Importance of Nonionic Signals for Glucose-Induced Biphasic Insulin Secretion

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Glucose induces biphasic insulin secretion by the islet β-cell. Based on recent knowledge on glucose signaling in the β-cell, the underlying mechanisms for this biphasicity could be envisaged as follows. Glucose-induced elevation of cytosolic free Ca^{2+} concentration, which is due to the electrophysiological events that originate in closure of the ATP-sensitive K^+ (K_{ATP}) channel, most likely triggers the first phase. The second phase is produced by gradual augmentation and potentiation of Ca^{2+}-triggered insulin release by the K_{ATP} channel–independent, nonionic signals. Protein acylation may be involved in the nonionic signaling. In patients lacking functional K_{ATP} channels, however, the first phase of glucose-induced insulin secretion is clearly retained, casting doubt on the simplistic view outlined above. In this pathological condition, the K_{ATP} channel–independent, most likely nonionic, glucose action alone is sufficient for the first-phase response. *Diabetes* 51 (Suppl. 1):S96–S98, 2002

Biphasic insulin release by the islet β-cell upon glucose stimulation has long been recognized (1). Although the pathophysiological significance of the biphasic response is not clearly established, diminution of the first-phase response in humans is considered a sign of β-cell failure (2). The underlying mechanisms for glucose-induced biphasic insulin release are not fully understood. Glucose generates many signals in the β-cell, and we have proposed a distinction between ionic and nonionic signals and discussed the importance of the latter to the second-phase response (3). Experimental data supporting our view and the problems left unanswered are reviewed here.

RECOGNITION OF BIPHASIC INSULIN RELEASE UPON A SQUARE-WAVE APPLICATION OF GLUCOSE

Glucose-induced biphasic insulin secretion was first recognized in pancreas perfusion experiments (4). Soon thereafter, it was found that a similar biphasic insulin release can be seen when isolated islets were tested in perifusion experiments (5). The biphasic response takes place when extracellular glucose concentration is abruptly raised from a substimulatory to a stimulatory level and kept at that high level. In contrast, when the extracellular glucose concentration is gradually raised, no biphasic response is elicited and insulin secretion is progressive (4). In normal subjects, fasting plasma glucose concentration is ~5 mmol/l, which is already stimulatory, and, postprandially, it rises to 8 mmol/l, at the very most, during a 30- to 60-min interval. A "glucose jump" from, e.g., 3 to 16.7 mmol/l often imposed in vitro, never occurs in normal subjects under physiological conditions. In that sense, a biphasic insulin release by the β-cell upon glucose stimulation, a salient feature of this cell type, is an experimental concept.

There are several thousands of islets in the pancreas, and the response of individual rat islets is highly heterogeneous. Some islets show a typical biphasic response, but many others show either predominant first- or second-phase responses (6). This finding agrees well with the electrophysiological heterogeneity of individual rat islets (7). When insulin secretion from many randomly selected islets is collectively analyzed, total response is clearly biphasic, as it is in the perfused pancreas. This potentially important fact, i.e., functional heterogeneity among islets, is not considered in the following discussion because the reason for such heterogeneity is unknown at present.

UNDERLYING SIGNALS FOR BIPHASIC INSULIN SECRETION

Elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) by a Ca^{2+} ionophore, A23187, produces a monophasic insulin response, whereas 12-*O*-tetradecanoyl-phorbol-13-acetate, an agonist of protein kinase C (PKC), causes gradually increasing insulin release, and a combination of the two elicits a biphasic release similar to that occurring in response to glucose (8). Therefore, it was speculated that the glucose-induced biphasic response might be due to an elevation of $[Ca^{2+}]_i$ and subsequent activation of PKC by elevated $[Ca^{2+}]_i$. By now, closure of ATP-sensitive K^+ (K_{ATP}) channels and subsequent electrophysiological events have been established as the basis for glucose-induced rise in $[Ca^{2+}]_i$ (9).

The biphasicity of insulin secretion upon glucose stimulation, especially a prominent second phase, is much more evident in rat than mouse islets (10–12). Recent studies on membrane potential and $[Ca^{2+}]_i$ changes in rat β-cells clearly revealed that the temporal profiles of ionic events during the second phase do not coincide with a gradually increasing pattern of insulin secretion (7). Namely, the depolarization, number of action potentials,

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$[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; CPTI, carnitine palmitoyl transferase I; K_{ATP} channel, ATP-sensitive K^+ channel; Kir6.2, inward-rectifying K^+ channel 6.2; LC-CoA, long-chain CoA; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; PKC, protein kinase C; SUR1, sulfonylurea receptor 1.

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TABLE 1
K_{ATP} channel-independent glucose action and the two phases of insulin secretion

Species	Status of the K_{ATP} channel	First phase	Second phase	Reference
Human				
PHHI*	Closed (SUR1 mutation)	+	−[a]	(26)
PHHI	Closed (SUR1 mutation)	ND	+	(27)
Normal	Open (diazoxide)	ND	+	(34)
Rat				
Normal	Closed (tolbutamide)	+	+	(31)
Normal	Open (diazoxide)	−	+	(12)
Mouse				
Normal	Closed (glyburide)	+	+	(30)
Normal	Closed (glyburide or tolbutamide)	+	+	(32)
Knockout	Closed (Kir6.2 knockout)	Trace	Trace	(28)
Knockout	Closed (SUR1 knockout)	ND	+	(29)
Normal	Open (diazoxide)	−	+	(13)

A list of the initial observations in which insulin secretion in response to glucose was reported in the β-cell with the K_{ATP} channels being closed or open by the gene mutation, pharmacological agents, or targeted disruption of the genes, as indicated in the parentheses. a, Likely due to decreased β-cell insulin content (27). ND, not determined. *This is an in vivo study where glucose was injected to the patients intravenously. Other studies were performed by using isolated pancreatic islets. Because existence of the first phase cannot be ascertained unless a perifusion experiment is performed, it was designated as ND when the perifusion was not done.

and elevation of $[Ca^{2+}]_i$ during the second phase all remained stable (6). Therefore, a biphasic insulin release upon glucose stimulation cannot be simply explained by the K_{ATP} channel closure and subsequent elevation of $[Ca^{2+}]_i$.

