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Synchronizing Ca²⁺ and cAMP oscillations in pancreatic β-cells: a role for glucose metabolism and GLP-1 receptors? Focus on “Regulation of cAMP dynamics by Ca²⁺ and G protein-coupled receptors in the pancreatic β-cell: a computational approach”

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THE HORMONAL ACTIVATION of cAMP production in pancreatic β-cells is accompanied by an increase of cytosolic [cAMP] that can be measured in real time through the use of a cAMP sensor (Epac1-camps) that exhibits a decrease of fluorescence resonance energy transfer (FRET) when it binds to cAMP (9, 15, 18). When this cAMP sensor is paired with the fluorescent Ca²⁺ indicator fura-2, it is possible to perform simultaneous measurements of cytosolic [cAMP] and [Ca²⁺] in single living β-cells (9). Studies performed in this manner have revealed oscillations in the levels of both second messengers, a phenomenon that can be imaged to evaluate the spatial distribution and temporal dynamics of cAMP and Ca²⁺ (15). Fridlyand et al. (6) now report a new mathematical model that seeks to explain how oscillations of cAMP and Ca²⁺ are generated. A primary focus of the model is glucagon-like peptide-1 (GLP-1), a blood glucose-lowering hormone that stimulates β-cell cAMP production and that also potentiates glucose-stimulated insulin secretion (4, 7, 12–14). An additional focus of the model concerns D-glucose, a metabolizable sugar that increases the cytosolic [Ca²⁺] of β-cells and that is generally considered to be the primary physiological stimulus regulating β-cell insulin secretion (11). The computational approach devised by Fridlyand et al. is of interest because it may help resolve a long-standing debate within the field of endocrinology: specifically, how do GLP-1 and glucose metabolism interact to stimulate insulin secretion from β-cells?

A prior study (24) has demonstrated a powerful synergistic interaction of GLP-1 and glucose to stimulate insulin secretion. Moreover, a wealth of data exists indicating that the insulin secretagogue actions of GLP-1 and glucose are secondary to their ability to stimulate an increase of cytosolic [cAMP] and [Ca²⁺] (5, 7, 12–14). GLP-1 stimulates cAMP production as a consequence of its binding to the β-cell GLP-1 receptor (22), a class II G protein-coupled receptor that activates G_s proteins and that stimulates transmembrane adenylyl cyclase (tmAC) (Fig. 1). In contrast, glucose must be metabolized by the β-cell for it to stimulate an increase of [Ca²⁺] (11) (Fig. 1). Glucose metabolism increases the cytosolic [ATP]-to-[ADP] ratio, which promotes the closure of ATP-sensitive K⁺ (K_{ATP}) channels and initiates bursts of Ca²⁺-dependent action potentials, causing oscillations of [Ca²⁺] (11, 19). The ensuing increase of cytosolic [Ca²⁺] triggers the fusion of insulin-containing secretory granules with the plasma membrane. Because cAMP facilitates the action of Ca²⁺

to stimulate secretory granule exocytosis in β -cells (20), GLP-1 potentiates glucose-stimulated insulin secretion. In view of the fact that pancreatic insulin secretion is stimulated after the ingestion of a meal when levels of GLP-1 and glucose increase in the systemic circulation (14), the model proposed by Fridlyand et al. predicts that the synergistic interaction of GLP-1 and glucose to stimulate insulin secretion results from synchronous and in-phase oscillations of β -cell [cAMP] and $[Ca^{2+}]$ (6).

The model of Fridlyand et al. proposes that the amplitude, periodicity, and phase characteristics of [cAMP] oscillations will be determined by two principal factors: 1) Ca^{2+} /calmodulin-dependent activation of cyclic nucleotide phosphodiesterase (PDE) and 2) Ca^{2+} /calmodulin-dependent activation of tmAC (Fig. 1). The computational approach of Fridlyand et al. provides an ingenious prediction: that when β -cells are exposed to elevated concentrations of glucose, the binding of GLP-1 to the GLP-1 receptor converts anti-phasic oscillations of [cAMP] and $[Ca^{2+}]$ into in-phase oscillatory activity and in so doing allows the concentrations of both cAMP and Ca^{2+} to rise simultaneously (Fig. 1). Once this in-phase oscillatory activity is established, the frequency and amplitude of each oscillation dictate the efficacy with which GLP-1 potentiates glucose-stimulated insulin secretion.

