Phospholipase C-γ Mediates the Hydrolysis of Phosphatidylinositol, but Not of Phosphatidylinositol 4,5-Bisphosphate, in Carbamylcholine-stimulated Islets of Langerhans*

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In pancreatic islets the activation of phospholipase C (PLC) by the muscarinic receptor agonist carbamylcholine (carbachol) results in the hydrolysis of both phosphatidylinositol 4,5-bisphosphate (PtdInsP2) and phosphatidylinositol (PtdIns). Here we tested the hypothesis that PtdIns hydrolysis is mediated by PLCγ1, which is known to be regulated by activation of tyrosine kinases and PtdIns 3-kinase. PtdIns breakdown was more sensitive than that of PtdInsP2 to the tyrosine kinase inhibitor, genistein. Conversely, the tyrosine phosphatase inhibitor, vanadate, alone promoted PtdIns hydrolysis and acted non-additively with carbachol. Vanadate did not stimulate PtdInsP2 breakdown. Carbachol also stimulated a rapid (maximal at 1–2 min) tyrosine phosphorylation of several islet proteins, although not of PLCγ1 itself. Two structurally unrelated inhibitors of PtdIns 3-kinase, wortmannin and LY294002, more effectively attenuated the hydrolysis of PtdIns compared with PtdInsP2. Adenovirally mediated overexpression of PLCγ1 significantly increased carbachol-stimulated PtdIns hydrolysis without affecting that of PtdInsP2. Conversely overexpression of PLCβ1 up-regulated the PtdInsP2, but not PtdIns, response. These results indicate that the hydrolysis of PtdIns and PtdInsP2 are independently regulated in pancreatic islets and that PLCγ1 selectively mediates the breakdown of PtdIns. The activation mechanism of PLCγ involves tyrosine phosphorylation (but not of PLCγ directly) and PtdIns 3-kinase. Our findings point to a novel bifurcation of signaling pathways downstream of muscarinic receptors and suggest that hydrolysis of PtdIns and PtdInsP2 might serve different physiological ends.

Many receptor tyrosine kinases and GPCRs1 couple to PLC that catalyzes the cleavage of PtdInsP2 and resultant generation of the two second messengers Ins(1,4,5)P3, which mobilizes Ca2+ from intracellular stores, and DAG, which activates protein kinase C (1–4). These pathways are well described in the regulation of insulin secretion following occupation of m3 and m1 muscarinic receptors on the surface of pancreatic β-cells (5–8). However we have shown that in addition to the classical pathway of PtdInsP2 hydrolysis, exposure of pancreatic islets to the muscarinic receptor agonist carbachol also promotes the hydrolysis of PtdIns (9, 10). The latter predominates quantitatively over, and is a precursor of, PtdInsP2. Although hydrolysis of either phosphoinositide species results in generation of DAG, only PtdInsP2 gives rise to a Ca2+ signal via Ins(1,4,5)P3 production. Therefore the two hydrolytic events may have different functional consequences. The inositol phosphate, corresponding to Ins(1,4,5)P3, that is derived from PtdIns, is Ins(1)P1. This has no biological function but can be used to quantify PtdIns hydrolysis, at least under the conditions verified in our previous studies (9).

The PLC family is comprised of four major groups (PLCs β, γ, δ, and ε). Members of the PLCβ family are activated by G-proteins in response to occupation of GPCRs (3, 4). The recently discovered protein kinase Ce is a component of ras signaling pathways (11–13). PLCβ is the most sensitive of the PLC family to Ca2+, and a rise in intracellular Ca2+ is thought to underly its activation in vivo (14, 15). The PLCγ family members (PLCγ 1 and 2) are classically activated downstream of receptor tyrosine kinases, but they also couple to muscarinic and other GPCRs in a manner secondary to stimulation of cytosolic tyrosine kinases (3, 4, 16, 17). Although PLCγ is a substrate for many tyrosine kinases there are indications, based both on activity assays (18–20) and experiments using whole cells (21, 22), that tyrosine phosphorylation of PLCγ itself is not always necessary for its activation. Indeed there is recent evidence that phosphatidylinositol 3,4,5-trisphosphate, generated by the enzyme PtdIns 3-kinase, binds to the pleckstrin homology domain of PLCγ and thereby stimulates its activity (23–26). The precise mechanism underlying this mode of activation is obscure, but it does not require tyrosine phosphorylation of PLCγ. This PLC is also of interest, because compared with the PLCβ and δ family members, which are relatively selective for PtdInsP2, PLCγ is also capable of efficiently using PtdIns as a substrate, at least in vitro (27).

