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Modeling analysis of inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} mobilization under the control of glucagon-like peptide-1 in mouse pancreatic β -cells

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Takeda Y, Shimayoshi T, Holz GG, Noma A. Modeling analysis of inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} mobilization under the control of glucagon-like peptide-1 in mouse pancreatic β -cells. *Am J Physiol Cell Physiol* 310: C337–C347, 2016. First published November 25, 2015; doi:10.1152/ajpcell.00234.2015.—Glucagon-like peptide-1 (GLP-1) is an intestinally derived blood glucose-lowering hormone that potentiates glucose-stimulated insulin secretion from pancreatic β -cells. The secretagogue action of GLP-1 is explained, at least in part, by its ability to stimulate cAMP production so that cAMP may facilitate the release of Ca^{2+} from inositol trisphosphate receptor (IP₃R)-regulated Ca^{2+} stores. However, a quantitative model has yet to be provided that explains the molecular mechanisms and dynamic processes linking GLP-1-stimulated cAMP production to Ca^{2+} mobilization. Here, we performed simulation studies to investigate how GLP-1 alters the abilities of Ca^{2+} and IP₃ to act as coagonists at IP₃R Ca^{2+} release channels. A new dynamic model was constructed based on the Kaftan model, which demonstrates dual steady-state allosteric regulation of the IP₃R by Ca^{2+} and IP₃. Data obtained from β -cells were then analyzed to understand how GLP-1 facilitates IP₃R-mediated Ca^{2+} mobilization when UV flash photolysis is used to uncage Ca^{2+} and IP₃ intracellularly. When the dynamic model for IP₃R activation was incorporated into a minimal cell model, the Ca^{2+} transients and oscillations induced by GLP-1 were successfully reconstructed. Simulation studies indicated that transient and oscillatory responses to GLP-1 were produced by sequential positive and negative feedback regulation due to fast activation and slow inhibition of the IP₃R by Ca^{2+} . The slow rate of Ca^{2+} -dependent inhibition was revealed to provide a remarkable contribution to the time course of the decay of cytosolic Ca^{2+} transients. It also served to drive and pace Ca^{2+} oscillations that are significant when evaluating how GLP-1 stimulates insulin secretion.

inositol 1,4,5-trisphosphate; glucagon-like peptide-1; Ca^{2+} mobilization; model stimulation

PANCREATIC β -CELLS PLAY a central role in the control of blood glucose homeostasis by generating bursts of action potentials when levels of blood glucose rise after a meal. This rhythmic bursting activity is accompanied by oscillations of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) that induce pulsatile insulin secretion (2; 17, 38). Glucagon-like peptide-1 (GLP-1) is also an insulin secretagogue (30), and it activates G protein-coupled

receptors (GPCRs) on β -cells to stimulate cAMP production so that cAMP signaling pathways may facilitate the inositol trisphosphate receptor (IP₃R)-dependent release of Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores (12, 13, 20, 24–27, 32, 37). This IP₃R-dependent ER Ca^{2+} release serves as a direct stimulus for insulin secretion (26), and it occurs when β -cells are simultaneously exposed to glucose and GLP-1 so that levels of intracellular IP₃, Ca^{2+} , and cAMP are elevated in concert (19, 31). In the present study, we report mathematical simulation studies that reveal how β -cell IP₃Rs act as molecular coincidence detectors so that their gating function is regulated not only by IP₃ and Ca^{2+} but also by cAMP.

Studies using microsomal vesicles containing the IP₃R derived from rat brain synaptosomes (14) provide a conceptual framework with which to evaluate how such coordinate regulation of ER Ca^{2+} mobilization is achieved. These studies indicate a positive feedback action of Ca^{2+} to potentiate IP₃-induced Ca^{2+} release that is measurable in living cells as a transient increase of $[\text{Ca}^{2+}]_i$. A subsequent decay of the Ca^{2+} transient is explained by Ca^{2+} -dependent inhibition (or possibly inactivation) of the IP₃R rather than depletion of Ca^{2+} stores. Collectively, these findings indicate that sequential positive and negative feedback regulation of the IP₃R by Ca^{2+} determines the time course of Ca^{2+} transients as well as Ca^{2+} oscillations. Still, it remains uncertain how Ca^{2+} , IP₃, and cAMP functionally interact to control IP₃R activity.

