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Immunohistochemical localization of eight phospholipase C isozymes in pancreatic islets of the mouse

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Abbreviations: PLC, phospholipase C; PBS, phosphate-buffered saline; DAB, 3,3-diaminobenzidine

Abstract

The possible involvement of phospholipase C (PLC) in the regulation of insulin secretion is not clearly understood and neither its isozymes expressed nor cellular localization in the pancreatic islets is known. By using specific monoclonal antibodies, we have investigated the expression and localization of eight different PLC isozymes, β1, β2, β3, β4, γ1, γ2, δ1, and δ2, in the pancreatic islets of adult mice. Immunohistochemical analysis carried out on paraffin embedded sections showed a distinct pattern of expression for each of the PLC isozymes. In the central part of the islets containing β cells, a high level of β4 and moderate levels of β3 and γ1 were expressed, whereas PLC-β1 and –γ1 were abundantly expressed in the exocrine pancreas. These results demonstrated the heterogeneity in expression of the phospholipase C isozymes in pancreatic islets. It is conceivable that these isozymes are coupled to different receptors and perform selective tasks in the regulation of insulin secretion for glucose homeostasis.

Keywords: pancreatic islet, β cell, insulin, isozymes, phospholipase C

Introduction

Glucose is the major stimulant for insulin secretion; however, the exact mechanism by which glucose induces insulin secretion still remains to be defined (Vadakekalam *et al*., 1997). Glucose can augment insulin secretion independently of K^+ channel closure, provided that the cytoplasmic free Ca^{2+} concentration is elevated (Gembal *et al*., 1992). A role for phospholipase C (PLC) in this phenomenon has been both claimed (Turk *et al*., 1993; Zawalich and Zawalich, 1997) and refuted (Gembal *et al*., 1993; Vadakekalam *et al*., 1997). Kelly *et al.* (1994) suggested that the enhanced phosphoinositide (PI) response was partially Ca^{2+}-dependent and might involve activation of distinct isozymes of PLC expressed in the islets. Nevertheless, scarce attention has been paid to determining whether PLC isozymes display a selective localization in different parts of the pancreatic islets. Information on the cellular localization of PLC proteins in an organ showing a remarkable cellular heterogeneity is essential for a better understanding of the specific functions of each isotype. However, current information about the cellular localization of PLC isozymes has so far been mainly limited to the brain and related organs (Ross *et al*., 1989; Peng *et al*., 1997). Therefore the pancreatic islet appears to be an interesting region in which to study the distribution of PLC isozymes mainly to provide clues to solving the controversy concerning the role of PLC isozymes in insulin secretion (Gembal *et al*., 1993; Vadakekalam *et al*., 1997). Moreover, several protein kinase C (PKC) isozymes, the downstream molecules for the PLC signaling pathway, were found from mouse pancreatic islets (Knutson and Hoenig, 1997). In addition, several reports suggested an active role of G proteins in the insulin regulation (Sharp, 1996). There is some scattered evidence available to suggest that pancreatic islets express certain PLCs (Kelly *et al*., 1995; Zawalich *et al*., 1995; Jonas and Henquin, 1996). However, detailed investigation is necessary to understand their precise localizations.

In the present studies we have investigated the expression and localization of eight different PLC isozymes, β1, β2, β3, β4, γ1, γ2, δ1, and δ2, in the pancreatic islets of adult mice using specific monoclonal antibodies. It was found that pancreatic islets expressed PLC-γ1, –β3, and –β4, by immunohistochemistry. To our knowledge, this is the first report to examine the complete set of PLC isozymes in pancreatic islets by immunohistochemistry.

Materials and Methods

Tissue preparation

Pancreas tissues were obtained from 8 week-old mice

of the wild type C57Bl/6J strain or mutant mice (for PLC-β1 or -β4) which were anesthetized and perfused transcardially with 4% paraformaldehyde in phosphate-buffered saline (PBS). The tissues were immediately fixed with 10% neutral buffered formalin, dehydrated in ethanol, and embedded in paraffin wax. For immunoblotting, pancreatic islets were obtained by picking from fresh pancreatic tissues on an inverted microscope. The PLC-β1 and the PLC-β4 knock out mice have been described previously (Kim *et al.,* 1997). For hormone studies, both wild type and knock out mice were sacrificed after 16-h fasting.

