Regulation of CCK-induced amylase release by PKC-δ in rat pancreatic acinar cells

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Li, Chenwei, Xuequn Chen, and John A. Williams. Regulation of CCK-induced amylase release by PKC-δ in rat pancreatic acinar cells. Am J Physiol Gastrointest Liver Physiol 287: G764–G771, 2004.—PKC is a group of phospholipid-dependent, serine/threonine kinases implicated in various biological functions, including proliferation, differentiation, and secretion (11, 27). The PKC isoforms in amylase secretion; our results indicate that PKC-δ is the PKC isoform involved with amylase secretion.

The major function of pancreatic acinar cells is to synthesize and secrete a variety of digestive enzymes. As a representative enzyme, amylase secretion can be physiologically activated by a variety of hormonal and neural stimuli, including CCK, secretin, and ACh. The receptors for these secretagogues are all G protein-coupled receptors, and their dominant-negative variants by means of adenoviral vectors. CCK stimulation caused translocation of PKC-α-, -δ, and -ε, but not -ζ, from soluble to membrane fraction. CCK-induced amylase release was inhibited ~30% by GF109203X, a broad spectrum PKC inhibitor, and by rotterlin, a PKC-δ inhibitor, but not by Go6976, a PKC-α inhibitor, at concentrations from 1 to 5 μM. Neither overexpression of wild-type or dominant-negative PKC-α affected CCK-induced amylase release. Overexpression of PKC-δ and -ε enhanced amylase release, whereas only dominant-negative PKC-δ inhibited amylase release by 25%. PKC-δ overexpression increased amylase release at all concentrations of CCK, but dominant-negative PKC-δ only inhibited the maximal concentration; both similarly affected carbachol and JMV-180-inhibited amylase release. Overexpression of both PKC-δ and its dominant-negative variant affected the late but not the early phase of amylase release. GF109203X totally blocked the enhancement of amylase release by PKC-δ but had no further effect in the presence of dominant-negative PKC-δ. These results indicate that PKC-δ is the PKC isoform involved with amylase secretion.

PKC is a group of phospholipid-dependent, serine/threonine kinases implicated in various biological functions, including proliferation, differentiation, and secretion (11, 27). The PKC family is divided into three subgroups on the basis of their structure and activation requirements: conventional PKCs (α, β, and γ), which are DAG and calcium-dependent; novel PKCs (δ, ε, η, and ζ), which are DAG-dependent but calcium-independent; and atypical PKCs (ζ, λ, τ, and μ), which are not activated by DAG (21, 25, 26, 29). In the four constant (C) domains of PKC, the C1 domain contains the binding site for DAG or phorbol ester, the C2 domain contains the calcium-binding site, the C3 domain contains the ATP-binding site, and the C4 domain contains the protein substrate recognition site (31). All PKC isoforms are primarily located in the cytoplasm, but on activation, translocate to a membrane environment. Different cell types express their own unique subset of PKC isoforms, and the expression of multiple isoforms regulating diverse functions within a single cell type suggest that individual PKC isoforms may have specific functions.

In pancreatic acini, PKC can be activated by a wide range of stimuli including several G protein-coupled receptors, such as those for CCK and carbachol (7, 32). Phorbol ester, a specific PKC activator, which has a DAG-like structure, can also stimulate amylase secretion and potentiates secretion stimulated by calcium in isolated acinar cells (3, 10). In various studies (6, 12, 23, 32, 35), PKC participation represents 30–50% of total amylase release. Various PKC inhibitors, including staurosporine, H-7, and, more recently, bisindolylmaleimide, have been shown to totally inhibit amylase secretion stimulated by phorbol ester and inhibit that induced by CCK and carbachol by 40 to 50% (6, 30). However, the specificity for PKC inhibition of some of those is not clear, and none discriminate between isoforms. Although the molecular targets of PKC action regulating secretion are unknown, PKC activity has been shown to be present in or to phosphorylate proteins in subcellular fractions enriched in zymogen granules (4, 36). Whereas these data suggest that PKC plays an important role in amylase release, which PKC isoform mediates this signal transduction pathway has remained unclear. In the present study, with the use of both chemical inhibitors and adenoviral-delivered PKC molecules, we compared the role of different PKC isoforms in amylase secretion; our results indicate that PKC-δ mediates amylase secretion.