Among the three subtypes of IP₃ receptors (IP₃R1, IP₃R2, and IP₃R3) identified so far, it is IP₃R1 that is predominantly expressed in mouse β -cells (29). The activity of IP₃R1 is determined by the occupancy of two major IP₃ binding sites; one with high affinity for IP₃, and another with low affinity ($K_d = 0.05 \sim 1.78$ and $10 \mu\text{M}$, respectively). Ca^{2+} acts as a coagonist, and the bell-shaped bimodal regulation of the steady-state channel open probability (P_o) by cytosolic Ca^{2+} is explained by two Ca^{2+} binding sites: one is for channel activation ($K_d = 0.8 \mu\text{M}$) and the other is for channel inhibition (K_d not yet determined) (6, 8, 22, 23, 35, 36, 40, 45). However, one limitation of earlier mathematical models of IP₃R1 regulation is that some models described a single high-affinity IP₃ binding site, usually omitting the low-affinity IP₃ binding site (9, 10, 16). Since these models predicted saturated channel activity at a basal cytosolic concentration of IP₃ concentration ($[\text{IP}_3]$; $\sim 2 \mu\text{M}$; Refs. 4, 21), they were not suitable for analysis of IP₃R1 channel activity at physiologi-

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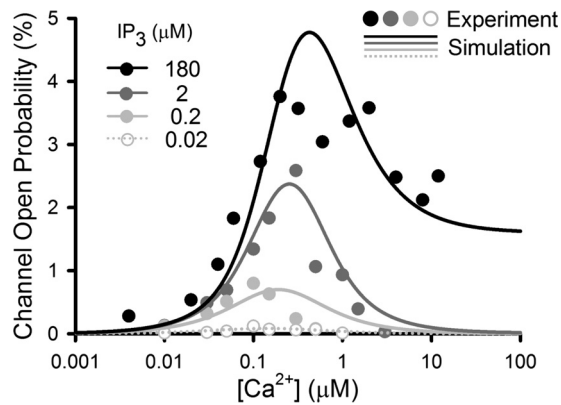


Fig. 1. Open probabilities of the inositol trisphosphate receptor (IP₃R) as a function of the Ca²⁺ concentration. Experimentally measured open probabilities of the IP₃R were prepared from canine cerebellar endoplasmic reticulum (ER) microsomes (where type 1 receptors are exclusively expressed) and studied in planar lipid bilayers. Data are plotted as filled circles as a function of the Ca²⁺ concentration. Ca²⁺-dependent results of the simulations obtained at different IP₃ concentrations are indicated in the corresponding colors [data are modified from Kaftan et al. (23), see MATERIALS AND METHODS]. Simulation results (solid and dotted lines) are superimposed upon experimental results.

cally relevant higher concentrations of IP₃. A significant improvement that allowed accurate simulation of IP₃R1 channel activity was the steady-state IP₃R1 model of Kaftan et al. (23) that considered both high- and low-affinity IP₃ binding sites, as well as two Ca²⁺-binding sites. Using experimental approaches, Kaftan et al. (23), demonstrated that Ca²⁺-dependent channel activity increased as levels of [IP₃] (0.02~180 μM) also increased. By taking into account both high- and low-affinity IP₃ binding sites, the model of Kaftan et al. (23) successfully simulated IP₃R1 channel activity over a wide range of IP₃ and [Ca²⁺].

In the mathematical simulation studies reported here, a new dynamic model of IP₃R Ca²⁺ channel activity is constructed based on the steady-state IP₃R1 model originally developed by Kaftan et al. (23). The model accurately reconstructs the steady-state open probability of the IP₃R over a wide range of [IP₃] and [Ca²⁺]. Results derived from the present theoretical studies indicate that for β-cells in which the activities IP₃R and sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) are enhanced through cAMP-dependent protein kinase (PKA) activation, the resultant transient changes of [Ca²⁺]_i are produced by sequential positive and negative feedback regulation due to fast activation and slow inhibition of the IP₃R by Ca²⁺. Interestingly, the model exhibited the ability to produce oscillatory Ca²⁺ mobilization when intracellular Ca²⁺ content was elevated. Remarkably, we find that the rate of Ca²⁺-dependent inhibition is critically important to the time course of the decay of the Ca²⁺ transient, and it also determines the frequency of Ca²⁺ oscillations.

MATERIALS AND METHODS

A dynamic IP₃R1 model was first developed on the basis of the model of Kaftan et al (23), which accurately reproduces steady-state activities of the channel that are known to be allosterically regulated by both [Ca²⁺] and [IP₃] (Fig. 1). Ca²⁺ transients and oscillations were then reconstructed in a minimal cell model, as fully described below. Simulation studies and mathematical analyses were subsequently applied to investigate quantitative mechanisms underlying the

IP₃R-mediated Ca²⁺-release events in pancreatic β-cells. Model development and simulation-based analyses were both performed on Microsoft Visual Studio, unless otherwise indicated. Time integration of the differential equations was conducted using the Euler method with a time step of 1 ms.

