Synthetic peptides containing a BXBXXXB(B) motif activate phospholipase C-β1

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We have recently shown that synthetic peptides of the effector domain of the low-molecular-mass GTP-binding protein Rab3 stimulate inositol 1,4,5-trisphosphate production in various permeabilized cells. To investigate the mechanism of the peptide-induced activation of phospholipase C (PLC) and to identify the PLC isoenzyme(s) targeted by these peptides, isolated pancreatic acinar membranes and cytosol were preincubated with anti-PLC antibodies before examination of PLC activity in response to the Rab3B/D effector-domain peptide (VSTVGIDFKVKTVYRH, peptide P1). Western blot analysis revealed the presence of PLC-β1, -β3, -γ1 and -δ1 in membrane and cytosolic fractions. P1 stimulated PLC activity in both membrane and cytosolic fractions. Anti-(PLC-β1) antibody inhibited P1-induced PLC activity in both subcellular fractions almost completely. Moreover, P1-induced amylase release in digitonin-permeabilized pancreatic acini was also inhibited. Other immunoneutralizing anti-PLC antibodies had no effect, suggesting that P1 activates PLC-β1 but not PLC-β3, -γ1 or -δ1. P1 also activated recombinant PLC-β1, indicating direct activation of PLC-β1 by Rab3 effector-domain peptides. To investigate further the structure–function relationship of the peptides, truncated peptides of P1 were tested for their ability to activate PLC in isolated pancreatic acinar membranes and to stimulate amylase release from digitonin-permeabilized pancreatic acini. Peptides containing a BXBXXXB(B) motif (where B represents a basic residue and X any residue) [KVKTVYRH (EC_{50} of 1 nM to stimulate amylase release) $\approx$ TVGIDFKVKTVYRH > TVGIDFKVKTVYR] were potent stimulators of amylase release and PLC activity, whereas deletion of the C-terminus (VSTVGIDF), of the two basic C-terminal amino acid residues (VSTVGIDFKVKTVY and KVKTVY), or destruction of the BXB motif (VKTVYR) resulted in inactive peptides. In conclusion, the results of the present study show that short peptides containing a BXBXXXB motif represent promising pharmacological agents to activate the PLC-β1 isoenzyme.

INTRODUCTION

Peptides of the effector domain of the low-molecular-mass GTP-binding protein Rab3A were originally designed to mimic the effect of cellular Rab3A protein [1]. The initial findings that these peptides stimulate exocytosis in various permeabilized cells were believed to support the contention that Rab3 proteins regulate exocytosis [2–10]. However, additional studies showed that the effects of these peptides are unlikely to be related to cellular Rab3 proteins because replacement of Thr^{35} by Ala in the effector domain of Rab3A protein inhibited the interaction of Rab3A with Rab3A-GAP, Rab3A-GEF and the putative Rab3A effector p85 [11], whereas the corresponding mutation in the Rab3A effector-domain peptide did not diminish the ability to stimulate exocytosis. Moreover, Rab3A effector-domain peptide does not interfere with the binding of p85 to Rab3A protein [11].

The finding that Rab3 effector-domain peptides stimulate inositol 1,4,5-trisphosphate production in various permeabilized cells indicates that these peptides activate phosphoinositide-specific phospholipase C (PLC) and thereby exocytosis [5,8,9,12]. The high potency of these peptides in stimulating inositol 1,4,5-trisphosphate production suggests that these peptides might represent useful pharmacological activators of PLC. The objective of the present study was to investigate the mechanism of activation of PLC, to identify the PLC isoenzyme(s) targeted by these peptides, and to determine the structural requirements of active peptides. The data show that the peptides stimulate PLC-β1 in both membrane and cytosolic fractions of pancreatic acini. A BXBXXXB(B) motif (where B represents a basic residue and X any residue) seems to be critical for activity of these peptides.

