Perspectives in Diabetes

Modulation of Insulin Secretion From β-Cells by Phosphoinositide-Derived Second-Messenger Molecules

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In isolated islets, the hydrolysis of membrane phosphoinositides (PI) participates in the transduction of both extracellular and intracellular signals into an effective insulin secretory response. A wide variety of potential second-messenger molecules are generated during the phospholipase C–mediated cleavage of these strategically situated membrane phospholipids. Several distinct but interrelated issues are addressed in this perspective. These include *1*) methodological approaches utilized to assess PI turnover, *2*) the synergistic relationship between PI-derived second messengers and cAMP, *3*) the contribution of changing PI turnover rates to the biphasic pattern of insulin output induced by 20 mM glucose, and *4*) the role played by PI turnover in the phenomenon of "memory" displayed by islets after prior stimulation with various agonists. The concept that events unique to PI turnover contribute to β-cell activation is well founded. Because of uncertainty regarding the exact nature of all PI-derived messengers, however, it is not yet possible to mold the available information into a comprehensive theory of β-cell activation. Future studies will have to address various important unresolved issues. *Diabetes* 37:137–41, 1988

There is virtual unanimity concerning the preeminence played by changing rates of glucose usage in the insulin secretory response to the hexose (1–4). However, despite 20 yr of intensive research effort, there is no agreement as to the precise nature of the metabolic intermediate(s) and/or cofactor(s) that actually function to trigger insulin output from these cells (5). Over the past several years, experiments with various excitable tissues have demonstrated that the transduction of an externally applied signal into an effective biologic response is accompanied by the hydrolysis of membrane phosphoinositides (PI) (6,7). The phospholipase C (PLC)-mediated cleavage of these membrane phospholipids is thought to be activated by the interaction of an extracellularly confined hormone or neurotransmitter with a specific membrane receptor (Fig. 1). This theory of cell activation provides a reasonable explanation as to how a cell might respond when confronted with a molecule confined to the interstitium.

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IS A SIGNAL GENERATED INTRACELLULARLY CAPABLE OF INCREASING PI TURNOVER RATES?

In the β-cell, at least, intracellular mechanisms also must exist to activate PI hydrolysis. This has been convincingly demonstrated for various small nutrient molecules (e.g., glucose) whose actions on the β-cell are thought to be mediated by their metabolic transformation and not via specific receptors (8–10). Although it may be premature to suggest that PI turnover is an essential pathway in stimulated secretion, regardless of the nature of the primary stimulant, results continue to accumulate supporting its importance. I do not intend to recapitulate all previous studies in this exciting and constantly evolving area of cell physiology. I focus on aspects of PI turnover that have been of primary interest over the past several years. From the outset, however, note that this is a most dynamic area of scientific investigation. The list of possible PI-derived second-messenger molecules continues to expand. Therefore, any comprehensive theory of cellular activation based on available evidence is probably premature and may require substantial modification. Many details remain to be elucidated.

Herein, the inositol-containing phospholipids can be considered storage forms for various intracellular second-messenger molecules (6; Fig. 1). For example, the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) results in the generation of the Ca^{2+}-mobilizing inositol-1,4,5-trisphosphate (IP_3) and the protein kinase C activator diacylglycerol (DG) (7,11). Phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol may be precursors for PIP_2 resynthesis or may themselves be subjected to PLC-mediated hydrol-

