

Phospholipid hydrolysis and insulin secretion: a step toward solving the Rubik's cube

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In a 1991 review article titled “The pancreatic islet as Rubik's cube. Is phospholipid hydrolysis a piece of the puzzle?” (26), Stewart Metz concluded that “an obligate role for phospholipase activation in glucose-induced insulin secretion is not yet rigorously established, despite tantalizing, inferential evidence.” This statement remained accurate for more than 15 years. Recent studies by Bao and colleagues (2, 5) and Jacobson et al. (13), including a recent article appearing in *AJP-Endocrinology and Metabolism* (2), provide key supportive evidence for a role of Group VIA phospholipase A₂ (iPLA₂β) in insulin secretion.

Defective insulin secretion from the pancreatic β-cell causes type 2 diabetes, a disease estimated to affect 6% of the world's population (11a). The molecular nature of the β-cell defect is still elusive, in part due to our incomplete understanding of the complex physiological mechanisms regulating insulin secretion. Glucose-stimulated insulin secretion (GSIS) involves a triggering pathway capable of inducing insulin release and an amplifying pathway that operates only when the triggering pathway is activated (11). The triggering pathway, also called ATP-sensitive potassium (K_{ATP}) channel-dependent pathway, involves an increase in ATP/ADP ratio following mitochondrial glucose metabolism, closure of the K_{ATP} channel, membrane depolarization, opening of the voltage-gated Ca²⁺ channel, influx of intracellular Ca²⁺, and insulin exocytosis. The nature of the amplifying pathway remains debated, probably because it involves several coupling factors (21–23, 27). In fact, signals derived from the malonyl-CoA/long-chain acyl-CoA pathway (29), NADPH (7, 20), citrate derived from cataplerotic reactions (8), glutamate (24), and GTP (15) have all been proposed as coupling factors for GSIS (Fig. 1). A role for lipid signaling molecules derived from phospholipase A₂-mediated hydrolysis of membrane phospholipids was first suspected some 25 years ago (16) but has remained uncertain until now.

Several lines of evidence support a role for phospholipid hydrolysis in insulin secretion. First, arachidonic acid (AA) represents ~30% of the fatty acid mass in islet glycerophospholipids (30). Second, glucose stimulates the release of AA in islets (34); blockade of AA release suppresses insulin secretion (31, 33), and both AA and lysophospholipids (released upon phospholipase A-mediated hydrolysis of phospholipids)

have well-documented signaling properties. Gross et al. (10) identified a cytosolic, Ca^{2+} -independent, and ATP-stimulated phospholipase A2 activity that prefers AA in the sn-2 position of the glycerol backbone, and Ma and colleagues (17, 19) cloned the 84-kDa isoforms of iPLA β in islets. Inhibition of the enzyme by its suicide substrate bromoenol lactone also inhibits insulin secretion (32). In addition, overexpression (18) or siRNA knockdown (1) of iPLA β in insulin-secreting cells respectively amplifies and reduces GSIS. Although clearly insightful, these studies did not provide conclusive evidence for a role of iPLA β in insulin secretion in physiologically relevant models, and interpretation of experiments using bromoenol lactone is confounded by the ability of this compound to inhibit other lipases (14).