MATERIALS AND METHODS

Materials

Cholecystokinin octapeptide (CCK8), collagenase type III, phosphatidylinositol 4,5-bisphosphate, phosphatidylethanolamine and soybean trypsin inhibitor were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{3}H]Phosphatidylinositol 4,5-bisphosphate (6 Ci/mmol) was from DuPont–New England Nuclear (Bad Homburg, Germany). Monoclonal antibodies against PLC-β1, -γ1 and -δ1 were from Upstate Biotechnology (Lake Placid, NY, U.S.A.). Polyclonal antibodies against PLC-β1, -β2, -β3, -β4, -γ2 and -δ2 were from Santa Cruz (Santa Cruz, CA, U.S.A.). The peptides were synthesized by using fluoren-9-ylmethoxycarbonyl chemistry and purified by C_{18} reverse-phase HPLC. Purity exceeded 95% in each case (Neosystem, Strasbourg, France). Enhanced chemiluminescence (ECL) reagents and films were obtained from Amersham (Braunschweig, Germany).

Preparation of pancreatic acini

Pancreatic acini were isolated from rat pancreas by collagenase digestion as described [13,14]. According to the Trypan Blue dye exclusion method, the viability of the cells exceeded 95%. The

Abbreviations used: CCK8, cholecystokinin octapeptide; ECL, enhanced chemiluminescence; G-protein, GTP-binding regulatory protein; PLC, phosphoinositide-specific phospholipase C; P1, VSTVGIDFKVKTVYRH.

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acini were then suspended in K^+-Krebs–Ringer Hepes buffer containing 145 mM KCl, 2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 0.01 mM $CaCl_2$, 10 mM glucose, 0.2% BSA, 0.01% (w/v) soybean trypsin inhibitor, 10 mM Hepes/NaOH, pH 7.4, and 10 μg/ml digitonin. This procedure led to the permeabilization of more than 95% of the acini, as estimated by exclusion of Trypan Blue, and does not damage the secretory apparatus [13].

Measurement of amylase release

The detection of amylase released into the medium from digitonin (10 μg/ml)-permeabilized rat pancreatic acini during a 30 min incubation at 37 °C under continuous supply of 100% O_2 was performed as described [13]. In some experiments permeabilized pancreatic acini were preincubated with anti-(PLC-β) antibody (0.1 μg/ml) or the appropriate amount of normal rabbit serum for 10 min at 37 °C before the determination of amylase release.

Preparation of subcellular fractions

The preparation of subcellular fractions was performed essentially as described [13]. Isolated pancreatic acini were homogenized in 6 vol. of ice-cold homogenization buffer containing 25 mM Hepes, pH 7.0, 0.3 M sucrose, 0.5 mM $MgCl_2$, 1 mM benzamidine, 0.5 mM PMSF, 0.01% (w/v) aprotinin, 0.01% (w/v) leupeptin and 0.01% (w/v) trypsin inhibitor by 20 strokes in a glass–Teflon Potter homogenizer. The homogenate was centrifuged at 8000 ***g*** for 15 min and the supernatants were pelleted by centrifugation at 50000 ***g*** for 25 min. The supernatant prepared by centrifugation of a homogenate at 100000 ***g*** for 1 h was considered to be the cytosolic fraction.

Immunoblotting

Pancreatic acinar membrane and cytosolic fractions (50 μg of protein) were boiled for 4 min in Laemmli buffer [15] and separated by SDS/PAGE under reducing conditions. Gel-resolved proteins were electrotransferred to nitrocellulose sheets. The nitrocellulose membrane was first washed for 15 min with Tris-buffered saline containing 0.05% (w/v) Tween-20 (TBST) and was then blocked with 5% (w/v) skimmed milk in TBST for 1 h. Subsequently, the nitrocellulose sheets were washed twice with TBST and incubated with the appropriate primary antibody in blocking buffer. Antigen–antibody complexes were revealed with the appropriate horseradish peroxidase-conjugated antibodies and the ECL system. Protein was measured by the method of Bradford [16] with BSA as standard.