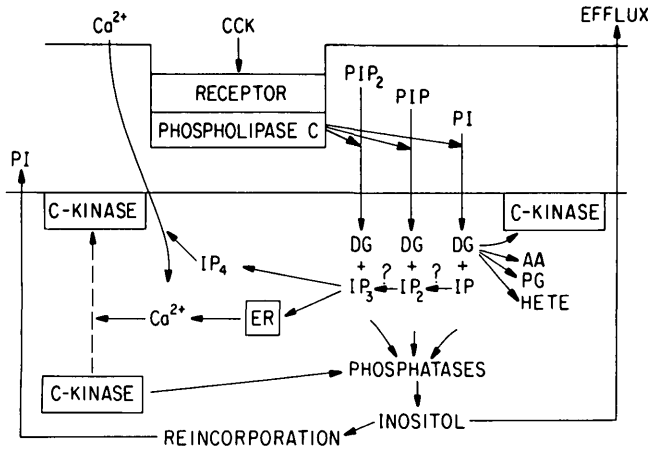

FIG. 1. Membrane phosphoinositide metabolism initiated by cholecystokinin (CCK). Schematic representation of events after CCK interaction with its β-cell membrane receptor. Interaction activates cellular phospholipase C. This enzyme is capable of hydrolyzing membrane phosphoinositides, including phosphatidylinositol (PI), phosphatidylinositol-4-monophosphate (PIP), and phosphatidylinositol-4,5-bisphosphate (PIP_2). Products generated during these reactions include inositol phosphates (IP, IP_2, and IP_3) and diacylglycerol (DG). IP_3 participates in the mobilization of intracellular Ca^{2+} from endoplasmic reticulum (ER). IP_3 may also be converted into IP_2 or IP_4 (inositol-1,3,4,5-tetrakisphosphate). IP_4 may influence Ca^{2+} gating and extracellular Ca^{2+} influx. Among other effects, this cation facilitates movement of cytoplasmic protein kinase C to membrane. Activation of this kinase depends on Ca^{2+} and DG. Kinase C may phosphorylate a phosphatase responsible for IP_3 degradation thereby increasing its activity. After removal of its phosphate moieties by phosphatase action, liberated inositol may be reincorporated into PI or efflux from cell (see Fig. 3). Inhibition of reuptake of labeled inositol improves ability to analyze PI turnover. Multiple metabolic fates exist for DG-derived products, e.g., arachidonic acid (AA), prostaglandins (PG), prostacyclins, and hydroxyeicosatetraenoic acids (HETE). These compounds may modulate stimulated secretion (17,18).

ysis. The latter metabolic fate results in the generation of DG and inositol compounds of various phosphate content. The inherent complexity of biochemical events associated with PI turnover is just being realized. For example, IP_3 may be phosphorylated to IP_4 (inositol-1,3,4,5-tetrakisphosphate), a compound with purported Ca^{2+}-gating properties (12). Novel IP_4 isomers have been identified in some tissues, although their actions are unknown (13). Whether IP_1 and IP_2 are possible precursors for more complex inositol phosphates or are physiologically inactive metabolites has not been established.

As complex as the generation of various inositol phosphates concomitant with PI turnover seems, the biochemical pathways activated by DG or DG-derived metabolites promise to be at least as difficult to define accurately. For example, DG itself activates the Ca^{2+}- and phospholipid-dependent kinase referred to as kinase C (11,14), an enzyme already identified in islets (15). DG may also participate in the activation of the phosphatases responsible for inositol phosphate inactivation (16). Multiple metabolic fates for the products of DG catabolism also exist. These include arachidonic acid, prostaglandins, prostacyclins, and leukotrienes (17). Many of these compounds or their derivatives have established modulatory effects on β-cell insulin secretory patterns (18). Measuring the levels of all possible PI-derived second messengers may therefore represent a monumental and prodigious task. Furthermore, the distinct possibility exists that a situation analogous to that concerning the precise identity of the metabolic trigger for glucose-induced insulin secretion will develop. In this case, an accurate marker to assess changes in PI turnover rates would seem most desirable. As an initial and reasonable first step, whether PI turnover is increased by various agonists and the quantitative extent of this activation must be assessed. This is analogous to using [^{3}H]glucose derivatives to monitor glucose usage rates by islets. Studies with these compounds have not revealed the identity of the intracellular metabolic trigger for secretion, but there is little doubt that studies with them have documented that increases in glucose metabolism regulate the insulinotropic action of the hexose (19,20).

WHAT EXPERIMENTAL APPROACHES HAVE BEEN UTILIZED TO MONITOR PI HYDROLYSIS IN RESPONSIVE TISSUES?

