control values [181 ± 22 pmol of [^{32}P]PtdIns(4)P/mg of protein]. Values are the mean ± standard error of five independent experiments. *, Significantly different from control values, as assessed by analysis of variance ($p < 0.05$).

Kinetic properties of PtdIns 4-kinase. To determine the properties of PtdIns utilization by PtdIns 4-kinase, acinar homogenates were pretreated with phospholipase C to deplete the stores of endogenous PtdIns before assay. Results of a representative experiment are presented as a double-reciprocal (Lineweaver-Burk) plot of initial velocity versus PtdIns concentration (Fig. 4A). The apparent K_m for PtdIns utilization (4.5 ± 0.2 μM) was not significantly altered after exposure to CCh (6.2 ± 0.9 μM). However, the apparent V_{max} for the reaction was significantly increased by CCh. The average V_{max} values from four separate experiments were 294 ± 26 and 375 ± 39 pmol of [^{32}P]PtdIns(4)P/mg of protein/min in the absence and presence of 10 μM CCh, respectively ($p < 0.05$).

Comparable findings were obtained when the kinetic properties for ATP utilization were determined in homogenates derived from acini exposed to CCh (Fig. 4B). The K_m values

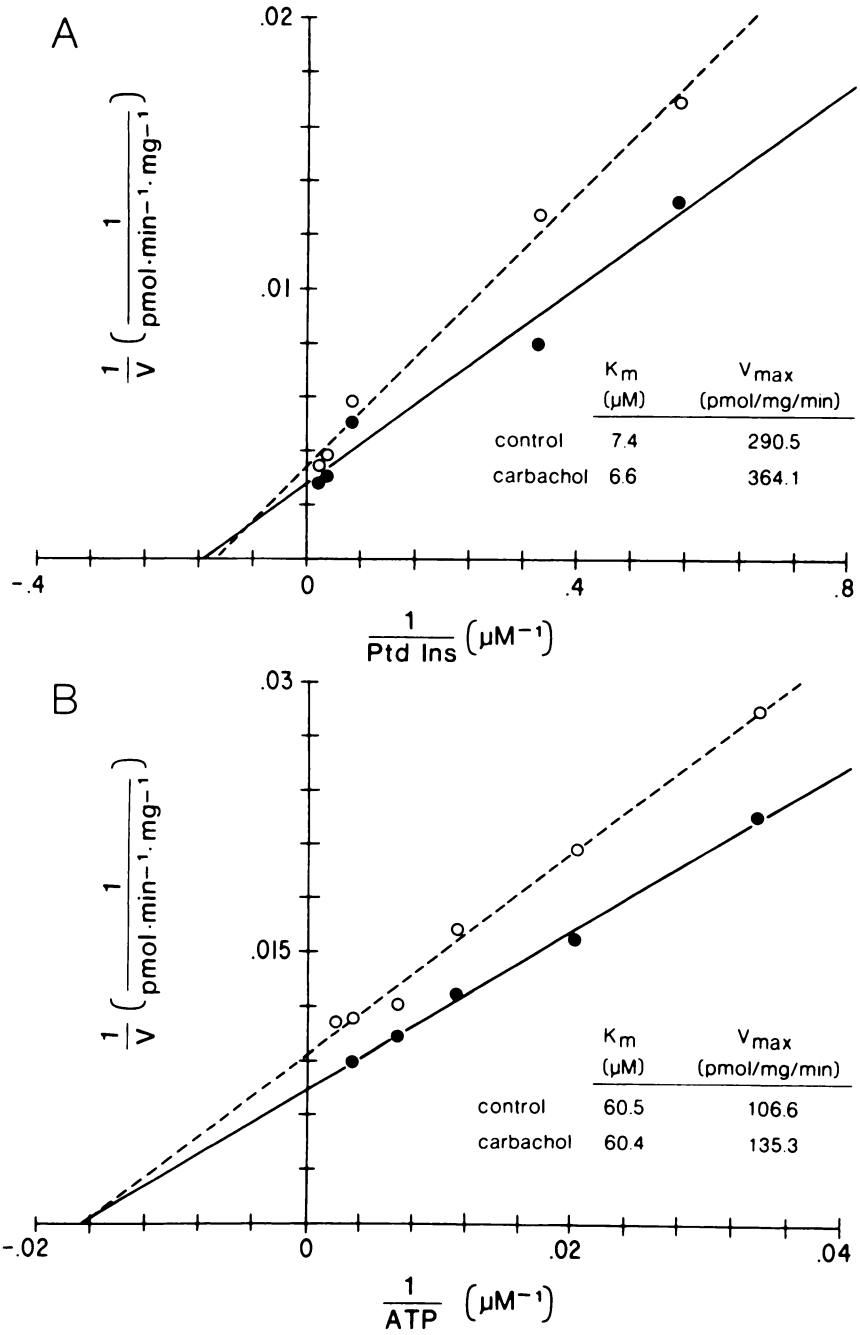

Fig. 4. Effect of CCh on the kinetic properties of PtdIns 4-kinase. Intact acini were incubated with (●) or without (○) CCh (10 μM) for 5 min, and homogenates were assayed in triplicate for PtdIns kinase activity. The data are plotted as 1/*V* versus the reciprocal of the PtdIns (A) or ATP (B) concentration. *Inset*, V_{max} and apparent K_m values for this representative experiment. These experiments were reproducible in their entirety.

for utilization of ATP by PtdIns 4-kinase measured in homogenates derived from control and CCh-treated cells were 55 ± 3 and 65 ± 4 μM, respectively. In contrast, CCh produced a highly significant increase in the apparent V_{max}, from 109 ± 3 to 136 ± 1 pmol of [^{32}P]PtdIns(4)P/mg of protein/min ($p < 0.005$; four experiments). These data suggest that CCh modifies enzyme activity independently of a change in affinity for either substrate.