We have previously provided limited evidence that hydrolysis of PtdIns and PtdInsP2 are independently regulated and hence potentially mediated by different PLCs. Thus, in pancreatic β-cells, a rise in intracellular Ca2+ levels is sufficient to cause breakdown of PtdInsP2 but not of PtdIns (10). Our current aim was to characterize further the mechanism underlying PtdIns hydrolysis, with particular reference to a potential role for PLCγ. Our results show for the first time that PLCγ

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‡ The abbreviations used are: GPCR(s), G-protein-coupled receptor(s); carbachol, carbamylcholine; KRB, Krebs-Ringer bicarbonate; PLC, phospholipase C; DAG, diacylglycerol; PtdIns, phosphatidylinositol; PtdInsP2, phosphatidylinositol 4,5-bisphosphate; BSA, bovine serum albumin; Ins(1,4,5)P3, inositol 1,4,5-trisphosphate; Ins(1)P1, inositol 1-monophosphate; Ins(4)P1, inositol 4-monophosphate; HPLC, high pressure liquid chromatography.

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mediates PtdIns hydrolysis in vivo and that the underlying mechanism appears to involve activation of PtdIns 3-kinase and alterations in the tyrosine phosphorylation of islet cell proteins although not of PLCγ itself.

**EXPERIMENTAL PROCEDURES**

**Materials**—The sources of all materials were as previously described (9, 10) except for tissue culture media which was from ICN Biomedicals (Seven Hills, NSW, Australia), radiochemicals from Amersham Pharmacia Biotech and scintillant from Canberra Packard (Sydney, NSW, Australia). All other biochemicals and specialized reagents were from Sigma except genistein, daidzein, LY294002, and wortmannin, which were purchased from Biomol (Plymouth Meeting, PA). Immunologicals were obtained from the following sources: anti-PLCγ1 and mixed monoclonal and rabbit antisera, Upstate Biotechnology, Lake Placid, NY; anti-PLCγ1, Santa Cruz Biotechnology, Santa Cruz, CA; RC20 anti-phosphotyrosine and anti-PLCγ1, Transduction Laboratories, San Diego, CA; goat anti-mouse IgG-Sepharose 4B conjugate and protein A-Sepharose 4B conjugate, Zymed Laboratories Inc., South San Francisco, CA.

**Islet Isolation and Culture**—Pancreatic islets were isolated following ductal flushing of collageinase into the exocrine pancreas, purified on a Histopaque 1077 gradient, and then handpicked under a binocular microscope (9). They were maintained in tissue culture flour (KRB) in Medium 199 containing 10% fetal calf serum, 14 mM NaHCO3, 11.1 mM glucose, 500 IU/ml penicillin, 100 µg/ml streptomycin, and 50 µg/ml gentamycin. For the inositol phosphate studies, 10 µCl/ml [2-3H]inositol was present throughout the culture period.