Various methodologies have been developed for this purpose. Many investigators have taken advantage of the fact that *myo*-[2-^{3}H]inositol is exclusively incorporated into PI (21). After washing to remove unincorporated label, islets can be stimulated, and several indices of PI hydrolysis can be monitored, including changes in total radioactive PI content, accumulation of labeled inositol phosphates, and ^{3}H efflux from stimulated tissue. Some shortcomings are inherent with each of these approaches. For example, the decline in total radioactive PI content is usually small, especially when measured over short periods, and not sensitive enough to measure minute-to-minute changes after agonist addition (22). Although inositol phosphate levels have been assessed in several studies (9,22–24), these experiments are usually conducted in the presence of lithium (9,22,23). Lithium inhibits glucose-induced insulin secretion (25), thus limiting the extrapolation of the data obtained in its presence to the dynamic insulin secretory response. If islets are similar to other tissues, the existence of novel inositol phosphate isomers poses another possible identification problem. Which are the most meaningful measurements to conduct? In regard to ^{3}H-efflux studies with isolated islets, the indirectness of these measurements as well as the apparent sluggishness (at least when compared to insulin secretion) of PI turnover are causes for concern (22,26). On the positive side, however, the functional integrity (insulin secretion) of the preparation can be determined in parallel with this dynamic, albeit indirect, barometer of PI turnover. This is a relevant concern particularly when correlating changing patterns of PI turnover with changing patterns of insulin output.

Our initial studies on PI metabolism were conducted with the gut hormone and the possible incretin and satiety factor cholecystokinin (the COOH-terminal 8-amino acid derivative sulfated on the tyrosine residue CCK-8S; 22,27). These studies were influenced by previous work demonstrating that in the perfused pancreas, CCK induced a glucose-dependent insulin secretory response (28,29). Best and Malaisse (9) had also demonstrated a positive impact of CCK-pancreozymin on PI turnover. Several different approaches were utilized in these studies, all indicating that PI turnover might play an important role in the capacity of this hormone to influence secretion (22). In *myo*-[2-^{3}H]inositol–prelabeled islets, CCK-8S decreases the content of labeled PI, increases the contents of labeled inositol phosphates, and increases ^{3}H efflux from perifused islets. Although the chem-

ical nature of the compounds present in the effluent was not analyzed in this study, a most recent report by Mathias et al. (30) indicated that with glucose or cholinergic stimulation of islets, >80% of the label is free [^{3}H]inositol derived from phosphatase action on labeled inositol phosphates.

IS A SECOND-MESSENGER MOLECULE GENERATED DURING PI TURNOVER THE INTRACELLULAR SIGNAL FOR STIMULATED SECRETION?

In attempting to ascribe a prominent role for PI turnover in stimulated secretion, regardless of the nature of the primary stimulant used, several problems have surfaced. First, CCK-8S stimulates PI turnover at low (2.75 mM) or high (7.0 mM) glucose levels but only increases insulin secretion at the higher hexose level. Second, the impact of CCK-8S on PI turnover is much greater than high (21.4 mM) glucose, even though insulin release due to this hexose level is far greater than that due to CCK-8S alone or in the presence of moderate glucose. A similar quantitative dissociation between PI turnover and secretion also appears to exist in cholinergically stimulated islets (31). The notion that PI turnover, and the generation of second-messenger molecules during this process, represents another complex component of the second-messenger roster present in islets deserves emphasis. The relative ineffectiveness of CCK-8S in activating the secretory apparatus despite dramatic changes in PI turnover, an event presumably accompanied by associated increases in second-messenger molecules, might be the result of at least several factors. First, in addition to increasing PI turnover, high glucose levels also elevate cAMP levels in islets (32). CCK has no such effect on cAMP (33). Because cAMP is known to potentiate glucose-induced secretion, the involvement of this nucleotide in CCK-8S–stimulated secretion was examined. In islets whose cAMP levels are elevated by treatment with 1 μM forskolin, the addition of CCK-8S is accompanied by a dramatic increase in insulin secretion even in the presence of moderate glucose levels (22). This augmented response is, however, most pronounced for the initial phase of secretion, and release rates return to basal values within 15–20 min despite the continued presence of CCK-8S. Whereas part of the so-called permissive effect of glucose may reside in its ability to increase islet levels of cAMP, some other as yet unidentified factors also play an important role. These other permissive components may include changes in cofactor patterns, redox state, phosphate potential, or other parameters that might be expected to fluctuate with changing patterns of glucose usage by the islet.