This model developed by Fridlyand et al. is an outgrowth of the earlier study of Landa and co-workers (15) in which synchronous but anti-phasic oscillations of [cAMP] and $[Ca^{2+}]$ were detected in an insulin-secreting cell line (MIN6) not treated with GLP-1. In that study, anti-phasic oscillations of cAMP and Ca^{2+} were observed after exposure of MIN6 cells to buffered saline containing glucose and a K^+ channel blocker (TEA). Under such conditions, the peak of [cAMP] coincided with the nadir of $[Ca^{2+}]$ for each oscillation, a finding that prompted Landa and co-workers to conclude that in the absence of GLP-1, anti-phasic oscillations of [cAMP] are induced by the periodic activation of Ca^{2+} -dependent PDEs (Fig. 1A) (15). Consistent with this concept, evidence exists for the expression of a Ca^{2+} /calmodulin-regulated PDE designated as PDE1C in β -cells (8, 15).

It is of interest to note that in the study of Landa et al., oscillations of $[Ca^{2+}]$ were induced by exposure of MIN6 cells to a solution that contained both glucose and TEA. However, in the absence of TEA, glucose failed to stimulate oscillations of $[Ca^{2+}]$ (15). This finding is understandable if TEA reduces a K^+ conductance that normally suppresses oscillations of membrane potential in this cell type. Thus, MIN6 cells differ substantially from primary β -cells, as evidenced by the fact that treatment of whole islets of Langerhans with glucose induces periodic membrane depolarization, bursting electrical activity, and oscillations of β -cell $[Ca^{2+}]$ (19). In an innovative manner, the model of Fridlyand et al. takes these unique features of β -cell physiology into account by simulating the glucose-dependent oscillations of membrane potential that generate anti-phasic oscillations of $[Ca^{2+}]$ and [cAMP] (Fig. 1A) and that are predicted to occur in the absence of GLP-1.

Fridlyand et al. also take advantage of findings generated in prior studies of R15, a neuron in the sea slug *Aplysia*. Although R15 is not glucose responsive, it exhibits oscillations of membrane potential that are sensitive to both cAMP and Ca^{2+} . In a computational study (25) of R15 electrical activity, a model was developed in which the activity of tmAC was considered to be stimulated or inhibited by the binding of Ca^{2+} to calmodulin. The model of Fridlyand et al. takes these predictions concerning Ca^{2+} /calmodulin-dependent regulation of tmAC into account when considering how Ca^{2+} influences β -cell cAMP production. Attention is focused on tmAC1 and tmAC8, isoforms of tmAC that are expressed in β -cells and that are known to act as molecular coincidence detectors (3, 15, 23). Available evidence indicates that tmAC1 and tmAC8 are synergistically activated by 1) $G\alpha_s$ proteins and 2) Ca^{2+} /calmodulin (2). In the model of Fridlyand et al., binding of GLP-1 to the GLP-1 receptor activates $G\alpha_s$ proteins, whereas glucose metabolism promotes the increase of

[Ca²⁺] that allows for the association of Ca²⁺ with calmodulin. Under conditions in which β-cells are exposed to both GLP-1 and glucose, tmAC1 and tmAC8 become synergistically activated, and cAMP production exceeds cAMP degradation. For this reason, [cAMP] and [Ca²⁺] oscillate in phase despite the fact that Ca²⁺ influx also generates periodic activation of PDE (Fig. 1B).

In summary, Fridlyand et al.'s model, supported by published data, demonstrates that cAMP oscillations are continuous, and entrained by, oscillations of [Ca²⁺]. Exactly how such oscillations of [Ca²⁺] are generated during the exposure of β-cells to glucose is a topic of substantial debate. In simulations of R15 electrical activity, oscillations of [Ca²⁺] can be generated as a consequence of Ca²⁺ and cAMP-dependent mechanisms controlling the activity of ion channels (25). Thus, there is reason to believe that oscillations of [Ca²⁺] in β-cells might have a similar ionic basis (19). However, β-cells differ from R15 in that the initiation of Ca²⁺ oscillations is contingent on the glucose metabolism-dependent reduction of plasma membrane K_{ATP} channel conductance. Furthermore, glucose stimulates both fast and slow oscillations of [Ca²⁺] in the β-cell, and it is the slow oscillations of [Ca²⁺] that Fridlyand et al. have incorporated into their model of cAMP and Ca²⁺ dynamics. With these points in mind, it seems reasonable to speculate that oscillations of [Ca²⁺] in β-cells might have a metabolic basis in addition to an ionic basis. Indeed, a dual oscillator model for β-cell Ca²⁺ handling has been described, one in which fast oscillations of [Ca²⁺] result from an ionic mechanism, whereas slow oscillations result from oscillations of metabolism (1). In the dual oscillator model, glucose metabolism generates glycolytic oscillations that are dependent on the activity of the allosterically regulated enzyme phosphofructokinase (21). Pulsatile delivery of pyruvate to the tricarboxylic acid (TCA) cycle leads to oscillations in the cytosolic [ATP]-to-[ADP] ratio, thereby generating oscillations of K_{ATP} channel activity, membrane potential, and [Ca²⁺]. It is important to note, however, that there is also evidence for an intramitochondrial source of oscillatory activity in the β-cell. Exposure of isolated β-cell mitochondria to pyruvate generates oscillations in the levels of TCA cycle intermediates (16, 17), whereas exposure of β-cells to the TCA cycle intermediates α-ketoglutarate or methyl-pyruvate (a membrane permeable analog of pyruvate) induces oscillations of [Ca²⁺] in the absence of glucose (10). Thus, it may be that oscillations of [Ca²⁺] and [cAMP] find their genesis in a complex interplay between ionic, glycolytic, and mitochondrial mechanisms of oscillatory control in β-cells.