Immunohistochemistry

Serial 5-μm sections were cut from paraffin embedded tissues. After deparaffinization and hydration, sections were incubated for 20 min with 10% normal goat serum to block nonspecific serum protein bindings. Immunohistological staining was done as described previously using monoclonal antibodies diluted 1:200 (Suh *et al.,* 1988; Lee *et al.,* 1993; Noh *et al.,* 1994). After incubation for 2 h, sections were washed three times in phosphate-buffered saline and incubated with streptavidin-biotin reagents from the Dako LSAB (labeled streptavidin biotin) Stain kit (Carpenteria, USA). Dako CSA (catalyzed signal amplification) kit was also used to amplify the intensity of staining for PLC-β1, -β2, -β3 and -δ2. In cases of negative control of PLC-γ1, -γ2 and -δ1, parallel sections were incubated with either PLC-γ1, γ2 and δ1 antibodies absorbed with corresponding peptides or PBS, substituting primary antibodies (PLC-β2, -β3, and -δ2). PLC-β1 and β4 knock-out mice were used as negative controls for the PLC-β1 and β4 staining. As positive controls, the immunohistochemical procedures were repeated using the most representative sections of the various tissues known for expression of each of the isozymes (Homma *et al.,* 1993; Watanabe *et al.,* 1998) (PLC-β1: pyramidal cells of cerebrum, PLC-β2: oligodendrocytes of brain, PLC-β3: myocardial cells of the heart, PLC-β4: Purkinje cells of cerebellum, PLC-γ1: well-differentiated colon cancer tissue, and PLC-γ2: human B cell lymphoma tissue, PLC-δ1: brain tissue). We did not perform staining on positive control tissue for -δ2. For staining of several hormones, basically the similar method was used. All sections were reacted with 3,3-diaminobenzidine (DAB, Dako), DAB with Nickel (Vector, Burlingame, USA), or 3-amino, 3-ethyl carbazole (Dako) as the chromogen and photographed under the Olympus BH2 light microscope.

Results

As shown in Figure 1, mouse pancreatic islets express certain PLC isozymes, but not all eight isozymes (Figure 1 A-H). The specificity of the immunoreaction was verified by using knock-out mice or by repeating the experiments with antibodies that had been pretreated with the corresponding peptide in the cases of PLC-β1, -β4, -γ1, -γ2 and -δ1 (no peptide was available for monoclonal antibodies to PLC-β2, -β3, and -δ2). After the pretreatment or in knock-out mice, any immunoreactivity was completely abolished in each case. Moreover we applied several negative and positive controls, as described in Materials and Methods, and results on the observed localization of the various PLC isozymes were in good agreement with the results available in the literature (Suh *et al.,* 1988; Ross *et al.,* 1989; Homma *et al.,* 1993; Lee *et al.,* 1993; Noh *et al.,* 1994; Peng *et al.,* 1997; Watanabe *et al.,* 1998).

The PLC-β1 immunoreactivity was observed in the exocrine pancreas but not in the islets of the wild type mice, whereas this was not observed in the PLC-β1 knock-out mice. We tried to amplify the signal using the Dako CSA kit, but this isozyme was still not detectable in pancreatic islet. PLC-β2 showed a negative result in the islets even with the amplification staining method, although the same antibody detected this enzyme in oligodendrocytes of the brain. PLC-β3 was negative in the islet by the routine staining method, whereas it was positive in the cardiac muscle cells. But when using the amplification staining method, this enzyme showed strong immunoreactivity in the pancreatic islet. Diffuse and intense expression of PLC-β4 was detected in the cytoplasm of islet cells even without the signal amplification. We used PLC-β4 knock-out mice as a negative control, and the definite and intense expression of PLC-β4 obtained in wild type mice was abolished in the pancreatic islet of the knock-out mice (Figure 2A, B). The PLC-β4 positive cells were located in the central part of the pancreatic islet, and were confirmed to be beta cells since they were also positive for insulin and negative for glucagon, somatostatin (Figure 3A-D), and pancreatic polypeptide (data not shown). PLC-γ1 was positive in both exocrine and endocrine pancreas. These positive reactions were abolished with the prior incubation of anti PLC-γ1 antisera with PLC-γ1 peptide (Figure 2C, D), indicating that the diffuse PLC-γ1 staining in the pancreas was specific. The PLC-γ2, -δ1, and -δ2 were not expressed in the pancreas even with the amplification method, although these enzymes were positively stained in B-lymphocytes (γ2) or neurons of the brain (δ1). In summary, we detected strong staining for PLC -β4, and moderate staining for PLC -β3 and -γ1 in the pancreatic islet, whereas β1 and γ1 predominated in the exocrine part.