Recent studies by Bao and colleagues have used genetic mouse models to further examine the functional roles of iPLA β in vivo. First, they generated iPLA β -knockout (KO) mice and reported reduced male fertility (4) and altered macrophage function (3). Second, they examined islet membrane phospholipid composition by electrospray ionization mass spectrometry and showed a virtually identical proportion of arachidonate-containing phosphatidylcholine species in iPLA β -KO and wild-type (WT) islets, arguing against a housekeeping role for iPLA β in regulating arachidonate incorporation into phosphatidylcholine (5). Third, they observed that isolated islets from iPLA β -KO mice have reduced insulin secretion in response to glucose and the cAMP-elevating agent forskolin (5). Fourth, although female iPLA β -KO mice had normal fasting and fed blood glucose levels, they developed more severe hyperglycemia upon administration of the β -cell toxin streptozotocin and became more glucose intolerant in response to high-fat feeding than WT mice (5). In a recent article appearing in *AJP-Endocrinology and Metabolism*, Bao et al. (2) complemented their loss-of-function strategy with a gain-of-function approach and generated iPLA β -transgenic (Tg) mice overexpressing iPLA β under the rat insulin 1 promoter. They demonstrated that iPLA β activity in iPLA β -Tg islets was elevated severalfold over that of the endogenous enzyme but that the morphology of the islets was not altered. Male iPLA β -Tg mice had higher circulating insulin and lower blood glucose levels than WT animals both in the fasting state and after glucose administration, yet insulin tolerance was similar in iPLA β -Tg and WT animals. Unexpectedly, insulin secretion in response to glucose was not increased in islets from iPLA β -Tg mice, although potentiation of GSIS by forskolin was greater in iPLA β -Tg islets. Conversely, as previously shown (5), forskolin potentiation of GSIS was reduced in islets from iPLA β -KO mice. GSIS in the absence of forskolin also tended to be reduced, although, in contrast to that study, the difference was not significant. Also in contrast to previous results using female mice (5), male iPLA β -KO animals had impaired fasting glucose and abnormal glucose tolerance. Neither the rate of incorporation of labeled arachidonic acid nor membrane phospholipid composition was significantly different between islets from iPLA β -Tg and -WT mice. The mechanism by which altered iPLA β activity affects GSIS appears to involve modulation of the delayed rectifier potassium channel Kv2.1. Kv2.1 is a voltage-gated channel that repolarizes the cell membrane after depolarization, thereby providing an “off” signal for insulin release (28). Previously, the same group of investigators (13) demonstrated that glucose inactivation of the Kv2.1 channel was mediated by AA. They now show that, in islets from iPLA β -Tg mice, inactivation of the Kv2.1 channel in response to glucose is more

pronounced, Kv currents are reduced, and the increase in cytosolic Ca^{2+} is more sustained. These changes are similar to those observed in islets from Kv2.1-KO mice (12). On the basis of these results, those authors proposed that glucose activates iPLA β -mediated AA release from membrane phospholipids, which restrains the activity of the Kv2.1 channel and results in amplification of intracellular Ca^{2+} influx and potentiation of insulin secretion (Fig. 1).

These results provide important information and also raise a number of questions. First, the role of iPLA β in the maintenance of glucose homeostasis under normal conditions is not entirely clear due to differences between males and females and between in vivo and in vitro situations (2, 5). In this regard, examining insulin secretion in vitro in dynamic perfusion studies may reveal differences in the phasic release of insulin that may not be apparent in static incubations. Since the mechanisms of action of iPLA β appear to involve the Kv2.1 channel, one might predict that first-phase insulin secretion will be prolonged or shortened in islets from iPLA β -Tg and -KO, respectively. Second, the relationship between iPLA β and the cAMP/protein kinase A pathway remains unclear. Alterations of forskolin potentiation of GSIS upon modulation of iPLA β activity might be interpreted either as a direct interaction between iPLA β and the cAMP/protein kinase A pathway, which remains to be characterized, or as an indirect consequence of altered GSIS. Examining whether the response to other amplifying agents and nonglucose secretagogues is also changed in iPLA β -KO and -Tg islets might help clarify this point. Third, it might be informative to assess the insulin response to glucose in iPLA β -KO and -Tg islets under conditions in which intracellular Ca^{2+} is elevated and the K_{ATP} channel is held open [i.e., in the presence of KCl and diazoxide (9)] to better ascertain the specific contribution of iPLA β to the K_{ATP} -independent pathway. Fourth, elucidating the exact mechanism(s) by which iPLA β /AA modulates the Kv2.1 channel may also be of interest, e.g., direct physical interaction to alter channel structure, intermediary factors, etc. Finally, although the assumption is that the changes in insulin secretion observed upon gain- or loss-of-function of iPLA β are mediated by changes in glucose-induced AA release and the resulting modulation of the Kv2.1 channel, intracellular AA levels were not directly measured in these studies. In this regard, the second product of the iPLA β -mediated phospholipid hydrolysis, lysophosphatidic acid, is known to also influence insulin secretion (25), and its potential contribution to the changes in insulin secretion in islets from iPLA β -KO and -Tg mice remains to be determined.