IS THERE ANY PHYSIOLOGICAL SIGNIFICANCE IN THE DRAMATIC POSITIVE INTERACTION BETWEEN THE SECOND-MESSENGER MOLECULE cAMP AND PI-DERIVED SECOND MESSENGERS?

The ingestion of a mixed meal is accompanied by the release of several gut factors, including but probably not confined to CCK and gastric inhibitory polypeptide. The release of the latter peptide hormone from the proximal digestive tract is primarily regulated by carbohydrate components of the diet, and it presumably influences insulin secretion by elevating islet cAMP levels (34). CCK release is most sensitive to the protein components of an ingested meal, although triglycerides may also weakly impact on its release (35). Because of their abilities to elevate levels of separate β-cell second-messenger molecules, we investigated whether these peptides might synergize in their insulinotropic influence on isolated perifused islets. The results demonstrate a dramatic positive interaction between these two postulated incretin factors on β-cell secretory responsiveness (Fig. 2). This synergistic action is abolished by lowering the hexose level to 2.75 mM or by blocking the interaction of CCK-8S with its membrane receptor by a recently developed CCK antagonist, L 364718 (36). Previous in vivo studies by Ahren et al. (37) also provide support for this synergistic arrangement.

DO CHANGING PATTERNS OF PI TURNOVER CONTRIBUTE TO CHANGING PATTERNS OF INSULIN OUTPUT?

The β-cell secretory response to an abrupt increase in the level of glucose bathing them is characterized by a brisk biphasic release of the hormone. To what extent do events associated with PI turnover contribute to this dynamic secretory pattern? When ^{3}H efflux rates from [2-^{3}H]inositol-prelabeled islets are used to monitor this process, an appreciable lag in PI turnover is evident, despite a rapid insulin secretory response (22,26). Results obtained with this methodology are, consequently, difficult to reconcile with the notion that the increase in PI turnover rates are responsible for