Modeling the steady-state open probability of the IP₃R at different agonist concentrations. On the basis of the model of Kaftan et al. (23), the steady-state open probabilities of the IP₃R over a wide range of [IP₃] and [Ca²⁺] (Fig. 1) were reconstructed to determine dissociation constants (K_d) for agonist binding sites. IP₃R forms a complex comprised of four subunits. Kaftan et al. (23) assumed that each subunit in the tetrameric complex contains two IP₃ binding sites (IP₃H: higher affinity site; and IP₃L: lower affinity site) and two Ca²⁺ binding sites (CaA: activation site; and CaI: inhibition site) according to experimental findings (6, 8, 22, 23, 35, 36, 40, 45). Since Ca²⁺ inhibits IP₃ binding to the channel due to an increase in the dissociation constant (K_d) of the IP₃ binding sites (22), Kaftan et al. assumed that the steady-state probability of IP₃ binding at IP₃H ($P_{IP_3HIp_3}$) was determined by two distinct K_d values (K_{dIP_3H} ; K_d when Ca²⁺ was not bound to CaI, and K_{dIP_3Hca} ; K_d when Ca²⁺ was bound to CaI) depending on the occupancy of the CaI site (Eq. 1). In contrast, the steady-state probabilities for IP₃ bound to IP₃L ($P_{IP_3Lip_3}$) as well as Ca²⁺ bound to CaA (P_{CaAca}) and CaI (P_{CaIca}) were simply calculated by Eqs. 2–4 with corresponding dissociation constants (K_{dIP_3L} , K_{dCaA} , and K_{dCaI} , respectively).

$$P_{IP_3HIp_3} = \frac{1}{1 + \frac{P_{CaI} \cdot K_{dIP_3H} + P_{CaIca} \cdot K_{dIP_3Hca}}{[IP_3]}} \quad (1)$$

$$P_{IP_3Lip_3} = \frac{1}{1 + \frac{K_{dIP_3L}}{[IP_3]}} \quad (2)$$

$$P_{CaAca} = \frac{1}{1 + \frac{K_{dCaA}}{[Ca]}} \quad (3)$$

$$P_{CaIca} = \frac{1}{1 + \frac{K_{dCaI}}{[Ca]}} \quad (4)$$

There are a total of 16 possible states of each monomer associated with every combination of agonist binding. Kaftan et al. (23) assumed that the overall single channel P_o of the tetrameric complex is determined by multiple combinations of agonist binding states of each subunit. The affinities for each agonist binding site in Kaftan et al. was estimated by model fitting to the experimental data for values of P_o over a wide range of [IP₃] and [Ca²⁺]. The Ca²⁺-dependent P_o curve obtained with a low ATP concentration (0.5 mM), however, was shown to shift to the left as [ATP] increased to a physiological concentration (~5 mM; Ref. 15). This effect of ATP is apparent when IP₃R activity is measured with higher [IP₃] (>10 μM) but not at basal levels of [IP₃] (~2 μM; Refs. 4, 21) (3). In the study reported here, K_{dIP_3H} , K_{dIP_3Hca} , K_{dIP_3L} , K_{dCaA} , and K_{dCaI} were thus reevaluated by taking into account the ATP effect in which the Ca²⁺-dependent P_o curve obtained with 180 μM IP₃ was left-shifted (Fig. 1). However, other experimental data with lower [IP₃] remained unmodified. All assumptions made for the steady-state IP₃R model in Kaftan et al. were adopted after reevaluating effects of ATP for the current model study. K_d values for each agonist binding site as well as P_i values [in Kaftan et al. (23)] were estimated by model fitting of the reevaluated data and are listed in Table 1.

Modeling dynamic properties of the IP₃R. On the bases of steady-state properties of the IP₃R, a new dynamic model was constructed. Key model parameter sets of the dynamic IP₃R model were estimated

Table 1. Parameter sets for the steady-state IP₃R model

Parameters	Experiments	Kaftan Model	Present Model
K_{dIP3H}	0.04–0.31 μ M (22, 23, 36, 45)	0.3 μ M	0.1 μ M
$K_{dIP3HCa}$	0.54–1.78 μ M (22, 23, 36)	1.5 μ M	0.4 μ M
K_{dIP3L}	~10 μ M (23)	10 μ M	800 μ M
K_{dCaA}	0.8 μ M (40)	0.03 μ M	0.27 μ M
K_{dCaI}		1.0 μ M	0.27 μ M
P_1		10 E-11	0.01
P_2		0.06	0.03
P_3		0.04	0.34
P_4		Not indicated	0.98