MATERIALS AND METHODS

Materials. Sulfated CCK octapeptide and JMV-180 were from Research Plus (Bayonne, NJ); carbachol chloride (carbachol), bombesin, and soybean trypsin inhibitor (SBTI) were obtained from Sigma (St. Louis, MO). Acinar isolation used either chromatograph-

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Adenoviruses were added at a specific H11003. After the specified time, the acinar suspension was centrifuged in HEPES-Ringer and then incubated with secretagogues. All chemical inhibitors were added to the acinar suspension 20 min before stimulation.

Overnight-cultured acini were washed and then resuspended in HEPES-Ringer supplemented with 11.1 mM glucose, 0.5 mg/ml SBTI, and 1 mg/ml BSA, equilibrated with 100% O2 and were studied within 2 h. In the viral infection experiments, isolated acini were resuspended in DMEM enriched with 0.5% fetal bovine serum and antibiotics and incubated overnight at 37°C in a 5% CO2 environment for 14–16 h. Adenoviruses were added at a specified titer, usually 5 × 10^8 pfu/ml, to the culture medium at the beginning of the overnight incubation. Overnight-cultured acini were washed and then resuspended in HEPES-Ringer for 30 min before stimulation.

Preparation of isolated pancreatic acini, short-term culture, and viral infection of pancreatic acini. Pancreatic acini were isolated by collagenase digestion of pancreas from male Sprague-Dawley rats weighing 125–150 g as previously described (34, 38). Acini were suspended in HEPES-Ringer supplemented with 11.1 mM glucose, Eagle’s minimal essential amino acids, 0.1 mg/ml SBTL, and 1 mg/ml BSA, equilibrated with 100% O2 and were studied within 2 h. In the viral infection experiments, isolated acini were resuspended in DMEM and divided into 100-mm petri dishes each containing 10 ml of DMEM enriched with 0.5% fetal bovine serum and antibiotics and incubated overnight at 37°C in a 5% CO2 environment for 14–16 h. Adenoviruses were added at a specified titer, usually 5 × 10^8 pfu/ml, to the culture medium at the beginning of the overnight incubation. Overnight-cultured acini were washed and then resuspended in HEPES-Ringer for 30 min before stimulation.

Analysis of amylase secretion. Freshly isolated and overnight-cultured acini were allowed to settle by gravity, resuspended in HEPES-Ringer and then incubated with secretagogues. All chemical inhibitors were added to the acinar suspension 20 min before stimulation. After the specified time, the acinar suspension was centrifuged for 30 s in a supernatant assayed for amylase activity using Phadebas reagent as described previously (34). Secretion was expressed as a percentage of initial acinar amylase total content.

Preparation of membrane fraction. Fresh, isolated rat pancreatic acini were washed in cold PBS and harvested in lysis buffer containing (in mM) 25 Tris, 5 EDTA, 150 NaF, 2 DTT, and 1 PMSF with 10 μg/ml leupeptin and 10 μg/ml aprotinin. The suspension was sonicated for 10 s twice and then centrifuged at 1,000 g for 8 min to remove debris. Supernatant was collected and centrifuged at 100,000 g for 50 min at 4°C, the supernatant was collected as a cytosol fraction, and the pellet was solubilized in the same volume of the above lysis buffer containing 1% Triton X-100 and sonicated for 10 s. The suspension was centrifuged at 15,000 g for 15 min at 4°C. The resulting supernatant was collected as the membrane fraction.

PKC measurement by Western blot analysis, translocation, and activity assays. Lysates of cultured acini were prepared in lysis buffer with 0.5% Triton X-100. Protein concentration was determined by using protein assay reagent (Bio-Rad, Hercules, CA). For Western blot analysis, an aliquot of sample was mixed with SDS stop solution, boiled, and 20 μg of protein/lane was loaded onto 12% SDS-PAGE (Bio-Rad). For PKC translocation studies, the same volume for each pair of cytosolic and membrane fractions solubilized as described was loaded. After gel electrophoresis, proteins were transferred to nitrocellulose membranes at 55 V for 2 h on ice. Western blot analysis was then carried out as previously described (5) by using different PKC antibodies overnight at 4°C. Enhanced chemiluminescence reagents were used to visualize the secondary antibody with attention paid to ensure film was not saturated. Films were scanned and band density quantitated by using Multi-analyst software (Bio-Rad). Activity of specific PKC isoforms was measured after immunoprecipitation from 0.5 mg protein acinar lysates with 2 μg antibody using the PepTag nonradioactive protein kinase assay from Promega (Madison, WI).