Discussion

Even though the presence of several PLC isozymes was confirmed in pancreatic islets (Jonas and Henquin,

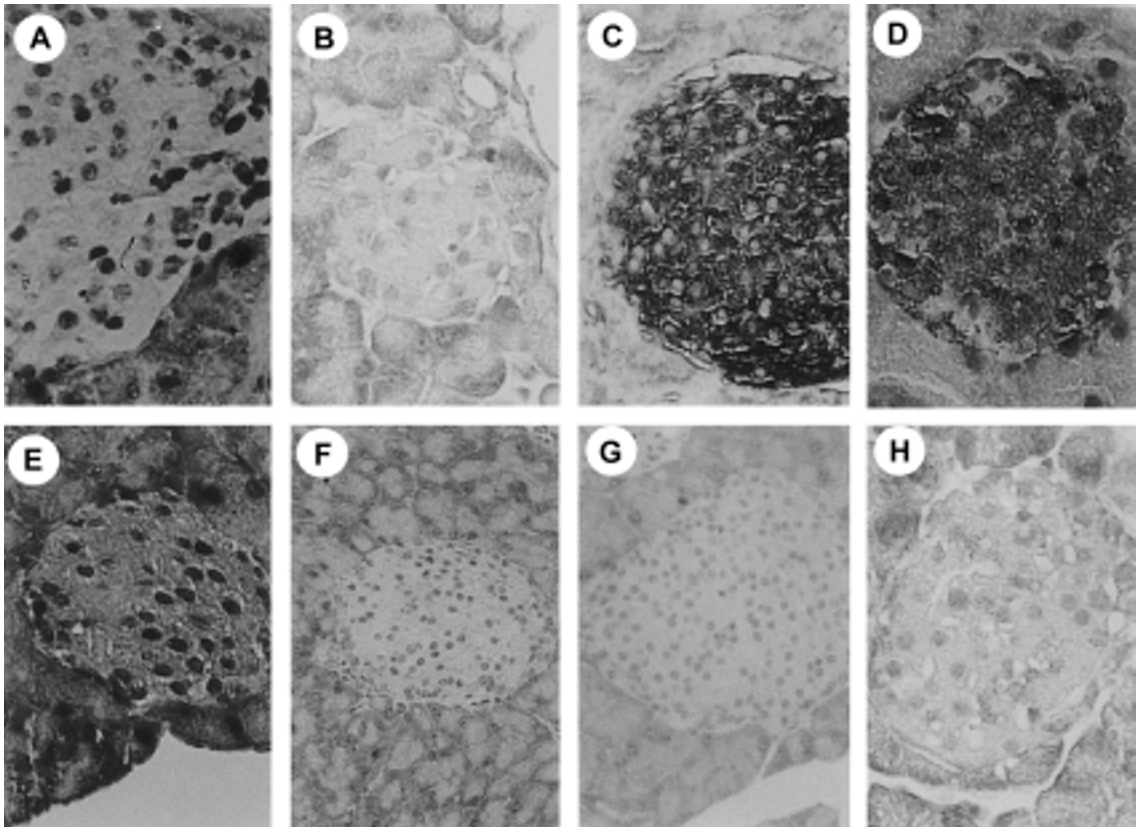

Figure 1. Immunohistochemistry of PLC isozymes in pancreatic islet: PLC-β3, β4, γ1 were expressed in islet, β1 and γ1 were in exocrine pancreas. A. PLC-β1, B. PLC-β2, C. PLC-β3, D. PLC-β4, E. PLC-γ1, F. PLC-γ2, G. PLC-δ1, H. PLC-δ2.

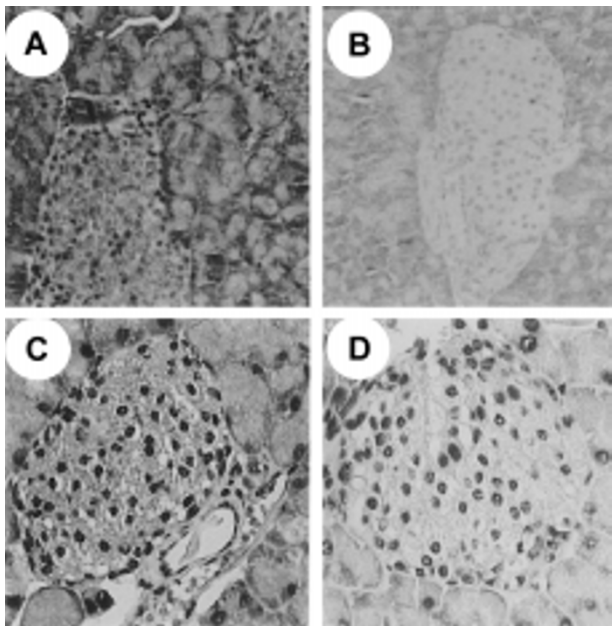

Figure 2. PLC-β4 and γ1 expression in pancreatic islet with negative controls: the expressions of PLC-β4 and γ1 were abolished in negative controls. A. PLC-β4 in wild type mice (+/+), B. PLC-β4 in knock-out mice (–/–), C. PLC-γ1 with monoclonal antibody, D. PLC-γ1 with preadsorption.