Parameter sets for the steady-state inositol trisphosphate receptor (IP₃R) model, as estimated by a comparison of the theoretical study of Kaftan et al. (23) and experimental findings (22, 23, 36, 40, 45). See text for definitions.

by accurately simulating the changes of [Ca²⁺] during the time course of Ca²⁺ transients that result from Ca²⁺-induced Ca²⁺ release (CICR) in mouse β -cells (25). To reproduce Ca²⁺ transients and oscillations generated in β -cells, the equilibrium equations for binding of Ca²⁺ to CaA (Eq. 3) and CaI (Eq. 4) were replaced by time-dependent processes (Eqs. 5 and 6). Chronological changes in P_{CaAca} and P_{CaIca} of the IP₃R were thus determined by time-based integration of Eqs. 5 and 6 where α and β represent overall rates for Ca²⁺ association and dissociation with respective binding sites followed by subsequent activation or inhibition of the channel.

$$\frac{dP_{CaAca}}{dt} = \alpha_{CaA} \cdot P_{CaA} \cdot [Ca^{2+}]_{nrs} - \beta_{CaA} \cdot P_{CaAca} \quad (5)$$

$$\frac{dP_{CaIca}}{dt} = \alpha_{CaI} \cdot P_{CaI} \cdot [Ca^{2+}]_{nrs} - \beta_{CaI} \cdot P_{CaIca} \quad (6)$$

The rate constants α_{CaA} , β_{CaA} , α_{CaI} , and β_{CaI} (Table 2) were estimated by reproducing Ca²⁺-induced Ca²⁺ release (Fig. 3) in a minimal β -cell model (Fig. 2), which is described below. Regulation of the IP₃R due to binding of IP₃, on the other hand, was approximated by an instantaneous equilibrium (see Eqs. 1 and 2) for simplicity.

Table 2. Best fit parameter sets for reproducing the transient Ca²⁺ response measured in response to UV flash photolysis

Parameters	Estimated Values
α_{CaA}	6 mM ⁻¹ ·ms ⁻¹
β_{CaA}	1.62 E-3 ms ⁻¹
α_{CaI}	1.5 E-2 mM ⁻¹ ·ms ⁻¹
β_{CaI}	4.05 E-6 ms ⁻¹
vol_{blk}	756.27 fl
vol_{ER}	280.0 fl
vol_{nrs}	7.73 fl
f_i	0.01
f_{ER}	0.025
P_{IP3R} (control)	13.65 fl/ms
P_{IP3R} (stimulated)	174.72 fl/ms
P_{ERleak}	0.24 fl/ms
P_{SERCA} (control)	0.0691 amol/ms
P_{SERCA} (stimulated)	0.23 amol/ms
P_{nrsblk}	817.31 fl/ms
P_{mleak}	6.0 fl/ms

Best fit parameter sets for reproducing the transient Ca²⁺ response (Ca²⁺-induced Ca²⁺ release) measured in response to UV flash photolysis (see arrows in Fig. 3) for mouse β -cells loaded with caged Ca²⁺ (NP-EGTA-AM) under conditions in which cells are either prestimulated or not stimulated with forskolin (25). See text for definitions.

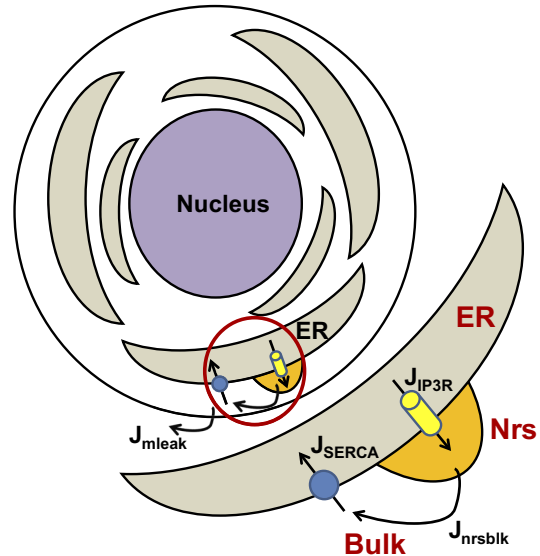


Fig. 2. Schematic diagram describing Ca²⁺ fluxes and Ca²⁺ compartments in the minimal cell model. Ca²⁺ fluxes through the IP₃R (J_{IP3R}) and sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) (J_{SERCA}), as well as Ca²⁺ diffusion from Ca²⁺ microdomains (“nrs”) to the bulk (“blk”) cytosolic compartment (J_{nrsblk}), are indicated as red circles within a simplified cellular model, and are expanded below. Membrane Ca²⁺ leak (J_{mleak}) was also included. Colors for Ca²⁺ compartments: luminal side of the ER, “nrs” microdomain site, and “blk” cytosolic space are indicated as filled beige, orange, and white, respectively.