Statistical analysis. The data presented are the means ± SE of three to six separate experiments. Statistical significance was calculated by the Student’s t-test with P < 0.05 representing significance.

RESULTS

CCK induced PKC-α, -δ, and -ε translocation from cytosol to membrane. We first determined which isoforms of PKC were present in rat isolated pancreatic acini. Twelve isoform-specific PKC antibodies were tested by using Western blot analysis samples from freshly isolated acini, and four PKC isoforms were identified, including PKC-α (conventional), -δ, and -ε (novel), and -ζ (atypical) (Fig. 1A). Preliminary experiments showed increased translocation from cytosol to total cellular membrane already at 2 min after CCK stimulation that was maximal by 10 min and tended to reverse slowly. CCK (100 pM), which had the maximal effect on amylase release, induced translocation of PKC-α, -δ, and -ε to the membrane fraction, but not -ζ (Fig. 1B). The average increase of PKC signal in the membrane fraction after 10 min was 2.5- to 3.3-fold. PMA (0.1 μM) also caused similar translocation of PKC-α, -δ, and -ε to membrane fraction, but not -ζ (data not shown). Because neither CCK nor PMA affected PKC-ζ, it was not studied further.

GF109203X and rottlerin inhibited CCK-induced amylase release. To determine whether PKC participated in CCK-induced amylase release in freshly isolated acini and which isoform could mediate it, we tested three different PKC inhib-
The broad-spectrum PKC inhibitor GF109203X, which blocks conventional and novel PKCs and therefore should inhibit α, δ, and ε, inhibited amylase release at concentrations from 1 to 5 μM with maximal effect of 30.7 ± 2.4% at 5 μM (Fig. 2A). The conventional PKC inhibitor Gö6976, which should inhibit α but not δ and ε showed no inhibitory effect at any concentration (Fig. 2B). By contrast, the specific PKC-δ inhibitor rottlerin inhibited amylase release by 26.3 ± 5.7% at 5 μM, and at 10 μM, the inhibition by rottlerin was 55.68 ± 3.2% (Fig. 2C); all three inhibitors had no effect on basal amylase release.

**Dominant-negative PKC-δ mutant inhibited CCK-induced amylase release, and wild-type PKC-δ potentiated it.** Adenovirus-mediated overexpression of various wild-type and dominant-negative PKCs was first examined by Western blot analysis using specific PKC antibodies. Preliminary experiments showed time- and titer-dependent expression of viral-introduced protein. When the incubation time was fixed at 14 h and with 5 × 10⁶ pfu/ml titer, all different wild-type and dominant-negative PKC expression were five- to tenfold higher than β-galactosidase (β-gal) control and showed no effect on expression of other PKC isoforms (Fig. 3). The dominant-negative PKC-α, -δ, and -ε cDNAs consist of a lysine-to-arginine mutation in the ATP binding domain at amino acid positions 368, 376, and 436, respectively (2, 18, 22). Dominant-negative PKC-δ inhibited 25.5 ± 2.7% of 300 pM CCK-induced amylase release, whereas wild-type PKC-δ enhanced amylase release by 28.4 ± 6.8% (Fig. 4B); dominant-negative PKC-ε showed no inhibitory effect on amylase release, whereas wild-type PKC-ε enhanced it by 30.9 ± 6%, similar to wild-type PKC-δ (Fig. 4C). Overexpression of wild-type and dominant-negative PKC-α had no effect on CCK-induced amylase release (Fig. 4A). Overexpression of wild-type and dominant-negative forms of all three PKCs had no significant effect on basal amylase release.