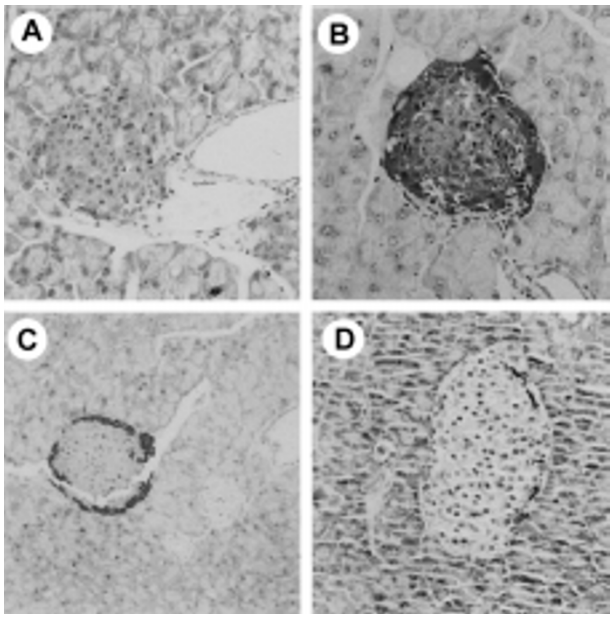

Figure 3. PLC-β4 expression in beta cells of pancreatic islet; the beta cells expressed PLC-β4. A. PLC-β4 immunostain in beta cells of pancreatic islet, B. Insulin immunostain in beta cells of pancreatic islet, C. Glucagon immunostain in alpha cells of pancreatic islet, D. Somatostatin immunostain in delta cells of pancreatic islet.

1996; Zawalich *et al.,* 1995; Kelly *et al.,* 1995), systematic classification has been difficult due to limited knowledge on their distribution. Zawalich and Zawalich (1997) demonstrated an augmentation of Ca^{2+}-induced inositol phosphate generation in rat islets when diazoxide is present. They further suggested that activation of the PLC-PKC pathway plays an important role in the fuel-induced insulin secretion. The present study aimed at the comprehensive examination on the localization of eight isozymes of PLC in pancreatic tissues.

PLC-β1 is known to mediate pertussis toxin-insensitive phosphoinositide hydrolysis (Rhee and Choi, 1992). Coupling of the enzyme to a cell-surface receptor is mediated by GTP-binding protein Gq (Rhee and Choi, 1992). In this study, PLC-β1 expression was not found in pancreatic islets and only at a low level in exocrine pancreas, as reported previously (Piiper *et al.,* 1997). No evidence of the PLC-β2 was found in both exocrine and endocrine pancreas even with the CSA amplification method. These findings suggest that PLC-β1 and -β2 do not participate in the insulin regulation signaling.

We found a moderate expression of PLC-β3 in pancreatic islets using the amplification method, whereas no expression was found with the routine ABC method, using the LSAB kit, indicating that pancreatic islets express PLC-β3 at a relatively lower level than any other organs such as the heart. Considering that PLC-β3 is present ubiquitously and PLC-β3 knock-out mice died at early embryo (Wang *et al.,* 1998), PLC-β3 may play some role in insulin secretion. Further investigation, however, is necessary to define its precise functional role during the insulin secretion process.

Recent reports implicated that PLC-β4 was activated by the a subunit of the Gq family of G proteins to mediate phosphoinositide hydrolysis (Ross *et al.,* 1989; Peng *et al.,* 1997). There are both selectivity and promiscuity in the G protein action inside the β cells (Sharp, 1996). Specifically, Gq and Gα11 protein, which are known to activate PLC-β4, are located in the islets (Verspohl and Herrmann, 1996; Shah BH, 1999). Our results suggest a possibility that PLC-β4 is an important constituent in the signaling pathway inside the β cells of the pancreatic islet.

PLC-γ1 was also expressed in the central part of the islets as well as in the exocrine pancreas. PLC-γ1 is known to be involved in the secretory function of the acinar cells (Wrenn *et al.,* 1996). Its location of expression suggests the PLC-γ1's role in mediating transduction events underlying trophic signals inside the insulin-secreting β cells. This idea is consistent with the observation that the γ1 isotype is involved in cellular response to growth factors.

PLC-γ2, -δ1 and -δ2 were not found in the pancreatic islet. Gasa *et al.* (1999) recently reported that PLC-β1, -β2, -β3, -γ1, and -δ1 were expressed in insulinoma cell line and rat islets. Also, the report by Kim *et al.* (2001) demonstrated that all four PLC-β isozymes and two PLC-δ were detected in the islets, but PLC-γ1 and -γ2 were not observed in rat pancreatic islets. The possible explanations for the discrepancy between the results of ours and that of others include the differences of species (mouse vs rat), specimens (tissue vs cell), methods (immunohistochemistry vs immunoblotting), and antibodies used.

In conclusion, we believe that our data add valuable insight into understanding the possible function of PLC isozymes in the regulation of insulin secretion. However, it still remains to be elucidated that these molecules in fact play inhibitory or stimulatory functions in insulin secretion.

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