Development of the minimal cell model. Schematic illustration of the mouse β -cell minimal cell model is shown in Fig. 2. The model consists of multiple ER Ca²⁺ compartments that contain IP₃R and SERCA pumps, as a minimum number of functional units for ER Ca²⁺ handling. Net membrane Ca²⁺ flux that signifies plasma membrane Ca²⁺ removal during the Ca²⁺-induced Ca²⁺ transient (Fig. 3A) is described as in J_{mleak} (amol/ms, Eq. 7), and Ca²⁺ flux across nuclear membrane was removed for simplicity. The resting [Ca²⁺]_{blk} was equilibrated at 0.0001 mM ([Ca²⁺]_{rest}) by J_{mleak} (Eq. 7).

$$J_{mleak} = P_{mleak} \cdot ([Ca^{2+}]_{rest} - [Ca^{2+}]_{blk}) \quad (7)$$

Steep gradients in cytosolic Ca²⁺ concentration are expected near the Ca²⁺ releasing site (nrs). For simplicity, the histological and functional composition of the Ca²⁺ releasing unit for every IP₃R was assumed to be uniform throughout the cell. Thus a single compartment containing ER, nrs, and the bulk cytosolic space was implemented for the minimal cell model. Volumes for nrs (vol_{nrs}) are indicated in Table 2, along with ER volume (vol_{ER}) and cytosolic bulk volume (vol_{blk}), as determined on the basis of ultrastructural morphometry (11).

Whole cell molar flux of Ca²⁺ through the IP₃R (J_{IP3R}) was defined by Eq. 8, where P_o is the open probability, determined by [Ca²⁺]_{nrs}.

$$J_{IP3R} = P_{IP3R} \cdot P_o \cdot ([Ca^{2+}]_{ER} - [Ca^{2+}]_{nrs}) \quad (8)$$

Background Ca²⁺ conductivity through the ER membrane (5) was described by Eq. 9.

$$J_{ERleak} = P_{ERleak} \cdot ([Ca^{2+}]_{ER} - [Ca^{2+}]_{nrs}) \quad (9)$$

Diffusion of Ca²⁺ from the nrs to blk space, J_{nrsblk} , was assumed as in Eq. 10.

$$J_{nrsblk} = P_{nrsblk} \cdot ([Ca^{2+}]_{nrs} - [Ca^{2+}]_{blk}) \quad (10)$$

P_{IP3R} , P_{ERleak} , and P_{nrsblk} in Eqs. 8–10 as well as P_{mleak} in Eq. 7 represent conductivities of corresponding Ca²⁺ fluxes with units of fl/ms. Finally, uptake of [Ca²⁺]_{blk} by ER through the SERCA pump (J_{SERCA}) was described by Eq. 11, where $K_d = 0.0002$ mM, and Hill