**Dominant-negative PKC-δ mutant inhibited CCK-induced amylase release only near the maximal-response dose, and wild-type PKC-δ potentiated it across the CCK dose-response curve.** It is well established that the CCK-dose-response curve is biphasic, with inhibition at high doses (7, 13, 32, 34). In overnight-cultured β-gal-infected acini, CCK started to have an effect on amylase release at 10 pM, reached a maximum at 300 pM, and showed supramaximal inhibition above 1 nM (Fig. 5A). Dominant-negative PKC-δ had an inhibitory effect at the concentration of CCK from 30 pM to 1 nM, but this was statistically significant only at the maximal CCK concentration (300 pM) in which it was 24.1 ± 5.5%; there was no significant inhibition at other concentrations. Wild-type PKC-δ potentiated amylase release across the CCK dose-response curve (Fig. 5A). Because the complex CCK dose-response curve is believed to result from the interaction of high- and low-affinity states of the CCK receptor, we made use of the CCK analog, JM1-180, which acts as an agonist on high-affinity and as an antagonist on low-affinity CCK receptors (7, 17). Overexpression of wild-type and dominant-negative PKC-δ affected amylase release stimulated by JM1-180 in a similar manner to that stimulated by CCK (Fig. 6) suggesting that PKC-δ is involved with high-affinity CCK receptor action. To determine whether the dominant-negative inhibition was receptor specific, we also studied the carbachol dose-response curve and the results were very similar to CCK dose-response curve. Dominant-negative PKC-δ inhibited across the carbachol dose-response curve, but this was statistically significant only at the maximal concentration of

![Fig. 2. Effects of GF1091203X, Gö6976, and rottlerin on 100 pM CCK-induced amylase release. Isolated, fresh rat pancreatic acini were incubated in HEPES-Ringer with different concentrations of 3 PKC inhibitors for 20 min and were then resuspended in fresh HEPES-Ringer. Basal (○) and CCK-induced (●) amylase release was measured over a 30-min period. Results shown are means ± SE for 3 to 5 separate experiments. *P < 0.05.](http://ajpgi.physiology.org/DownloadedFrom/10.22033.1 On October 21, 2017)
carbachol (3 μM); overexpression of wild-type PKC-δ increased secretion across the carbachol dose-response curve (Fig. 5B). Wild-type and dominant-negative PKC-δ also affected amylase release stimulated by bombesin in experiments paralleling the studies with JMV-180 (data not shown).

Dominant-negative PKC-δ mutant inhibited, whereas wild-type enhanced CCK-induced amylase release during late phase of secretion. It is well established that the time course of CCK-induced amylase release has two phases with an early phase of ~5 min and a late phase that can last 60 min (20). Overexpression of dominant-negative PKC-δ had no effect on the early phase, but inhibited late-phase amylase release (from 5 to 30 min) by 33.9 ± 8.2% (Fig. 7A); wild-type PKC-δ also showed no effect on the early phase, whereas it increased the later-phase secretion by 53.9 ± 8.2% (Fig. 7B). Neither dominant-negative nor wild-type PKC-δ had an effect on basal amylase secretion.

Dominant-negative PKC-δ mutant can inhibit amylase secretion induced by wild-type PKC-δ. If the effects of overexpressing wild-type PKC-δ are mediated by its PKC-δ activity, it should be possible to competitively inhibit the effects of dominant-negative PKC-δ. To do this it is necessary to express excess dominant-negative PKC-δ. We first established that increasing the viral titer of control β-gal virus to 3 × 10^7 pfu/ml did not inhibit amylase secretion. We then combined the usual 5 × 10^6 pfu/ml wild-type virus with 2.5 × 10^7 pfu/ml dominant-negative virus and found significant inhibition of the effect of wild-type PKC-δ overexpression (Fig. 8A).

GF109203X abolished the enhancement induced by wild-type PKC-δ but showed no effect on dominant-negative PKC-δ. To also verify the PKC specificity of these adenoviruses, we studied the combination effect of GF109203X and overexpression of dominant-negative and wild-type PKC-δ. GF109203X at 5 μM totally abolished the enhancement induced by wild-type PKC-δ but showed no effect on the reduced secretion in response to the dominant-negative PKC-δ (Fig. 8B). These data support the conclusion that overexpression of PKC-δ and its dominant-negative variant is only affecting the PKC component of intracellular signaling in amylase secretion.
PKC by Western blot analysis in purified pancreatic acini with minimal islet contamination and detected \( \alpha \), \( \delta \), \( \epsilon \), and \( \zeta \), but no other PKC isoforms. It is well accepted that translocation to a membrane fraction is tightly associated with activation of PKCs. Most PKC in a resting cell is in the cytoplasm. PKCs translocate to specific subcellular sites after activation through direct interaction with docking proteins named receptors for activated C kinases (RACKs) (19), which allow specific substrate recognition and signal transduction. Different PKC isoforms have their own RACKs (19). In fresh pancreatic acini, 100 pM CCK stimulated the translocation of PKC-\( \delta \)WT, -\( \epsilon \), and -\( \zeta \) but not -\( \alpha \) and -\( \delta \) (1, 23, 35). Our preliminary data showed that CCK at concentrations <100 pM did not cause measurable translocation of any PKCs in freshly isolated acini, although 10 pM CCK can induce amylase secretion. We also tested PMA, and observed similar effects on translocation (data not shown). These data indicate that CCK can activate PKC-\( \alpha \), -\( \delta \), and -\( \epsilon \), but probably