**Generation of Adenovirus and Islet Infection**—The two PLCs were present in slightly different adenosiviral vectors necessitating the use of different control viruses. Rat PLCγ1 (28) was subcloned into pShuttle-CMV and recombined with pAdEasy-1 as described (29). Control virus was generated by recombination of pShuttle-CMV and pAdEasy-1. Virus-encoding rat PLCγ1 (30) was a generous gift from Dr. C. B. Newgard (Dallas, TX). Here the appropriate control virus, not containing any transgene, was based on the pJM17 adenosiviral vector (31) and was provided by Dr. L. Carpenter. After islets were prelabeled in tissue culture for 44 h as described above, the [3H]inositol-containing medium was removed and retained. Groups of 400–600 islets were incubated in 140 µl of inositol-labeled culture media with 5% polyethylene glycol, to which 20 µl of concentrated adenovirus stock was added (2–5 × 105 plaque-forming units/µl stock). Islets were incubated with virus for 1 h at 37 °C with gentle agitation every 15 min. Infected islets were washed eight times with fresh media without labeled inositol before two washes in reserved inositol-labeled culture media, and recombiant protein was allowed to express during a further 3-h culture in reserved inositol-labeled culture media.

**Inositol Phosphate Studies**—Groups of 50 islets were preincubated at 37 °C for 15 min in 500 µl of a modified KRB medium containing 5 mM NaHCO3, 2.8 mM glucose, 1 mM CaCl2, 0.5% BSA, and 10 mM Hepes, pH 7.4. Premaxed stimulated solutions were added as double concentrated 500-µl stocks in the same medium. Reactions were terminated after 1 min by the addition of 110 mM sodium orthovanadate and, following a 15-min preincubation, stimulated for 1 min in KRB medium containing 0.5 mM carbachol (Carb) where indicated. In some instances 10 or 100 µM genistein (Gen) or 100 µM daidzein (Daid) were added 15 min prior to stimulation. Hydrolysis of PtdIns and PtdInsP2 was quantitated as Ins(1)P1 and Ins(4)P1, respectively. Inositol monophosphate isomers were separated by HPLC using an ammonium phosphate gradient and a Partisphere PAC column. For further details see "Experimental Procedures." Results are presented as percentages of the carbachol-stimulated increment in either Ins(1)P1 or Ins(4)P1, and are means ± S.E. of eight to ten individual determinations. As calculated using Student's t test, * denotes p < 0.05, ** denotes p < 0.001 versus the corresponding value for carbachol alone, and § denotes p < 0.05 as indicated.

**Carbachol-stimulated Tyrosine Phosphorylation**—Incubations and stimulations were as for inositol phosphate studies except that groups of islets were incubated in modified KRB without BSA. Reactions were terminated by immediate removal of the incubation medium and addition of 50 µl of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.6, 5 mM EGTA, 5 mM EDTA, 1% Triton X-100, 2 mM phenylmethylsulfonylfluoride, 5 µg/ml aprotinin, 2 µg pepstatin A, 2 mM benzamidine, and 1 mM EDTA in orthophosphate and transferred to ice. Islets were sonicated using a Branson Sonifier 250 (20 pulses at 10% duty cycle; setting 1) and mixed with 50 µl of 2× Laemmli sample buffer, boiled for 5 min, and centrifuged for 5 min prior to carrying out SDS polyacrylamide gel electrophoresis on 10% gels. Proteins were then transferred to a nitrocellulose membrane at 500 mA for 2 h and blocked for 1 h in a solution of 20 mM Tris-HCl, 137 mM NaCl, and 0.1% Tween 20, pH 7.5, containing 5% milk powder. The membranes were then incubated with 1% BSA for 1 h. Tyrosine-phosphorylated proteins were detected using the anti-phosphotyrosine antibody RC20 for 1 h and visualized directly by enhanced chemiluminescence.

**PLC Expression**—Islet extracts were prepared from 50 islets by addition of 100 µl of 1× Laemmli sample buffer, boiled for 5 min, and centrifuged for 5 min prior to carrying out SDS polyacrylamide gel electrophoresis on 10% gels. The proteins were then transferred to a nitrocellulose membrane at 500 mA for 2 h and blocked for 1 h in a solution of 20 mM Tris-HCl, 137 mM NaCl, and 0.1% Tween 20, pH 7.5, containing 5% milk powder. The membranes were then incubated with 1% BSA for 1 h with an anti-PLC antibody before incubating for 1 h with a horseradish peroxide-conjugated secondary antibody and detection of immunoreactive proteins using the enhanced chemiluminescence system.