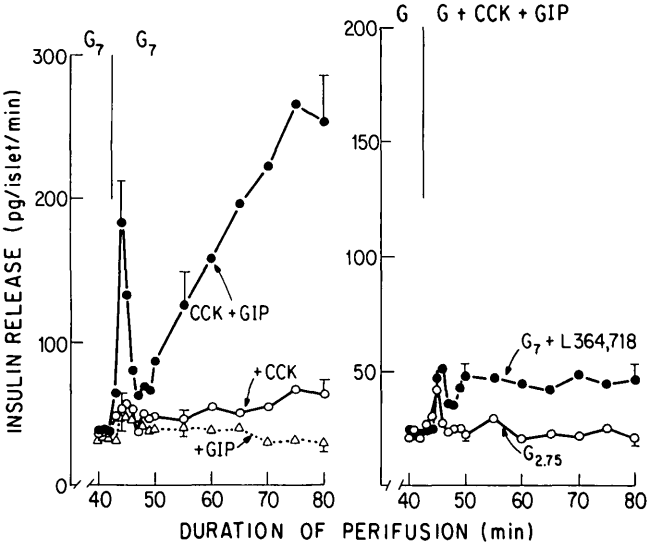

FIG. 2. Influence of cholecystokinin (CCK) and gastric inhibitory polypeptide (GIP) on insulin release from isolated rat islets. *Left*: batches of freshly isolated islets were perifused for 40 min with 7 mM (126 mg/dl) glucose to establish basal, stable insulin secretory rates. They were then stimulated for 40 min with CCK (5 nM) alone, GIP (50 ng/ml) alone, or combination of CCK plus GIP at same levels. Values are means ± selected SEs. At least 4 experiments were performed under each condition. Figure has been corrected for dead space in perifusion system (~2.5 ml or 2.5 min with flow rate of 1 ml/min). *Right*: batches of islets were perifused for 40 min with either 2.75 mM (50 mg/dl) or 7 mM (126 mg/dl) glucose. Islets maintained with 2.75 mM glucose were then exposed to combination of CCK (5 nM) and GIP (50 ng/ml) for 40 min in continued presence of 2.75 mM glucose. Islets maintained for 40 min with 7 mM glucose were also exposed to CCK plus GIP. In addition, however, L 364718 (10 nM), a competitive antagonist of CCK binding to its membrane receptor (36), was also included in perifusion medium for 10 min before peptide stimulation and for entire 40-min stimulation period. Four experiments were performed under each experimental condition. Note change in ordinate scale for insulin-release values.

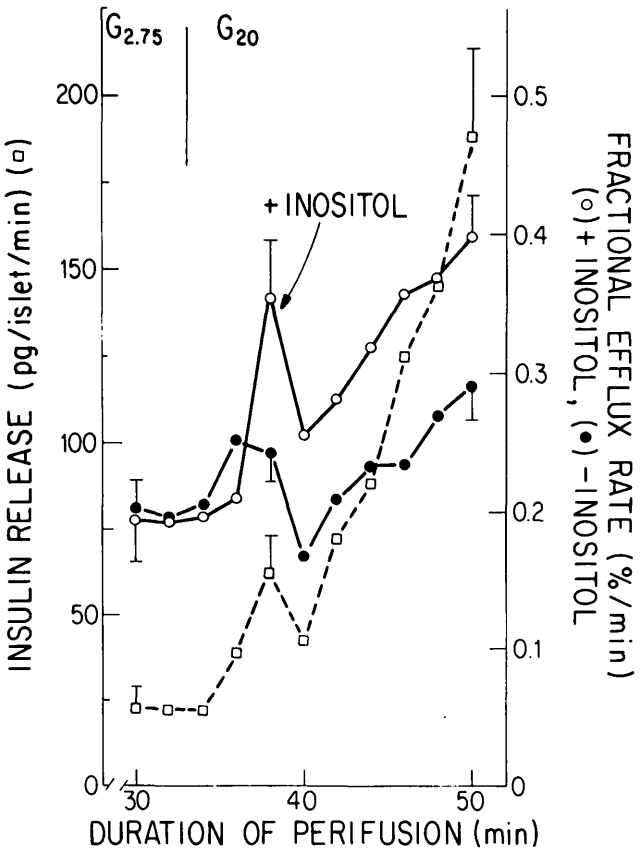

FIG. 3. Fractional ^3H efflux rates in response to glucose stimulation: effect of cold inositol. Isolated islets were incubated for 2 h with *myo*-[2-^3H]inositol and then perifused. After 30-min stabilization period, they were exposed to 20 mM glucose ± 1 mM inositol. Insulin secretion was biphasic, and inclusion of cold inositol had no effect on this response. ^3H-efflux patterns (calculated as fractional efflux rates; ref. 38) were, however, markedly influenced by cold inositol. A biphasic pattern was observed in its presence. A small but statistically insignificant response was initially observed in its absence. Fractional efflux rates slowly increased in these islets during course of perifusion with 20 mM glucose. We attribute this blunted efflux pattern noted in absence of cold inositol to reincorporation of free [^3H]inositol (formed by phosphatase action on inositol phosphates generated during PI turnover) back into PI.