Fig. 5. Effect of PKC-\( \delta \) on CCK and carbachol-stimulated amylase release over the concentration-response curve. Isolated rat acini were incubated with 5 \( \times \) 10\(^6\) pfu/ml control \( \beta \)-gal, WT, or DN PKC-\( \delta \) adenoviruses overnight, resuspended in fresh HEPES-Ringer, and incubated with various concentrations of CCK (A) or carbachol (B) for 30 min. Results shown are means \( \pm \) SE for 4 separate experiments. \( *P < 0.05 \).

Fig. 6. Effect of PKC-\( \delta \) on CCK- and JMV-180-stimulated amylase release. Isolated rat acini were incubated overnight with adenovirus expressing \( \beta \)-gal, WT, or DN PKC-\( \delta \) as for Fig. 5 and then were incubated with 300 pM CCK or 1 \( \mu \)M JMV-180. Results shown are means \( \pm \) SE for 4 separate experiments. \( *P < 0.05 \).

Fig. 7. Effect of overexpression of PKC-\( \delta \) on the time course of amylase secretion. Isolated rat acini were incubated overnight with 5 \( \times \) 10\(^6\) pfu/ml control \( \beta \)-gal, WT, or DN PKC-\( \delta \) adenoviruses, resuspended in fresh HEPES-Ringer, and incubated with (circles) or without (triangles) 300 pM CCK for the indicated period of time. Results shown are means \( \pm \) SE for 3 separate experiments. \( *P < 0.05 \).
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were incubated with 5M CCK for 30 min. Results shown are means ± SE for 3 separate experiments. *Compared with the CCK response in the β-gal control; + compared with WT PKC-δ. Both P < 0.05.

not PKC-ζ. It also may indicate that translocation by cell fraction is a relatively insensitive approach to PKC activation.

A number of increasingly specific chemical PKC inhibitors have been synthesized and applied to studies of pancreatic secretion as well as other cells. It has been reported that staurosporine inhibits 40 to 50% amylase release stimulated by CCK and carbachol (6, 30), but staurosporine is now known to be not specific in inhibiting PKC. We used a more specific broad-spectrum bisindolylmaleimide PKC inhibitor, GF109203X. The inhibitory effects of all chemical PKC inhibitors are due to an interaction with the ATP-binding site of PKC (8, 16). At 1 μM concentration, GF109203X is known to totally block PKC-α, -δ, and -ε activity but has almost no effect on PKC-ζ (16). The 30% inhibition by GF109203X on amylase release induced by CCK in fresh acini was therefore most likely mediated by the PKC-α, -δ, or -ε. GF109203X had no effect on PKC translocation (data not shown) as expected, because it acts at the ATP binding site. Several PKC inhibitors have been developed that appear specific for conventional PKC isoforms. G66976, which inhibits PKC-α at nM concentration, had no effect on amylase secretion, even at 10 μM. It is therefore likely that PKC-α does not mediate amylase secretion. The PKC-δ inhibitor rottlerin inhibited 30% of amylase secretion at 5 μM and inhibited >50% at 10 μM. Rottlerin’s IC50 for δ is 3–6 μM and for other PKC isoforms, is from 30 to 100 μM (8). Recent data (28), however, have questioned the specificity of rottlerin on pancreatic acini. The effect of 10 μM rottlerin may well be a nonspecific effect. Thus the use of rottlerin by itself cannot allow a definitive determination.