**RESULTS**

**In the following experiments breakdown of PtdIns and PtdInsP2 were assessed as increases in Ins(1)P1 and Ins(4)P1 levels, respectively. This approach has been previously verified on the basis of time course and inhibitor studies, as well as flux measurements (9). Changes in steady-state levels of these inositol phosphates at 1 min post-stimulation, as performed here, are not as pronounced as in end point accumulation assays, such as following prolonged stimulation in the presence of LiCl to inhibit inositol phosphate breakdown (30). For technical reasons, however, the rationale underlying measurement of PtdIns hydrolysis as an increase in Ins(1)P1 does not necessarily hold at longer time points nor in the presence of LiCl, which precluded use of these alternative protocols (9). As a first means of addressing whether different PLCs might be involved in mediating hydrolysis of PtdIns and PtdInsP2, we made use of the tyrosine kinase inhibitor, genistein, which has been widely demonstrated to inhibit activation of PLCγ (16, 17). As shown in Fig. 1, genistein reduced carbachol-stimulated PtdIns hydrolysis in a dose-dependent manner with 70% inhibition occurring at 100 µM genistein. Most importantly this inhibition was significantly greater than that seen with an equivalent dose of daidzein, a genistein analogue that does not affect tyrosine kinase activity. However the effects of genistein and daidzein were identical on PtdInsP2 hydrolysis suggesting that, in contrast to PtdIns hydrolysis, this is not specifically

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influenced by tyrosine kinase inhibition.

Although the results above are suggestive of a selective requirement for tyrosine kinases in the PtdIns response, the high nonspecificity of genistein complicated further analysis. Similar nonspecificity has been seen on other parameters when using a range of other tyrosine kinase inhibitors in islet cells (32). We therefore turned to the converse approach of using sodium orthovanadate, a tyrosine phosphatase inhibitor that augments tyrosine phosphorylation of several islet cell proteins (Ref. 33 and not shown). This potently stimulated PtdIns hydrolysis to ~75% of the maximal carbachol response (Fig. 2). The combination of carbachol plus vanadate exerted no greater effects than those because of carbachol alone. In marked contrast, vanadate alone caused only a very modest increase in PtdInsP<sub>2</sub> hydrolysis and actually potentiated the response to carbachol. This is probably explained by the ability of vanadate to stimulate PtdInsP<sub>2</sub> hydrolysis in islets secondary to Ca<sup>2+</sup> influx (34) whereas we have previously shown that Ca<sup>2+</sup> influx is insufficient to promote PtdIns hydrolysis (10). The current experiments therefore suggest that increased tyrosine phos-

phorylation is sufficient to activate PtdIns hydrolysis that occurs via a mechanism that is non-additive (and thus potentially similar) to that of carbachol. Taken together the findings of the genistein and vanadate experiments implicate tyrosine phosphorylation as a component of the mechanism by which carbachol activates PtdIns hydrolysis. Because there should be no requirement for activation of tyrosine kinases in activation of PLC<sub>β</sub> and PLC<sub>δ</sub>, in contrast to PLC<sub>γ</sub> (3, 4), our results also suggest that hydrolysis of PtdIns and PtdInsP<sub>2</sub> are mediated by different PLCs and that PLC<sub>γ</sub> is implicated in the PtdIns response.

We next analyzed the ability of carbachol to increase tyrosine phosphorylation of PLC<sub>γ</sub> and other islet cell proteins. Looking first at islet cell lysates, carbachol increased the tyrosine phosphorylation of a number of proteins, including prominent species at ~30, 32, 36, 38, 64, and 125 kDa (Fig. 3). These responses were rapid in onset (maximal at 1–2 min) but remained elevated over the 30 min of stimulation. Although these experiments established that carbachol could activate tyrosine kinases, there was no marked increase in phosphorylation in substrates around the 145-kDa region, corresponding to PLC<sub>γ</sub>. To address this issue directly, PLC<sub>γ</sub> was immunoprecipitated from lysates of 600 carbachol-treated and untreated islets. Although PLC<sub>γ</sub> was detectable in the immunoprecipitates, its phototyrosine content was extremely low under basal conditions and not increased by prior carbachol stimulation (results not shown).