stimulated secretion and not simply a result of it. However, the experimental basis for using this prelabeling protocol (incubating islets in radioactive inositol) to monitor PI metabolism is the fact that islets rapidly incorporate inositol into PI. The possibility therefore exists that any [2-^3H]inositol labilized as a result of an increase in PI turnover might be quickly reincorporated into PI. This scenario would obviously limit the appearance of label in the effluent and contribute to an underestimation of PI hydrolysis. To circumvent this possible shortcoming, we have included cold inositol in the medium in some experiments to compete with labeled free inositol (produced as a result of agonist-induced increases in inositol phosphates and their subsequent catabolism to free inositol by islet phosphatases) for reuptake. This manipulation has no effect on glucose-stimulated insulin secretion. Some of the results obtained with this methodology are shown in Fig. 3. For comparison, efflux results obtained without the addition of cold inositol are also shown. In the absence of inositol, a significant increase in fractional efflux rates of the isotope is not apparent during the early minutes of 20 mM glucose stimulation. A small increase does occur, but this does not achieve statistical significance. In contrast to this efflux response, the inclusion of 1 mM inositol together with 20 mM glucose in the perifusion medium results in a biphasic ^3H efflux, a response that bears a striking resemblance to the brisk biphasic insulin secretory response in timing and amplitude. Interestingly, we have more recently established that the levels of IP_3 also change in a biphasic pattern and that the kinetics of this response precede both ^3H efflux and insulin output (W.S.Z. and K.C. Zawalich, unpublished observations). In any event, it appears from these studies that reincorporation of radioactive inositol into PI greatly influences the appearance of label in the perifusate. The inclusion of cold inositol circumvents this problem, demonstrates the rapidity of PI hydrolysis in glucose-stimulated islets, and reinforces the validity of using [^3H]inositol efflux patterns to monitor PI turnover in islets.

Finally, I address the possible involvement of PI turnover in the phenomenon of "memory" displayed by islets. It has been established that prior glucose stimulation of islets either in vivo or in vitro sensitizes them to a subsequent provocation (39,40). The ensuing insulin secretory response is much greater after priming with glucose. Data obtained with various agonists, including CCK-8S, glucose, glyceraldehyde, tolbutamide, and α-ketoisocaproate, have documented three important points. *1*) All of the agonists increase PI hydrolysis in isolated perifused islets. *2*) This stimulatory effect, monitored by a persistent increase in ^3H efflux from [^3H]inositol-labeled islets, is sustained after agonist removal even though insulin secretory rates rapidly subside. *3*) The duration of this increase in PI hydrolysis parallels the duration of sensitization. In other words, if islets are restimulated during this period of sustained PI turnover, an amplified secretory response ensues. Although the precise mechanisms responsible for this sustained impact of various agonists on PI turnover have not been elucidated, it seems logical to conclude that they may contribute to the induction and maintenance of memory in islets. Thus, PI turnover may have not only an acute effect on release but may also play a long-term modulatory role in stimulus-secretion response coupling. From a clinical perspective, the involvement of PI turnover may assume added importance, because hyperinsulinemia may result in an acquired deficit—insulin resistance (41). The contribution of changing patterns of PI turnover to the initiation and persistence of hyperinsulinemia is an issue with important clinical overtones.

In conclusion, several important points have emerged. First, PI turnover is clearly increased in islets by various agonists, and this response at least partly regulates the exocytosis of insulin from these cells. The impact of PI turnover on stimulated secretion is greatest, however, when accompanied by parallel increases in islet glucose usage rates and cAMP levels. Second, ^3H efflux from *myo*-[2-^3H]inositol–prelabeled islets is an accurate barometer of PI turnover rates, particularly if reincorporation of the label is prevented by the inclusion of nonradioactive inositol. Note that this approach allows the functional integrity of the islet preparation, an index of β-cell function often overlooked in previous studies, to be established as well. Finally, events associated with PI hydrolysis exert a chronic effect on insulin output, because once PI turnover rates are increased by agonist presentation,

they only slowly return to prestimulatory values. Restimulation during this period of persistent PI turnover is accompanied by an amplified secretory response. Future studies designed to assess the adaptive nature (e.g., influence by excess caloric intake) of this metabolic pathway and its contribution to various states characterized by a hyporesponsive or hyperresponsive β-cell are indicated.

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