Role of PKC in PtdIns 4-kinase regulation. Muscarinic receptor agonists activate PKC in rat pancreatic acinar cells (27), thus suggesting that the observed effects of CCh on PtdIns 4-kinase activity may be mediated by this pathway. A 20-min exposure of intact acini to the cell-permeable diacylglycerol analogues diC_8 or OAG caused a concentration-dependent increase in PtdIns 4-kinase activity (Fig. 5). Moreover, a 3-min pretreatment with the putative PKC inhibitor staurosporine (10 nM) reduced the stimulatory effect of 200 μM diC_8 by 74 ± 7%. Attempts to block the CCh-mediated increase in PtdIns kinase activity with staurosporine were hampered by the ability of the vehicle (ethanol or dimethylsulfoxide) to blunt the agonist-mediated response.

To delineate further the mechanism by which PKC regulates PtdIns kinase activity, the kinetic parameters with respect to ATP and PtdIns utilization were investigated. A representative double-reciprocal plot of the initial velocity versus the PtdIns concentration is shown in Fig. 6A. The apparent K_m for PtdIns utilization (3.8 ± 0.3 μM) was not significantly altered after a 20-min exposure to 200 μM diC_8 (4.9 ± 0.8 μM). However, the

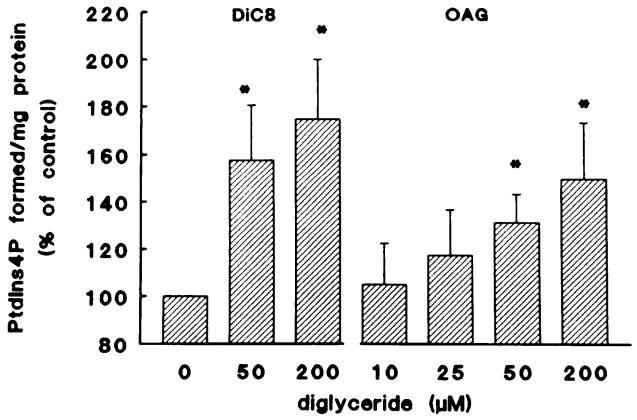

Fig. 5. Concentration-dependent effects of cell-permeable diglyceride analogues on PtdIns 4-kinase activity. Intact acini were incubated for 20 min with increasing concentrations of diC_8 or OAG, and homogenates were assayed for enzyme activity. Values are the percentage of controls [87 ± 14 pmol of [^{32}P]PtdIns(4)P/mg of protein]. Values are the mean ± standard error of eight independent experiments. *, Significantly different from control, as assessed by analysis of variance.

apparent V_{max} for the reaction (285 ± 37 pmol/mg of protein/min) was significantly increased by diC_8 (340 ± 45 pmol/mg/min), as assessed by paired Student's t test ($p < 0.02$; five experiments). When the concentration of ATP was varied, the apparent K_m for PtdIns utilization (92 ± 13 μM) was not significantly altered by diC_8 (96 ± 13 μM) (Fig. 6B). In contrast, diC_8 produced a highly significant increase in the apparent V_{max} of the reaction, raising it from 192 ± 14 to 258 ± 26 pmol of PtdIns(4)P/mg/min ($p < 0.01$; six experiments).