There is now ample evidence that tyrosine phosphorylation of PLC<sub>γ</sub> itself is not always necessary for its activation. One alternative activation mechanism is via stimulation of PtdIns 3-kinase activity and subsequent generation of phosphatidylinositol 3,4,5-trisphosphate, which binds to the pleckstrin homology domain of PLC<sub>γ</sub> (23–26). This mechanism was investigated in experiments examining the effects of well documented, structurally diverse, PtdIns 3-kinase inhibitors (35) on carbachol-stimulated phosphoinositide hydrolysis. As shown in Fig. 4, 100 nM wortmannin and 50 μM LY294002 inhibited PtdIns hydrolysis by more than 40 and 70%, respectively. Corresponding effects on PtdInsP<sub>2</sub> hydrolysis were much less pronounced, with wortmannin exerting no significant effect and LY294002 inhibiting by less than 30%. Thus PtdIns hydrolysis exhibits a selective requirement for PtdIns 3-kinase, consistent with a role for that enzyme as an upstream regulator of PLC<sub>γ</sub>.

To examine the involvement of PLC<sub>γ</sub> more directly we used
FIG. 4. Effect of PtdIns 3-kinase inhibitors on carbachol-stimulated hydrolysis of PtdIns and PtdInsP. Incubations and experimental analyses were performed as described in the legend to Fig. 1 except that where indicated 100 nm wortmannin (Wart) or 50 nm LY294002 (LY) were added 15 min prior to stimulation with 0.5 mM carbachol (Carb). Hydrolysis of PtdIns and PtdInsP was quantified as Ins(1)P1, and Ins(4)P1, respectively. For further details see “Experimental Procedures.” Results are presented as percentages of the carbachol-stimulated hydrolysis of PtdIns and PtdInsP, respectively, and are means ± S.E. of six to eight individual determinations. As calculated using Student’s t test, * denotes p < 0.01, and ** denotes p < 0.001 versus the corresponding value for carbachol alone.

stimulated phosphoinositide turnover in A-431 cells, now known to involve PLCγ, demonstrating a transient rise in inositol trisphosphate but a persistent increase in total inositol monophosphate (38). Although the latter was interpreted as evidence of PtdIns hydrolysis, it is not clear that this increase actually represented Ins(1)P1 derived from PtdIns. Nevertheless it would now be of interest to re-examine the A-431 cells more closely for direct evidence of PtdIns hydrolysis, using the rigorous analytical criteria we established previously for pancreatic islets (9).

A corollary of our major finding is that PLCγ appeared not to play a role in carbachol-stimulated PtdInsP2 hydrolysis in islet cells. This is consistent with studies demonstrating that PtdInsP2 is targeted in pancreatic islets and β-cell lines by PLCβ1, PLCβ3, and PLCβ1 (30, 36). However it is clearly inconsistent with a host of earlier data from other cell types demonstrating that PLCγ does hydrolyze PtdInsP2 (3, 4). The most obvious explanation for this apparent contradiction is tissue differences, especially because the great majority of previous studies were undertaken with transformed cell lines, instead of the terminally differentiated primary tissue used here. An alternative explanation is that under conditions following occupation of GPCRs, where both PLCβs are activated, PLCβ might compete more effectively than PLCγ for access to PtdInsP2. On the other hand receptor tyrosine kinases, which would not be expected to couple to PLCβ, could stimulate PtdInsP2 hydrolysis through the activation of PLCγ alone. This proposal would need to be supported by direct evidence but is attractive in that it might help to explain the necessity for PLCγ activation, which is presumably not to stimulate PtdInsP2 hydrolysis, because this would be effectively mediated by PLCβ. Another potential explanation is that the substrate preference of PLCγ might be conditioned by its activation mechanism. For example PtdInsP2 is likely to be