We (12) and others (13) found that glucose strongly augments Ca^{2+}-triggered insulin release in a K_{ATP} channel–independent manner, and we consider that the second-phase response is produced mostly though this signaling pathway. Because glucose-induced ionic events linked to insulin exocytosis originate in the K_{ATP} channel closure, and signals involved in the K_{ATP} channel–independent pathway are not related to electrophysiological events in the β-cell, we proposed a separation of the K_{ATP} channel–dependent and –independent signalings on the basis of their ionic or nonionic nature (3).

Thus, an abrupt rise in extracellular glucose concentration causes an elevation of $[Ca^{2+}]_i$ through the ionic signaling as outlined above, and insulin release is triggered by Ca^{2+}-dependent processes. This corresponds to the first phase. Ca^{2+}-triggered insulin release is then gradually augmented/potentiated by the K_{ATP} channel–independent signals, and a prominent second phase is generated (14,15).

MOLECULAR BASIS OF NONIONIC SIGNALS

Until now, phospholipase A_2 (a Ca^{2+}-independent, ATP-dependent subtype), guanosine triphosphate, ATP, and glutamate were proposed as possible conveyer(s) of the K_{ATP} channel–independent glucose signaling (16–20). We have recently suggested a role of protein acylation (21,22). Namely, cerulenin, an inhibitor of protein acylation, selectively obliterated nutrient-induced insulin release without metabolic perturbation in the islet cells (21). Based on this observation, we considered that it is likely that glucose causes fatty acylation of protein or proteins engaged in exocytosis of insulin. This idea stemmed from the malonyl-CoA hypothesis originally proposed by Corkey et al. (23). According to this hypothesis, citrate derived from the tricarboxylic acid cycle is accumulated in the cytosol and converted to malonyl-CoA, which suppresses carnitine palmitoyl transferase I (CPTI) on the mitochondrial outer membrane. Because CPTI is a rate-limiting enzyme for mitochondrial transport of long-chain acyl CoAs (LC-CoAs), LC-CoAs are accumulated in the cytosol as a result of CPTI suppression. Especially, accumulation of palmitoyl-CoA will then facilitate fatty acylation of protein(s) (24) involved in insulin exocytosis, leading to augmentation and potentiation of exocytosis. On the other hand, overexpression of malonyl-CoA decarboxylase in insulin-secreting INS-1 cells eliminated glucose-induced accumulation of malonyl-CoA, yet glucose-induced insulin secretion remained unaltered (25). Thus, in INS-1 cells, the validity of the malonyl-CoA hypothesis is questioned.

NEWER INSIGHTS FROM THE FINDINGS IN THE K_{ATP} CHANNEL–DEFICIENT β-CELL

Recently, insulin secretion has been studied in patients or animals without functional K_{ATP} channels (26–29). The patients lacking K_{ATP} channels because of mutations in the genes encoding the sulfonylurea receptor 1 (SUR1), a subunit of the K_{ATP} channel, suffer from severe neonatal hypoglycemia. The disease is called persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (26,27). In contrast, mice with targeted disruption of the genes encoding either SUR1 or the other subunit of the K_{ATP} channel, inward-rectifying K^+ channel 6.2 (Kir6.2), remain euglycemic under regular feeding (28,29).

From the conventional viewpoint that the first phase is mostly, if not entirely, due to glucose-induced closure of the K_{ATP} channels eventually causing a sharp rise in $[Ca^{2+}]_i$ (see above), it is rather surprising that the first-phase insulin secretion was clearly elicited in PHHI subjects upon intravenous bolus injection of glucose (26). The second phase appears absent, probably because of a markedly reduced β-cell insulin content. Straub et al. (27) examined the function of islets isolated from such patients and found that application of glucose alone elicited a clear-cut insulin release: the basal level of $[Ca^{2+}]_i$ in β-cells from these patients was normal and did not increase upon glucose stimulation (27). These observations indicate that the K_{ATP} channel–independent nonionic signals alone can trigger insulin release by the β-cell, provided the channel

is closed: the absence of functional K_{ATP} channels appears to be equivalent to persistent closure of the channel.

When islets from normal rodents are stimulated with glucose in the presence of sulfonylurea (a K_{ATP} channel–closer), both first and second phases are elicited (30–32). For unknown reasons, islets from SUR1 or Kir6.2 knockout mice lack such rapid response to glucose (28,29) (Table 1). Such a difference may explain why mice without functional K_{ATP} channels, unlike humans with the similar genetic abnormality, do not suffer from persistent hyperinsulinemic hypoglycemia.

CONCLUSION

Glucose-induced biphasic insulin secretion by pancreatic islets is a characteristic feature of β-cell physiology. As far as we are aware, biphasic hormone release as seen in the β-cell, especially the prominent second-phase secretion, upon a square-wave application of physiological stimuli, has not been reported in other endocrine or neuronal cells. For example, thyrotropin-releasing hormone elicits a sharp, distinct first-phase hormone release followed by a gradually decreasing tiny second phase from GH_4C_1 pituitary tumor cell lines (33). Understanding of the molecular basis of this unique response of the β-cell will hopefully contribute to the treatment of patients with diabetes through development of newer insulinotropic agents.

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