Discussion

In this study we have described a CCh-sensitive PtdIns 4-kinase in homogenates derived from intact acini. Our evidence indicates that this response is mediated via muscarinic receptors, because the stimulation was sensitive to inhibition by the potent muscarinic receptor antagonist *n*-methylscopolamine and was mimicked by muscarine. CCh and muscarine, which have comparable efficacies in terms of amylase secretion (26), also had comparable efficacies in terms of PtdIns 4-kinase activation. Moreover, the fact that peak stimulation of enzyme activity was attained with a lower concentration of muscarine (10 μM) than CCh (50 μM) supports their rank order of potency for muscarinic cholinergic receptors of exocrine pancreas (26). The time course for CCh-induced activation of PtdIns 4-kinase activity paralleled the time course and concentration dependence for activation of amylase secretion (28, 29), thus substantiating the physiological relevance of these findings. Furthermore, the increase in PtdIns kinase activity elicited by muscarinic agonists is similar in magnitude to the observed CCh-mediated hydrolysis of polyphosphoinositides (8, 15). Thus, the relatively modest increase in polyphosphoinositide synthesis observed in this study is adequate for the replenishment of the agonist-sensitive pool of PtdIns(4)P.

The broad pH profile, with only minor variations in activity in the pH range of 6–8, that was observed with pancreatic PtdIns 4-kinase resembles the pH profile observed in other test systems, including a purified enzyme preparation (4, 6, 30). Also, the K_m value of 60 μM for ATP is similar to that of previously studied PtdIns 4-kinase activities, including a preparation of membrane-bound PtdIns 4-kinase purified to apparent homogeneity (21, 31, 32). The somewhat lower K_m value for PtdIns (4 μM), compared with corresponding kinetic constants obtained in these same systems, may be ascribed to our novel experimental protocol, which included the elimination of endogenous PtdIns before kinetic analysis (see also Ref. 14).

Certain properties of the PtdIns 4-kinase of acinar homogenates are shared by the enzyme derived from purified zymogen granule membranes. In both preparations, the enzyme exhibited a broad pH spectrum, with a neutral pH optimum, and was stimulated by CCh. Our finding that the relative specific activity of PtdIns 4-kinase in granule membranes is approximately 6 times that detected in fragment homogenates affirms that the granule membrane represents a highly active source of the enzyme. In bovine adrenal medulla, the highest specific activity of PtdIns 4-kinase was likewise observed in the granule fraction (7). An in-depth consideration of the potential physiological role of an agonist-stimulatable PtdIns 4-kinase associated with zymogen granule membranes is a subject for future investigation; however, relevant to this point, phosphoinositides have been directly implicated in the exocytotic process (33).

Our novel approach for completely depleting endogenous PtdIns in acinar homogenates using PtdIns-specific phospholipase C allowed us to control substrate levels by adding known amounts of PtdIns. Thus, any change in PtdIns levels (i.e., substrate availability) in response to CCh was eliminated as a possible cause of augmented PtdIns 4-kinase activity. Additionally, the fact that PtdIns levels decrease, rather than increase, after CCh stimulation of pancreatic acini (34) provides further proof that substrate availability cannot play a significant role in the activation of PtdIns 4-kinase by CCh. Finally, the fact that CCh stimulation is associated with an increase in the V_{max}, with no change in the K_m of the enzyme, suggests that CCh does not alter substrate affinity.

The physiological basis for the regulation of PtdIns 4-kinase by PKC seemingly stems from the ability of CCh to generate diacylglycerol from the hydrolysis of polyphosphoinositides and phosphatidylcholine (35, 36). The time course for PtdIns 4-kinase activation by CCh is consistent with that for diacylglycerol production in pancreatic acinar cells (37), as well as activation of PKC (27). Our finding that treatment of acini with cell-permeable diglycerides elicits a stimulatory effect on PtdIns 4-kinase that is blocked by the putative PKC inhibitor staurosporine further supports a possible role for PKC in the regulation of this enzyme. This interpretation implies a positive feedback regulation of PtdIns 4-kinase by a PKC-mediated pathway.