FIG. 5. Effect of overexpression of PLCγ1 (A) on carbachol-stimulated hydrolysis of PtdIns and PtdInsP. Isolated islets were cultured as described in the legend to Fig. 1 except that recombinant adenovirus encoding PLCγ1 or control virus (Shuttle) was added for 1 h, 3 h prior to conducting the experiment. A, lysates from islets infected with control adenovirus or PLCγ1 adenovirus were immunoblotted with anti-PLCγ1 antisera. B, following a 15-min incubation, islets were stimulated or not for 1 min with 0.5 mM carbachol (Carb), and PtdIns and PtdInsP were quantified as Ins(1)P1, and Ins(4)P1, respectively. For further details see “Experimental Procedures.” Results are presented as percentages of the carbachol-stimulated increment in either Ins(1)P1 or Ins(4)P1, and are means ± S.E. of six to eight individual determinations. As calculated using Student’s t test, * denotes p < 0.005 versus carbachol control.

recombinant adenovirus to overexpress this enzyme in pancreatic islets, as demonstrated in Fig. 5A. Most importantly this virus caused a 40% increase in carbachol-stimulated PtdIns hydrolysis (Fig. 5B). In marked contrast it did not affect PtdInsP2 hydrolysis at all. The specificity of these findings was confirmed in control experiments using a PLCβ1 adenovirus previously documented to increase PtdInsP2 hydrolysis by ~50% in pancreatic islets treated for 10 min with carbachol in the presence of LiCl (30). Under the conditions of our assay (1-min stimulation, no LiCl) the agonist-stimulated PtdInsP2 response was increased to 119.3 ± 4.7% versus 100 ± 2.3% in carbachol-treated, control virus-infected islets (n = 12, p < 0.005), whereas PtdIns hydrolysis was not significantly affected; 106.5 ± 10.2 versus 100 ± 4.8 (n = 12). Basal responses were not affected by the PLCβ1 virus (not shown).

DISCUSSION

The major finding of the current study is that carbachol-stimulated PtdIns hydrolysis in pancreatic islets is mediated by PLCγ. This conclusion was based partially on the differential sensitivities of the PtdIns and PtdInsP2 responses to genistein, vanadate, wortmannin, and LY294002, which are consistent with the known mechanisms of activation of PLCγ (3, 4). This was confirmed directly by the evidence that overexpression of PLCγ1 but not PLCβ1 resulted in a selective increase of agonist-stimulated PtdIns breakdown. Although we have not formally assessed the PLCβ family members in this context, we consider their involvement highly unlikely. As is the case in other cell types (14, 15), PLCβ is known to be activated in β-cells as a direct consequence of a rise in intracellular Ca2+ (36). Although this is sufficient to promote PtdInsP2 hydrolysis in pancreatic islets (37), we have previously shown this was not the case for PtdIns breakdown (10). Our current data are also supported by earlier findings that PLCγ efficiently utilizes both PtdInsP2 and PtdIns as substrates in activity assays in vitro, whereas the PLCβ and δ and families show a much higher degree of specificity for PtdInsP2 (20). However our results are the first to demonstrate directly that PLCγ acts on PtdIns in a more physiological setting using intact cells. This might seem surprising, but in practice PtdIns hydrolysis would be difficult to detect unless specifically addressed. There is one earlier study of epidermal growth factor
sequenced in vivo by binding proteins such as profilin, which have much lower affinities for PtdIns(39). In principle, therefore, PtdIns should be easier for PLCγ to access than PtdInsP2, and indeed there is some evidence that tyrosine phosphorylation of PLCγ is necessary for displacement of profilin (40). Consistent with this, our findings would suggest that PLCγ does not need to be tyrosine-phosphorylated to hydrolyze PtdIns, although there was at least a partial requirement for activation of an upstream tyrosine kinase. In addition, PtdIns hydrolysis was dependent on the activation of PtdIns 3-kinase. Although a well documented route for stimulation of PLCγ, this has only been addressed previously in the context of PtdInsP2 hydrolysis (23–26). Likewise there is very little information on whether other potential mechanisms for activation of PLCγ, such generation of phosphatidic acid (19) or arachidonic acid (20), might be also be involved in regulation of PtdIns hydrolysis.