Nevertheless, these studies provide a significant advancement to our understanding of the role of iPLA β in insulin secretion. First, they demonstrate that iPLA β has a signaling function rather than a housekeeping role in β -cells. Second, although iPLA β appears dispensable for the maintenance of blood glucose levels under normal conditions, it is one of the (probably many) mechanisms by which the β -cell mounts a compensatory response to the increased secretory demand imposed, for example, by high-fat feeding. Third, the mechanism of action of iPLA β involves modulation of the Kv2.1 channel activity and Ca^{2+} currents. These findings provide a positive answer to Stewart Metz's question (26) by demonstrating that, indeed, phospholipid hydrolysis is a piece of the Rubik's cube. Although an obligate role for phospholipase activation in GSIS is still uncertain, the results of Bao and colleagues (2, 5) clearly demonstrate that iPLA β belongs to the growing list of intracellular pathways contributing to the regulation of insulin secretion.

Acknowledgments

Many thanks to Dr. Marc Prentki (Montreal Diabetes Research Center, University of Montreal, Montreal, QC, Canada) for critical reading of the manuscript.

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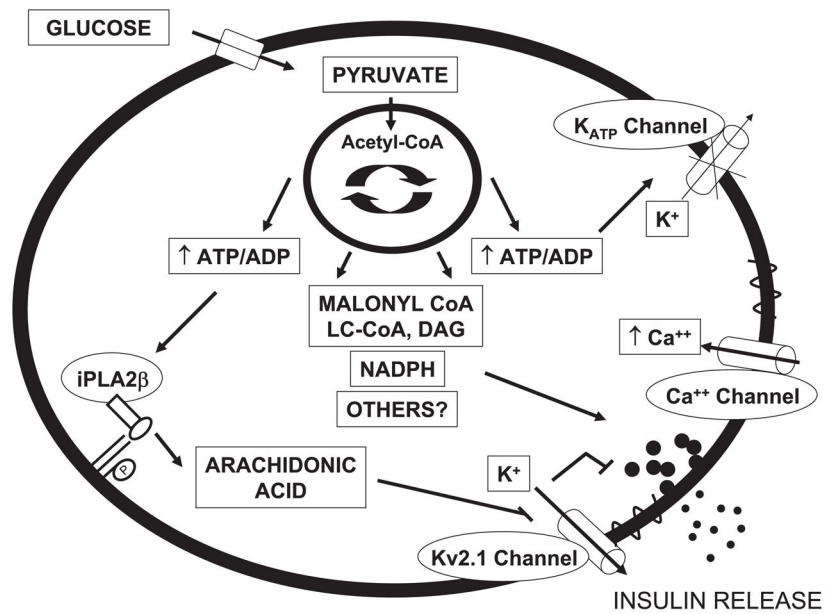


Fig. 1.

Schematic representation of stimulus-secretion coupling mechanisms in the pancreatic β -cell. Mitochondrial metabolism of glucose generates coupling factors that all contribute to stimulating insulin release. The triggering pathway of insulin secretion involves an increase in ATP/ADP ratio, closure of the ATP-sensitive potassium (K_{ATP}) channel, membrane depolarization, opening of the voltage-gated Ca^{2+} channels, Ca^{2+} influx, and insulin exocytosis. The voltage-gated delayed rectifier $Kv2.1$ channel contributes to repolarizing the cell membrane and provides an “off” signal for insulin release. Mitochondrial signals, such as those derived from metabolism of long-chain acyl-CoA (LC-CoA), NADPH, and others, amplify the triggering pathway and potentiate insulin secretion. Bao and colleagues (2, 5) and Jacobson et al. (13) now provide evidence for an additional amplifying pathway involving Group VIA phospholipase A_2 ($iPLA_2\beta$)-mediated hydrolysis of membrane phospholipids, arachidonic acid release, and inhibition of the $Kv2.1$ channel. DAG, diacylglycerol.