The fact that activation of PtdIns 4-kinase attained in intact acini or fragments was retained in homogenates and a purified zymogen granule membrane fraction that took several hours to isolate suggests that CCh stimulation causes a stable covalent modification of the enzyme. Previous reports of agonist stimulation of PtdIns 4-kinase in intact cells that persists after isolation of subcellular fractions complement these results (6, 32). Indeed, the ability of putative activators of PKC to elevate PtdIns 4-kinase activity supports the contention that activation of this enzyme is associated with protein phosphorylation-mediated events. This regulation may involve phosphorylation/dephosphorylation of sites on either PtdIns 4-kinase or a modulatory protein. Conclusive evidence for the covalent modification of PtdIns 4-kinase awaits its purification and the dem-

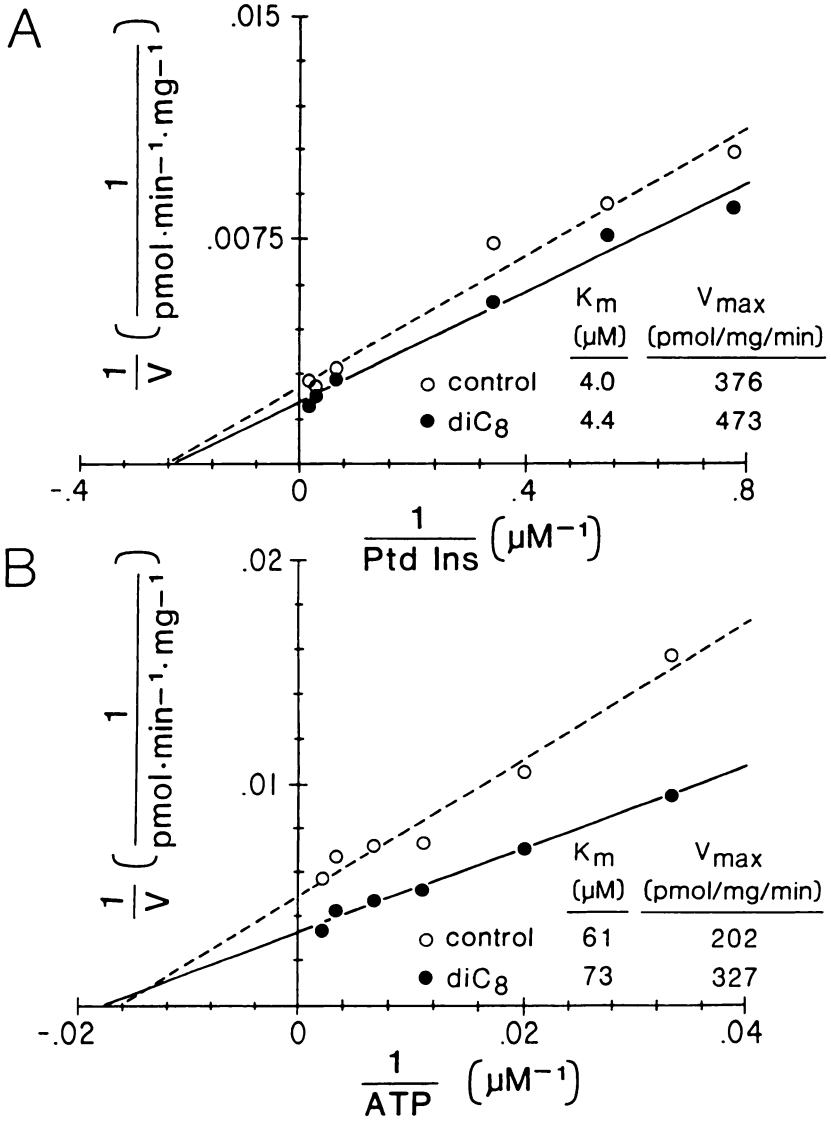

Fig. 6. Effect of diC_8 on the kinetic properties of PtdIns 4-kinase. Intact acini were incubated with (●) or without (○) 200 μM diC_8 for 20 min, and enzyme assay was performed in triplicate on homogenates. The data are plotted as $1/V$ versus the reciprocal of the PtdIns (A) or ATP (B) concentration. *Inset*, V_{max} and apparent K_m values for this representative experiment. This experiment was reproducible in its entirety.

onstration of altered ^{32}P incorporation into this enzyme after CCh stimulation. In any event, the demonstrated ability of CCh and PKC activators to stimulate PtdIns 4-kinase, as shown in this study, implies that phosphoinositide synthesis, like hydrolysis, is under tight control that can be regulated by physiological stimulation of the acinar cell.

Acknowledgments

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