Increases in protein phosphotyrosine content in response to carbachol or other GPCRs have not been demonstrated previously using pancreatic islets. In one earlier study (43) carbachol increased tyrosine phosphorylation of a single, unidentified protein of 125 kDa in a transformed insulin-secreting cell line, whereas it had been reported previously that 15-min muscarinic stimulation of pancreatic islets was without effect (33). These results are clarified and extended by our findings of an early (maximal at 1–2 min) incorporation of phosphate into tyrosine residues of a number of proteins, including one around 125 kDa. Tyrosine phosphorylation can therefore be added to the other known responses of pancreatic islets to muscarinic receptor occupation. In addition to the hydrolysis of both PtdIns and PtdInsP2 (5–10), these include activation of protein kinase C (44, 45), mobilization of diacylglycerol in response to stimulation of a variety of tissues (41, 42, 48). In addition, hydrolysis of PtdIns, but not of PtdInsP2, is directly influenced by the prevailing membrane potential (10) and might therefore serve as a means of integrating signals arising from different external stimuli. Direct experimental support for these proposals will await a better understanding of the mechanisms underlying the two processes, especially of the tyrosine kinases and their substrates that control PtdIns hydrolysis. However this would be more conveniently addressed in a model system that is more amenable to molecular intervention and biochemical analysis than are isolated pancreatic islets.