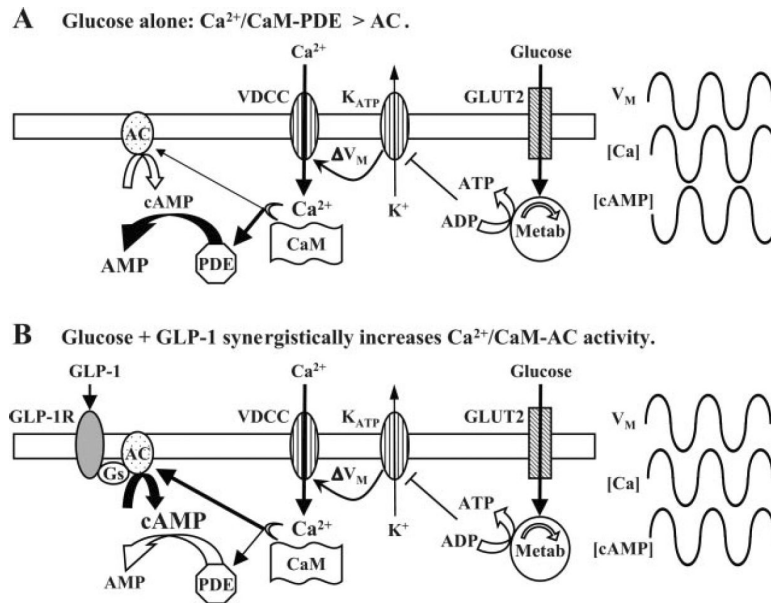
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**Fig. 1.**

Changes in the synchrony of $[\text{Ca}^{2+}]$ and $[\text{cAMP}]$ induced by treatment of β -cells with glucose or glucose plus glucagon-like peptide-1 (GLP-1). *A*: in the presence of glucose alone, metabolism (Metab) of the sugar induces ATP-sensitive K^+ (K_{ATP}) channel closure, membrane depolarization [change in membrane voltage (ΔV_{M})], electrical bursting activity, and periodic influx of Ca^{2+} . This Ca^{2+} influx activates calmodulin (CaM), which weakly stimulates transmembrane adenylyl cyclase (AC) 1/8 (tmAC1/8) but strongly stimulates phosphodiesterase 1C (PDE1C). Since PDE1C activity exceeds that of tmAC1/8, cAMP levels fall when $[\text{Ca}^{2+}]$ increases, and these oscillations are antiphasic. Because glucose alone has little effect on basal $[\text{cAMP}]$, $[\text{cAMP}]$ oscillates at levels close to the basal concentration. VDCC, voltage-dependent Ca^{2+} channel; GLUT2, glucose transporter 2. *B*: exposure of β -cells to GLP-1 in the presence of glucose results in the synergistic activation of tmAC1/8 by G_{α_s} and $\text{Ca}^{2+}/\text{CaM}$, whereas PDE1C is also activated by $\text{Ca}^{2+}/\text{CaM}$. When $[\text{Ca}^{2+}]$ increases, the rate of cAMP production exceeds the rate of cAMP hydrolysis so that $[\text{cAMP}]$ oscillates in phase with the increase of $[\text{Ca}^{2+}]$. Since GLP-1 is a strong stimulus for cAMP production under conditions of elevated cytosolic $[\text{Ca}^{2+}]$, $[\text{cAMP}]$ oscillates at levels greatly in excess of basal $[\text{cAMP}]$. GLP-1R, GLP-1 receptor. Note that these two models of cAMP and Ca^{2+} dynamics predict that oscillations of $[\text{cAMP}]$ will either have little effect (*A*) or a strong stimulatory effect (*B*) on glucose-stimulated insulin secretion.