Expression of recombinant PLC-β1 and -β2

Recombinant PLC-β1 and -β2 were produced in baculovirus-infected *Spodoptera frugiperda* (Sf9) cells as previously described [17].

Assay of PLC activity

Phosphatidylinositol 4,5-bisphosphate-specific PLC activity in pancreatic acinar membranes or isolated recombinant PLC was assayed essentially as previously described [17,18]. Briefly, 30 μg of pancreatic acinar membrane protein was incubated for 20 min at 30 °C in a final volume of 70 μl containing 28 μM [^{3}H]phosphatidylinositol 4,5-bisphosphate (5 Ci/mol), 280 μM phosphatidylethanolamine, 50 mM Tris/HCl, pH 7.4, 2.8 mM EGTA, 80 mM KCl, 10 mM LiCl, 10 mM 2,3-bisphosphoglycerate, 1.2 mM sodium desoxycholate, and $CaCl_2$ to obtain 1 μM free Ca^{2+}.

For determination of the activity of recombinant PLC, soluble proteins (0.5 μg per sample) of Sf9 cells infected with recombinant baculovirus encoding PLC-β1 or -β2 were incubated with increasing concentrations of the Rab3 effector-domain-related peptide. The incubation was performed for 60 min at 30 °C as described [17,18].

Reactions were terminated by adding 350 μl of $CHCl_3$/CH_3OH/HCl containing 5 mM EGTA (500:500:3). Radioactivity released into the upper aqueous phase was quantitated by liquid-scintillation counting. PLC activity is expressed as pmol of phosphatidylinositol 4,5-bisphosphate hydrolysed/min per μg of protein.

Statistical analysis

The results presented are means ± S.E.M. for at least three separate experiments with assays performed in triplicate. Statistical analysis was performed with Student's *t* test for paired values.

RESULTS

Effect of Rab3B/D peptide and antibodies against PLC-β, -γ and -δ on PLC activity in subcellular fractions of pancreatic acini

We have recently shown that Rab3 effector-domain-derived peptides stimulate inositol 1,4,5-trisphosphate production in digitonin-permeabilized pancreatic acini [5,8,9], suggesting that these peptides activate phosphoinositide-specific PLC. To investigate which PLC isoenzyme(s) might be activated by Rab3

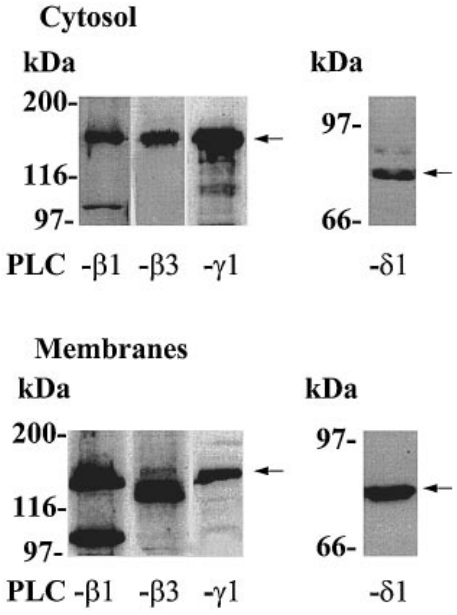

Figure 1 Presence of PLC isoenzymes in pancreatic acinar cytosol (upper panels) and membranes (lower panels)

Western blots of pancreatic acinar membranes or cytosol (50 μg of protein per lane) were probed with polyclonal antibodies against PLC-β1, -β2, -β3, -β4, -γ1, -γ2, -δ1 and -δ2, and immune complexes were revealed by peroxidase-conjugated secondary antibodies and the ECL system. Sf9 cell-expressed PLC-β1 co-migrated with the upper 145 kDa band visible in anti-(PLC-β1) immunoblots. Antibodies against PLC-β2, -β4, -γ2 and -δ2 did not recognize a substrate in pancreatic acinar cytosolic or membrane fractions (results not shown). The arrows indicate the positions of the bands representing PLC isoenzymes. The data shown are representative for four independent experiments. The positions of molecular mass markers are shown at the left of each panel.