Because of the uncertain specificity of chemical inhibitors and because there is no PKC-ε inhibitor available at present, we utilized an adenoviral delivery system encoding wild-type and dominant-negative cDNA of PKC-α, -δ, and -ε to clarify the role of specific PKC isoforms. The dominant-negative PKC mutants consist of a lysine-to-arginine substitution in ATP binding domain. Although they are inactive, they still can interact with endogenous RACKs, thereby blocking the action of endogenous PKC isoforms to interact with the same RACKs (2). Adenoviral infection has been shown to be a highly efficient method of gene transfer to pancreatic acinar cells with nearly 100% efficiency (5, 9). In this study, a 14- to 16-h incubation was used to obtain adequate expression of PKC protein, and also to preserve, as much as possible, the acinar polarity and secretory responsiveness. Various adenoviral titers were tested and 5 × 106 pfu/ml was used as standard to obtain a five- to tenfold increase of protein expression comparing to the β-gal control, which showed no effect on expression of other PKC isoforms. Overexpressed PKC translocated to a membrane fraction after CCK stimulation, leading to an increase in membrane-associated PKC similar to control cells (data not shown).

There are two important changes regarding amylase release of overnight-cultured mouse acini: 1) reduced amylase release and 2) a threefold increase in the concentration of CCK required for maximal amylase release (5). In our study of rat acini, we saw similar changes, but there was adequate remaining amylase release to study the contribution of PKC. The concentration of CCK required for maximal secretion had also increased from 100 to 300 pM. Dominant-negative PKC-δ inhibited amylase release by 25%, whereas wild-type PKC-δ increased it by 28%, which is consistent with earlier results (6, 30) and our own data showing PKC contribute ~30–40% of total CCK stimulation. Wild-type PKC-ε also enhanced amylase release by 30%. Although we do not fully understand why the wild-type PKC-ε has this enhanced effect, it may be replacing the role of PKC-ζ, because dominant-negative PKC-ε had no inhibitory effect on CCK-induced amylase release. Both PKC-δ and -ε overexpression increased PKC activity after...
immunoprecipitation, whereas each dominant-negative variant inhibited endogenous activity of that isoform (data not shown). Dominant-negative PKC-δ was able to inhibit the action of overexpressed wild-type PKC-δ to enhance amylase secretion. Thus we believe that PKC-δ but not ε is the major isoform participating in amylase release.

It is well established that the biphasic shape of the CCK dose-response curve is characterized by supramaximal inhibition, involving binding of CCK to low-affinity receptors (7, 13). Our data showed that dominant-negative PKC-δ did not alter the biphasic shape of the amylase release curve and inhibited amylase release only at maximal concentration of CCK. JMV-180 acts on high-affinity CCK receptor and does not induce a biphasic dose response. Whereas there has been uncertainty whether JMV-180 activates PKC, overexpression of the wild-type and dominant-negative PKC-δ affected amylase release stimulation by JMV-180 and CCK very similarly. To show that this effect was not specific to the CCK receptor, we used carbachol, which acts on a muscarinic cholinergic receptor to activate PKC. The dominant-negative PKC-δ also inhibited carbachol-stimulated amylase release at maximal concentration of carbachol, this suggests that the effect of dominant-negative PKC-δ is general and not receptor specific.

In pancreatic acinar cells, two phases of amylase secretion have been proposed: an early phase, which is completed within 5 min of stimulation with a high rate of amylase secretion, and a late phase, which is sustained for a duration of stimulation with slow amylase release (20). Both dominant-negative and wild-type PKC-δ affected the late phase, but had no effect on the early phase. These results were similar to observations using GF109203X on fresh acini (data not shown). Our data indicate that the high-rate secretion early phase is not mediated by PKC but presumably is by calcium; the slow-rate secretion late phase is mediated by PKC-δ. This is consistent with previous reports (3, 20). When we added GF109203X to wild-type PKC-δ-infected acinar cells, the enhancement effect was totally abolished, whereas GF109203X did not show further inhibition on dominant-negative PKC-δ infected acinar cells. These data confirm that the PKC-δ is the isoform that is inhibited by GF109203X.

In conclusion, the present study demonstrates that PKC-δ plays an important role in regulating pancreatic amylase release by affecting the late phase of secretion. Further experiments are required to determine the downstream substrates of PKC-δ and the role of PKC-δ in supermaximal inhibition. In pancreatic acini, it seems that each PKC isoform has its unique function. Whereas PKC-δ, appears involved in amylase release, PKC-ε appears to mediate the activation of ERK through an MEK-dependent pathway (15). The role of PKC-α is not yet clear, but in other cell types it is involved in proliferation. Whether this is true in acinar cells will require further study. The multiplicity of PKC isoforms in acinar cells appears related to a role in regulation of diverse physiological effects.

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