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REFERENCES

1. Berridge, M. J. (1993) Nature 361, 315–325
2. Irvine, R. F. (1998) Curr. Biol. 8, 557–559
3. Rhee, S. G., and Bae, Y. S. (1997) J. Biol. Chem. 272, 15045–15048
4. Suh, P. G., Bae, Y. S., and Rhee, S. G. (1999) Chem. Phys. Lipids 98, 3–11
5. Zawalich, W. S., and Rasmussen, H. (1990) Mol. Cell. Endocrinol. 70, 119–137
6. Iismaa, T. P., Kerr, E. A., Wilson, J. R., Carpenter, L., Sims, N., and Biden, T. J. (2000) Diabetes 49, 382–388
7. Morgan, N. G., Runford, G. M., and Montague, W. (1985) Biochem. J. 226, 713–718
8. Wollheim, C. B., and Biden, T. J. (1986) J. Biol. Chem. 261, 8314–8319
9. Biden, T. J., Pringe, M. E., and Davison, A. G. M. (1992) Biochem. J. 285, 541–549
10. Biden, T. J., Davison, A. G. M., and Pringe, M. L. (1993) J. Biol. Chem. 268, 17226–17232
11. Song, C., Hu, C.-D., Masago, M., Kariya, K., Yamawaki-Kataoka, Y., Shibaotohe, M., Wu, D., Satoh, T., and Kataoka, T. (2001) J. Biol. Chem. 276, 2755–2767
12. Lopez, I., Mak, E. C., Ding, J., Hamm, H. E., and Lomasney, J. W. (2001) J. Biol. Chem. 276, 2758–2765
13. Kelley, G. G., Reks, E. S., Orndrake, J. M., and Smrcka, A. V. (2001) EMBO J. 20, 743–754
14. Banno, Y., Okano, Y., and Nozawa, Y. (1994) J. Biol. Chem. 269, 15846–15852
15. Kim, Y.-H., Park, T.-J., Lee, Y. H., Baek, J. K., Suh, P.-G., Rhy, S. H., and Kim, Y. S. (1999) J. Biol. Chem. 274, 30127–30134
16. Guosouy, F., Leuders J. E., Kohn, E. C., and Felder, C. C. (1993) J. Biol. Chem. 268, 7768–7772
17. Marrero, M. B., Paxton, W. G., Duff, J. L., Berk, B. C., and Bernstein, K. E. (1994) J. Biol. Chem. 269, 10935–10939
18. Hernandez-Sotomayor, S. M. T., and Carpenter, G. (1993) Biochem. J. 293, 507–511
19. Jones, G. A., and Carpenter, G. (1993) J. Biol. Chem. 268, 20845–20850
20. Hwang, S. C., Hong, D.-K., Bae, Y. S., Kim, J. H., and Rhee, S. G. (1996) J. Biol. Chem. 271, 18342–18349
21. Yeo, E.-J., Provost, J. J., and Exton, J. H. (1997) Biochim. Biophys. Acta 1256, 308–320
22. Baldassare, J. J., Henderson, P. A., Tarver, A., and Fisher, G. J. (1997) J. Biol. Chem. 272, 283–288
23. Fiala, M., Logan, S. K., Lehto, V. P., Baccante, G., Lemmon, M. A., and Vogelstein, B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2519–2524
24. Gasa, R., Trinh, K. Y., Yu, K., Wilkie, T. M., and Newgard, C. B. (1999) Diabetes 48, 1035–1044
25. Becker, T. C., Noel, R. J., Coats, W. S., Gomez-Foix, A. M., Alam, T., Gerard, W. H. (1988) Biochem. Biophys. Res. Commun. 161, 1189–1195
26. Jonas, J.-C., Plant, T. D., Gilon, P., Dietmary, P., Nenquin, M., and Henquin, J.-C. (1995) Br. J. Pharmacol. 114, 872–880
27. Jonas, J.-C., and Henquin, J.-C. (1996) J. Biol. Chem. 271, 12313–12319
28. Zhang, A., Gao, Z.-Y., Gilon, P., Nenquin, M., Drews, G., and Henquin, J.-C. (1991) J. Biol. Chem. 266, 21649–21656
29. Davies, S. P., Reddy, H., Caivan, M., and Cohen, P. (2000) Biochem. J. 351, 103–105
30. Ishiihara, H., Wada, T., Kizuki, N., Asano, T., Yaraki, Y., Kikuchi, M., and Oka, Y. (1999) Biochem. Biophys. Res. Commun. 254, 77–82
31. Biden, T. J., Peter-Risch, B., Schlegel, W., and Wollheim, C. B. (1987) J. Biol. Chem. 262, 3567–3571
32. Tilly, B. C., Van Paridon, P. A., Verlaan, L., De Laat, S. W., and Moelenar, W. H. (1988) Biochem. J. 252, 857–863
33. Goldschmidt-Clermont, P. J., Machesy, L. M., Baldassare, J. J., and Pollard, T. D. (1990) Science 247, 1575–1578
34. Goldschmidt-Clermont, P. J., Kim, J. W., Machesy, L. M., Rhee, S. G., and Pollard, T. D. (1991) Science 251, 1241–1243
35. Nakashima, S., Suganuma, A., Matsui, A., and Nozawa, Y. (1991) Biochem. J. 275, 355–361
36. Tokuwa, Y., Takuwa, N., and Rasmussen, H. (1986) J. Biol. Chem. 261, 265–269
43. Konrad, R. J., Dean, R. M., Young, R. A., Billings, P. C., and Wolf, B. A. (1996) *J. Biol. Chem.* **271**, 24179–24186
44. Persaud, S. J., Jones, P. M., Sugden, D., and Howell, S. J. (1989) *Biochem. J.* **264**, 753–758
45. Easom, R. A., Landt, M., Colea, J. R., Hughes, J. H., Turk, J., and McDaniel, M. (1990) *J. Biol. Chem.* **265**, 14938–14946
46. Henquin, J.-C. Garcia, M.-C., Bezem, M., Hermans, M. P., and Nenquin, M. (1988) *Endocrinology* **122**, 2134–2142
47. Gilon, P., Nenquin, M., and Henquin, J.-C. (1995) *Biochem. J.* **311**, 259–267
48. Griendling, K. K., Rittenhouse, S. E., Brock, T. A., Ekstein, L. S., Gimbrone, M. A., and Alexander, W. A. (1986) *J. Biol. Chem.* **261**, 5901–5906
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