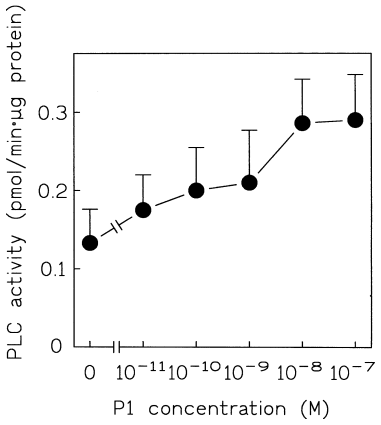

Figure 2 Effect of P1 on PLC activity in pancreatic acinar membranes

Pancreatic acinar membranes (30 μg of protein) were incubated with the indicated concentrations of P1 and assayed for PLC activity with [^{3}H]phosphatidylinositol 4,5-bisphosphate as substrate. PLC activity was estimated as described in the Materials and methods section. The results shown are means $\pm$ S.E.M. for three different experiments.

Table 1 Effect of anti-PLC antibodies on PLC activity induced by P1, carbachol and EGF in pancreatic acinar membranes (30 μg of protein)

Pancreatic acinar membranes were preincubated with the indicated polyclonal anti-PLC antibody (at a concentration of 10 μg/ml IgG) for 2 h at 4 °C before assessment of PLC activity induced by P1 (1 μM), carbachol (50 μM) or EGF (85 nM). PLC activity was estimated as described in the Materials and methods section. The results shown are means $\pm$ S.E.M. for at least four independent experiments. Asterisks indicate significant inhibition (**$P < 0.01$, ***$P < 0.001$) of stimulus-induced PLC activation.

		PLC activity (pmol/min per μg of protein)			
Antibody	Stimulant ...	None	P1	Carbachol	EGF
None (control)		0.12 $\pm$ 0.02	0.29 $\pm$ 0.04	1.03 $\pm$ 0.05	0.74 $\pm$ 0.02
Anti-(PLC-β1)		0.10 $\pm$ 0.04	0.16 $\pm$ 0.02**	0.94 $\pm$ 0.04	0.70 $\pm$ 0.03
Anti-(PLC-β3)		0.12 $\pm$ 0.04	0.25 $\pm$ 0.05	0.70 $\pm$ 0.02**	0.76 $\pm$ 0.04
Anti-(PLC-γ1)		0.09 $\pm$ 0.03	0.30 $\pm$ 0.05	1.08 $\pm$ 0.10	0.27 $\pm$ 0.03***
Anti-(PLC-δ1)		0.10 $\pm$ 0.05	0.29 $\pm$ 0.06	1.01 $\pm$ 0.08	0.72 $\pm$ 0.07

effector-domain peptides, we have determined the PLC isoenzymes present in pancreatic acinar subfractions by Western blotting. As illustrated in Figure 1, antibodies against PLC-β1, -β3, -γ1 and -δ1 recognized the respective PLC isoenzymes in pancreatic acinar cytosolic and membrane fractions, whereas PLC-β2, -β4, -γ2 and -δ2 were undetectable. PLC-β1, -β3 and -δ1 isoenzymes were enriched in the membrane fraction, whereas PLC-γ1 was more prevalent in the cytosolic fraction.

In isolated pancreatic acinar membranes the Rab3 effector-domain-derived peptide VSTVGIDFKVKTVYRH (P1) caused a dose-dependent increase in PLC activity (Figure 2). P1 (1 μM)-induced PLC activation was, however, only one-third of the effect of a high dose of carbachol (50 μM) (Table 1). Preincubation of the membranes with anti-(PLC-β1) antibody caused the inhibition of P1-induced PLC activity, whereas other anti-PLC antibodies had no significant effect (Table 1). The immunoneutralizing capacity of anti-(PLC-β3) and anti-(PLC-γ1) antibodies was confirmed because the anti-(PLC-β3) and anti-(PLC-γ1) antibodies inhibited the activation of PLC by carbachol (50 μM) and epidermal growth factor (85 nM) respectively (Table 1), without influencing basal PLC activity.