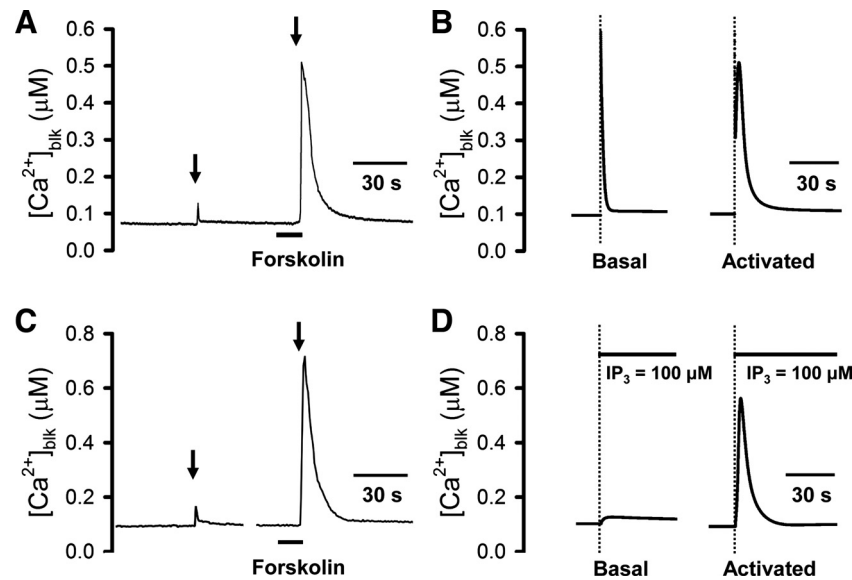


Fig. 3. Simulation of Ca²⁺ transients triggered by an abrupt increase of [Ca²⁺] and [IP₃] in β -cells. **A**: experimental data (25) in which a mouse β -cell was loaded with fura-2-AM and caged Ca²⁺ (NP-EGTA-AM) so that Ca²⁺ could be uncaged by UV flash photolysis of NP-EGTA. The duration of the UV flash was 600 μ s. Uncaging of Ca²⁺ was performed under control conditions in the absence of forskolin (*left arrow*) or under test conditions in which forskolin (2 μ M) was applied directly to the cell (*right arrow*). Due to the relatively slow fura-2 sampling rate (0.5 Hz), the amplitude of the Ca²⁺ transient measured under control conditions is an underestimate of the true amplitude measured immediately after the 600 μ s UV stimulus. **B**: simulation of the Ca²⁺ transients induced by the uncaging of Ca²⁺, for comparison with **A**. To initiate Ca²⁺ transients, UV flash photolysis of NP-EGTA was simulated mathematically by injecting Ca²⁺ (dotted lines) at a rate of 40 fmol/s for 1 ms under control conditions (Basal) or under test conditions that mimic the action of forskolin (Activated). [IP₃] was held at 2 μ M throughout the simulation experiments. **C**: experimental data (25) in which Ca²⁺ transients were measured in a mouse β -cell after UV flash photolysis of caged IP₃ (ci-IP₃/PM) for control conditions in the absence of forskolin (*left arrow*) or test conditions in the presence of 2 μ M forskolin (*right arrow*). **D**: simulation of the Ca²⁺ transients induced by the uncaging of IP₃ for comparison with **C**. To initiate Ca²⁺ transients under control conditions in the absence of forskolin (Basal) or under test conditions in the presence of forskolin (Activated), the [IP₃] (black bars) was increased from 2 to 100 μ M in a step-wise manner (dotted lines indicate start of stimulus). For simulations in **B** and **D**, $P_{IP3R} = 13.65$ fl/ms and $P_{SERCA} = 0.069$ amol/ms were set for Basal conditions, whereas $P_{IP3R} = 174.72$ fl/ms and $P_{SERCA} = 0.23$ amol/ms were set for Activated conditions, respectively.

coefficient $n_H = 2$. P_{SERCA} the maximum molar flux of SERCA with a unit of amol/ms.

$$J_{SERCA} = P_{SERCA} \cdot \frac{1}{1 + \left(\frac{K_d}{[Ca^{2+}]_{blk}} \right)^{n_H}} \quad (11)$$

The rate of changes in $[Ca^{2+}]_{blk}$, $[Ca^{2+}]_{nrs}$, and $[Ca^{2+}]_{ER}$ were described by *Eqs. 12, 13, and 14*, respectively, where vol_x is the volume of the corresponding Ca²⁺ compartment x . In contrast, f_i and f_{ER} (Table 2) are fractions of free Ca²⁺ in cytosol and in ER, respectively, considering buffering capacity of Ca²⁺ in each compartment (5). J_{flash} in *Eqs. 12 and 13* indicates the flux of Ca²⁺ by Ca²⁺ injection applied in *Figs. 3 and 4*.

$$\frac{d[Ca^{2+}]_{blk}}{dt} = \frac{f_i}{vol_{blk}} \cdot (J_{nrsblk} - J_{SERCA} + J_{mleak} + J_{flash}) \quad (12)$$

$$\frac{d[Ca^{2+}]_{nrs}}{dt} = \frac{f_i}{vol_{nrs}} \cdot \left(J_{IP3R} - J_{ERleak} + J_{nrsblk} + J_{flash} \cdot \frac{vol_{nrs}}{vol_{blk}} \right) \quad (13)$$

$$\frac{d[Ca^{2+}]_{ER}}{dt} = \frac{f_{ER}}{vol_{ER}} \cdot (J_{SERCA} - J_{IP3R} + J_{ERleak}) \quad (14)$$

In these mathematical analyses, J_{mleak} was set to zero for simplicity. Thus intracellular total Ca²⁺ content (Ca^{2+}_{tot}) was kept constant. This approach revealed mechanisms of Ca²⁺ mobilization that are intrinsic to ER function and that are determined by activities of the IP₃R and SERCA. Redundancy in calculating Ca²⁺ concentrations at all subcellular compartments was avoided by omitting *Eq. 14* but applying the Ca²⁺ conservation below (*Eq. 15*).

$$[Ca^{2+}]_{ER} = \frac{f_{ER}}{vol_{ER}} \cdot \left(Ca^{2+}_{tot} - \frac{vol_{blk}}{f_i} [Ca^{2+}]_{blk} - \frac{vol_{nrs}}{f_i} [Ca^{2+}]_{nrs} \right) \quad (15)$$