Table 2 Effect of anti-PLC antibodies on P1-induced PLC activation in pancreatic acinar cytosol (30 μg of protein)

Pancreatic acinar cytosol was preincubated with the indicated anti-PLC antibody (at a concentration of 10 μg/ml IgG) for 2 h at 4 °C before assessment of P1 (1 μM)-induced PLC activity. PLC activity was estimated as described in the Materials and methods section. The results shown are means $\pm$ S.E.M. for at least four different experiments. ***$P < 0.001$; significant effect of anti-PLC-β1 antibody.

		PLC activity (pmol/min per μg of protein)	
Antibody	Stimulant ...	None	P1
None (control)		0.13 $\pm$ 0.02	0.33 $\pm$ 0.04
Anti-(PLC-β1)		0.12 $\pm$ 0.06	0.15 $\pm$ 0.03***
Anti-(PLC-β3)		0.15 $\pm$ 0.08	0.32 $\pm$ 0.07
Anti-(PLC-γ1)		0.13 $\pm$ 0.06	0.34 $\pm$ 0.04
Anti-(PLC-δ1)		0.16 $\pm$ 0.05	0.33 $\pm$ 0.05

Table 3 Effect of anti-PLC antibodies on P1-, CCK8- and carbachol-induced amylase release in digitonin-permeabilized pancreatic acini

Digitonin-permeabilized pancreatic acini were preincubated without or with anti-PLC antibodies (1 μg/ml IgG) for 10 min. Thereafter, amylase release in response to vehicle (water), P1 (30 nM), CCK8 (0.1 nM) or carbachol (10 μM) was measured as described in the Materials and methods section. Results are expressed as means $\pm$ S.E.M. for at least three different experiments. *$P < 0.05$; significant inhibitory effect of anti-PLC antibody. Abbreviation: n.d., not determined.

		Amylase release (% of total amylase content of the acini)			
Antibody	Stimulant ...	None	P1	CCK8	Carbachol
None (control)		9.4 $\pm$ 1.3	19.8 $\pm$ 2.8	22.3 $\pm$ 2.3	21.2 $\pm$ 2.9
Anti-(PLC-β1)		7.8 $\pm$ 2.2	11.3 $\pm$ 3.3*	16.7 $\pm$ 3.4*	20.4 $\pm$ 3.2
Anti-(PLC-β3)		9.1 $\pm$ 2.4	18.6 $\pm$ 3.5	21.3 $\pm$ 2.6	17.6 $\pm$ 2.7*
Anti-(PLC-γ1)		8.8 $\pm$ 1.7	19.4 $\pm$ 2.4	n.d.	n.d.
Anti-(PLC-δ1)		9.3 $\pm$ 2.0	18.3 $\pm$ 3.1	n.d.	n.d.

In the cytosolic fraction P1 caused an increase in PLC activity (Table 2). Preincubation of the cytosol with polyclonal anti-PLC antibodies revealed that anti-(PLC-β1) antibody abolished P1-induced PLC activation, whereas the antibodies against PLC-β3, -γ1 and -δ1 had no effect (Table 2).

Monoclonal anti-(PLC-β1) antibody inhibited P1-induced PLC activity in both cytosol and membrane fractions, as did the respective polyclonal antibody (results not shown). Anti-PLC antibodies that did not recognize a PLC substrate in pancreatic acinar subfractions (antibodies against PLC-β2, -β4, -γ2 and -δ2) had no effect (results not shown).

Effect of antibodies against PLC-β1, -β3, -γ1 and -δ1 on P1-induced amylase release from digitonin-permeabilized pancreatic acini

If P1 activates amylase release through the activation of PLC-β1, anti-(PLC-β1) antibody should specifically inhibit P1-induced amylase release. Therefore digitonin-permeabilized pancreatic acini were preincubated with antibodies against PLC-β1, -β3, -γ1

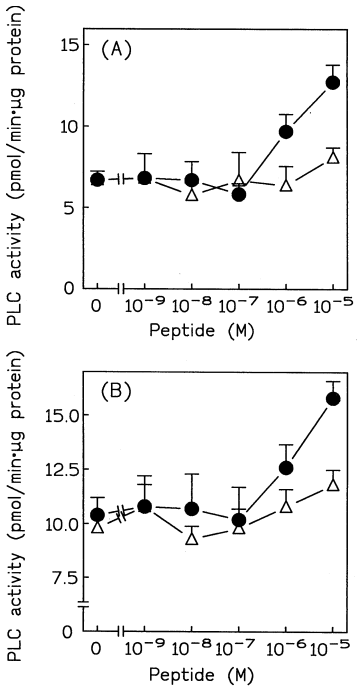

Figure 3 Effect of P1 and a derived peptide on the activation of recombinant PLC-β1 and -β2

Soluble proteins (0.5 μg per sample) of Sf9 cells infected with recombinant baculovirus encoding PLC-β1 (**A**) or PLC-β2 (**B**) were incubated with increasing concentrations of P1 (●) or a C-terminally truncated peptide (VSTVGIDFKVKTVY) (△). The results shown are means ± S.E.M. for three independent experiments.

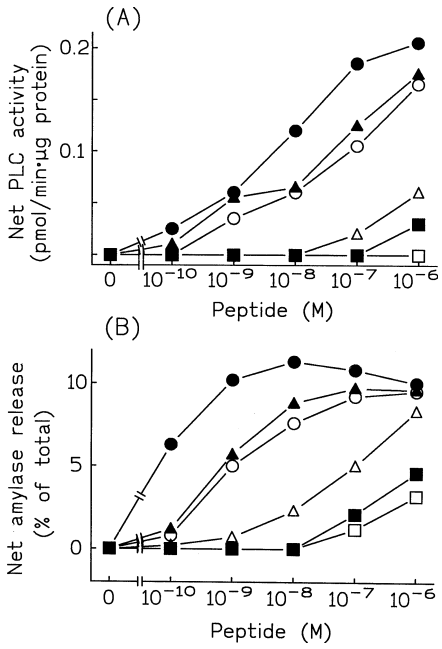

Figure 4 Effect of P1 and derived peptides on PLC activity in isolated pancreatic acinar membranes and release of amylase from digitonin-permeabilized acini

(**A**) For determination of peptide-induced PLC activity pancreatic acinar membranes (30 μg of protein) were incubated with various concentrations of P1 or truncated peptides of P1 and assayed for PLC activity with [^{3}H]phosphatidylinositol 4,5-bisphosphate as substrate. PLC activity was estimated as described in the Materials and methods section. (**B**) For measurement of the effect of the peptides on amylase release, rat pancreatic acini were permeabilized with digitonin (10 μg/ml) and amylase release was determined during a 30 min incubation at 37 °C. Symbols in both panels: ●, P1; ▲, KVKTVYRH; ○, VSTVGIDFKVKTVYR; △, VSTVGIDFKVKTVY; ■, KVKTVYR; □, KVKAAAR.

and -δ1 for 10 min before the determination of amylase release induced by P1, CCK8 and carbachol.

As shown in Table 3, anti-(PLC-β1) antibody inhibited P1 (30 nM)-induced amylase release completely without significantly altering basal amylase release. The inhibitory effect of this antibody on CCK8 (0.1 nM)-stimulated amylase release agrees well with recently published data [19]. The anti-(PLC-β1) antibody had no effect on amylase release in response to carbachol (10 μM), which has recently been shown to activate PLC-β3, but not PLC-β1, in pancreatic acinar membranes [20]. The anti-(PLC-β3) antibody inhibited carbachol-induced amylase release (Table 3). The antibodies against PLC-β3, -γ1 and -δ1 had no effect on P1- or CCK8-induced amylase release.