P_{IP3R} and P_{SERCA} (Table 2) were determined on the basis of passive Ca²⁺ leak and J_{SERCA} , respectively, for the full β -cell model describing the nonbursting period (5). V_{nrs} , as well as P_{ERleak} , P_{nrsblk} , and P_{mleak} , were estimated by reproducing the resting $[Ca^{2+}]_{blk}$, $[Ca^{2+}]_{ER}$, and the time course of the decay of released Ca²⁺ under control conditions (see *Fig. 3A*). Parameters for V_{nrs} and P_{nrsblk} , however, are not unique in satisfying the resting cellular conditions. Decreasing V_{nrs} in concert with increasing P_{nrsblk} , for example, would provide the analogous result. P_{IP3R} as well as P_{SERCA} in the presence of forskolin, a cAMP-elevating agent, were also estimated by reproducing the Ca²⁺-induced Ca²⁺ transient (in *Fig. 3A*).

Bifurcation analysis. Bifurcation analysis is a mathematical approach to investigate changes in model behavior (whether it converges, diverges, or oscillates) initiated by continuous alteration in the value of a given model parameter. More specifically, this analysis examines bifurcations of stationary and periodic steady-state solutions of the model; equilibrium points (EPs) and limit cycles (LCs), respectively. For the model described here, each of the key parameters (Ca^{2+}_{tot} , P_{IP3R} , P_{SERCA} , α_{CaA} , and α_{CaI}) was systematically varied with the objective to determine how the $[Ca^{2+}]_{nrs}$ transitions from a stable stationary behavior (at the EP) to a spontaneously generating oscillatory pattern (within a LC). For this analysis, the positions and stabilities of the steady states were automatically obtained by directly finding roots of the model equation system using XPPAUT, a computational tool for bifurcation analysis.