Effect of P1 on the activity of recombinant PLC-β1 and -β2

To investigate whether P1 directly activates PLC-β isoenzymes, the effect of P1 on the activity of human recombinant PLC-β1 and PLC-β2 was examined. As shown in Figure 3, P1 markedly activated PLC-β1. In contrast, a peptide in which the two C-terminal basic amino acid residues of P1 had been deleted was almost completely inactive. A similar result was obtained by investigating the effects of these peptides on PLC-β2 activity (Figure 3).

Sequence–function relationship of P1-related peptides

To investigate the essential sequence motif of the P1 peptide to activate PLC, truncated P1-related peptides were synthesized and tested for their ability to activate PLC in isolated pancreatic acinar membranes and to stimulate amylase release from digitonin-permeabilized pancreatic acini.

An N-terminal peptide of P1 (VSTVGIDF) and a peptide lacking the basic two C-terminal amino acid residues (VSTVGIDFKVKTVY) were less active towards the activation of PLC in isolated pancreatic membranes and the stimulation of amylase release in digitonin-permeabilized pancreatic acini, whereas a peptide lacking only the histidine residue at the C-terminus (VSTVGIDFKVKTVYR) retained activity (EC_{50} of 1 nM for amylase release compared with 0.1 nM of P1) (Figure 4). These results show that a basic amino acid residue at the C-terminus of P1 is required for activity.

The C-terminal peptide of P1 containing all basic amino acid residues (KVKTVYRH) was only slightly less active than P1 with respect to its ability to activate PLC and to stimulate amylase release (Figure 4). Thus the N-terminal eight amino acid residues of P1 are not essential for activity but increase the potency of these peptides. Deletion of the C-terminal histidine residue caused a decrease in the activity of KVKTVYRH. KVKTVY, a peptide that lacks the two basic C-terminal amino acid residues similar to VSTVGIDFKVKTVY, was completely inactive (results not shown). Peptides lacking the KVK motif of KVKTVYRH (VKTVYR, TVYRH) were also inactive (results not shown). Replacement of the neutral amino acid residues by alanines (KVKAAAR) did not significantly alter the activity of the peptide (Figure 4). Taken together, these results indicate that

a BXBXXXB motif (where B represents a basic residue and X any residue) is required for the stimulation of PLC activity and amylase secretion in pancreatic acinar cells.

DISCUSSION

Peptides corresponding to the putative effector domain of the low-molecular-mass GTP-binding protein Rab3 have been shown to stimulate exocytosis and PLC activity in a variety of cells [2–10,13]. In the present study immunoneutralizing antibodies raised against various PLC isoenzymes were used to identify the particular PLC isoenzyme(s) activated by Rab3-related peptides. The results show that anti-(PLC-β1) antibody selectively inhibited Rab3 effector-domain-derived peptide P1-induced PLC activation in pancreatic acinar cytosol and membrane fractions. Moreover, P1 activated recombinant PLC-β1, indicating the direct activation of PLC-β1 by Rab3 effector-domain-derived peptide P1. In mast cells Rab3A effector-domain peptide seems to stimulate exocytosis via the activation of pertussis toxin-sensitive GTP-binding regulatory proteins (G-proteins) similar to mastoparan [21], a direct activator of G-proteins [22], suggesting that Rab3A effector-domain peptides might activate G-proteins. Preincubation of pancreatic acinar membranes with an antibody raised against the C-terminus of $G_{q/11}\alpha$ had a small inhibitory effect on P1-induced PLC activation, whereas anti-$G_i\alpha$ antibodies had no effect (results not shown). However, if P1 stimulates $G_{q/11}$-proteins directly, both PLC-β1 and -β3 should be activated. Because we have no evidence that P1 activates PLC-β3, we favour the interpretation that in pancreatic acinar cells, P1 acts by the direct activation of PLC-β1 rather than the activation of G-proteins.

A peptide comprising the sequence CGGLPSPEDLRGKILIKNKK, thus containing a BXXXBXB motif similar to the peptides of the present study, has been shown to stimulate recombinant PLC-β2 with a similar potency [17,23]. The present study shows that P1 also stimulated human recombinant PLC-β2 in addition to recombinant PLC-β1. The structural similarity between CGGLPSPEDLRGKILIKNKK and P1 suggests that both peptides activate PLC-β1 and -β2 by a similar mechanism. It seems possible that charge distribution within the peptides might be more critical than orientation (BXBXXXB compared with BXXXBXB). CGGLPSPEDLRGKILIKNKK activated a C-terminal deletion variant of PLC-β2 lacking F^{819}–E^{1166} [17] necessary for $G_{q/11}\alpha$-dependent stimulation of PLC-β [24]. This indicates that the mechanism of activation of PLC-β2 by this peptide is different from that by the $G_{q/11}\alpha$ subunit and involves binding to a domain proximal from F^{819}. Our recent finding that Rab3-related peptide and CCK8 show additive effects on PLC activity [8] supports the hypothesis that CGGLPSPEDLRGKILIKNKK and P1 activate PLC-β1 and -β2 by similar $G_{q/11}\alpha$-independent mechanisms. Although this could not be tested directly in recombinant PLC-β3 protein because a full-length cDNA isolate of PLC-β3 was not available, the finding in the present study that antibodies against PLC-β3, -γ1 and -δ1 had no effect on P1-induced PLC activity suggests that P1 does not activate these PLC isoenzymes. The immunoneutralizing activity of the anti-(PLC-β3) and anti-(PLC-γ1) antibodies was demonstrated because these antibodies selectively inhibited the activation of PLC in pancreatic acinar membranes by carbachol and epidermal growth factor respectively (Table 1).

The minimal structural requirement of active peptides was a BXBXXXB sequence motif. Further deletions resulted in almost or totally inactive peptides. Thus our results show that short peptides can activate PLC-β1; they might be useful pharmacological tools as direct activators of PLC. The molecular mechanism by which these peptides stimulate PLC-β1 is not yet clear. It has recently been demonstrated that the related LPS-PEDLRGKILIKNKK peptide alters its conformation in the presence of phosphatidylinositol 4,5-bisphosphate, suggesting a physical interaction of this peptide with phosphatidylinositol 4,5-bisphosphate [23]. Furthermore it was suggested that the peptide stimulates PLC-β2 by binding to and offering the substrate to the catalytic domain of the enzyme in a more favourable configuration [23]. Thus it seems possible that P1 and related peptides are capable of binding to phosphatidylinositol 4,5-bisphosphate and that this is related to the ability of these peptides to stimulate PLC-β1 and -β2.

The present study shows that anti-(PLC-β1) antibody inhibited the P1-induced amylase release. Thus it seems that P1-induced exocytosis occurs via activation of PLC-β1 and is unrelated to Rab3 protein function. Cross-linking experiments have shown that P1 binds to a 75 kDa protein in pancreatic acinar cells [9]. However, the identity of this protein is still unknown and the question whether this protein is related to P1-induced activation of PLC-β1 cannot yet be answered.

The dose–response curves of the peptides for activation of PLC activity were always shifted to the right compared with their effect on amylase release. This is similar to the effect of secretagogues such as CCK8, carbachol and bombesin, which stimulate amylase release from pancreatic acini at considerably lower doses than PLC activity [25]. One possibility is that other second messenger systems than that following the activation of PLC mediate the secretory response [26]. Another possibility is that small changes that do not elicit a measurable increase in the inositol 1,4,5-trisphosphate level are capable of stimulating amylase release.

In conclusion, the present study shows that small peptides containing a BXBXXXB motif activate PLC-β1 and might represent promising pharmacological tools to activate PLC isoenzymes selectively.

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