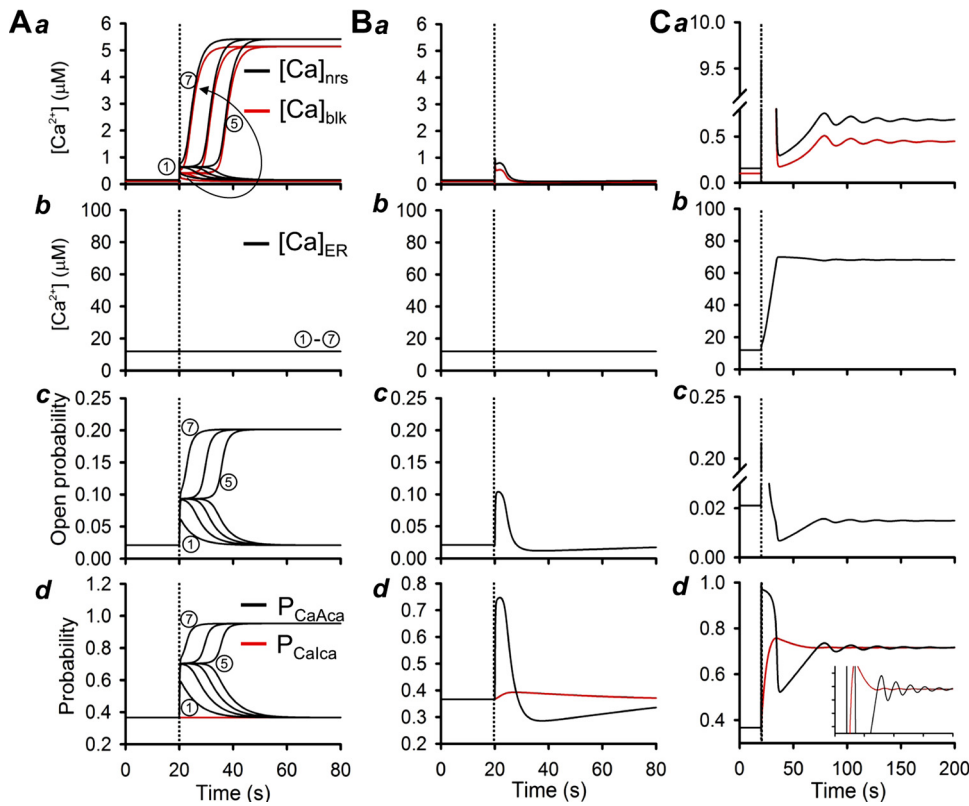


Fig. 4. Ca²⁺-dependent positive and negative feedback regulation of the IP₃R during dynamic Ca²⁺ mobilization. A–C: [Ca²⁺]_{nrs} and [Ca²⁺]_{blk} (a) were plotted along with [Ca²⁺]_{ER} (b) and P_o (c), including P_{CaAca} and P_{CaIca} of the IP₃R (d), before and after Ca²⁺ injections. As in the simulation study of Fig. 3 (B), Ca²⁺ was injected at a rate of 40 fmol/s for 1 ms (IP₃ = 2 μM). A, Effects of gradual increases in the intensities of Ca²⁺ injection, from 40 to 60 fmol/s (①: 40; ②: 54; ③: 54.45; ④: 54.50; ⑤: 54.52; ⑥: 54.7; and ⑦: 60 fmol/s as indicated by an arrow), while P_{CaIca} and [Ca²⁺]_{ER} were both fixed at the corresponding resting levels. Responses to the Ca²⁺ injection at 60 fmol/s (⑦ in A) were compared with those simulated without fixing P_{CaIca} at the resting level (B). C: responses to Ca²⁺ injection at 650 fmol/s. Changes in P_{CaIca} and [Ca²⁺]_{ER} were both calculated for this simulations. Trace in Cd between probabilities of 0.65–0.75 is expanded at bottom.

Instantaneous equilibrium analysis. To examine mechanisms that contribute to dynamic IP₃R-mediated Ca²⁺ oscillations, we applied instantaneous equilibrium (IE) analysis (39) to a minimal β-cell model. IE analysis is a simulation-based analysis, and it can be used to quantify the contributions of all differential variables (i.e., P_{CaAca}, P_{CaIca}, and [Ca²⁺]_{blk}) to changes in [Ca²⁺]_{nrs} in the minimal cell model (Fig. 6). This is obtained by calculating an IE point of [Ca²⁺]_{nrs} (IE[Ca²⁺]_{nrs}) at each moment. IE[Ca²⁺]_{nrs} is the [Ca²⁺]_{nrs} when the rate of change in [Ca²⁺]_{nrs} (J_{IP3R} + J_{ERleak} - J_{nrsblk}) described by Eq. 13 equals 0 for a condition where every variable but [Ca²⁺]_{nrs} is fixed at a every time point. IE[Ca²⁺]_{nrs} was determined by Eq. 16 (see Ref. 39 for the derivation).

$$IE[Ca^{2+}]_{nrs} = \frac{P_{IP3R} \cdot P_o \cdot [Ca^{2+}]_{ER} + P_{ERleak} \cdot [Ca^{2+}]_{ER} + P_{nrsblk} \cdot [Ca^{2+}]_{blk}}{P_{IP3R} \cdot P_o + P_{ERleak} + P_{nrsblk}} \quad (16)$$

Eq. 13 can be deformed into the following equation (J_{flash} was not included for oscillation studies).

$$\frac{d[Ca^{2+}]_{nrs}}{dt} = \frac{f_i}{Vol_{nrs}} (P_{IP3R} \cdot P_o + P_{ERleak} + P_{nrsblk}) (IE[Ca^{2+}]_{nrs} - [Ca^{2+}]_{nrs}) \quad (17)$$

Since IE[Ca²⁺]_{nrs} is a momentary equilibrium concentration of [Ca²⁺]_{nrs}, IE[Ca²⁺]_{nrs} always moves in advance of [Ca²⁺]_{nrs}, and thus its time derivative, dIE[Ca²⁺]_{nrs}/dt drives the change in [Ca²⁺]_{nrs}. The contribution (c) of a variable of interest, i, to the rate of change in IE[Ca²⁺]_{nrs} can be calculated by multiplying the partial derivative of IE[Ca²⁺]_{nrs} with respect to the variable of interest, i, and the rate of change in the parameter (Eq. 18). Summation of the contributions, c, from every variable in the cellular model made up the dIE[Ca²⁺]_{nrs}/dt.

Consequently, c represents an i-component of dIE[Ca²⁺]_{nrs}/dt, or a quantified contribution of the variable of interest i to changes in [Ca²⁺]_{nrs}. See Shimayoshi et al. (39) for additional details.

$$c = \frac{\partial IE[Ca^{2+}]_{nrs}}{\partial_i} \cdot \frac{di}{dt} \quad (18)$$

RESULTS

Simulation studies of Ca²⁺- and IP₃-induced ER Ca²⁺ release in mouse β-cells. Kang et al. (25) reported that for mouse β-cells loaded with a membrane permeable caged Ca²⁺ (NP-EGTA-AM), it was possible to use short-duration, low-intensity UV flash photolysis to uncage Ca²⁺ to examine how Ca²⁺ and cAMP functionally interact to facilitate CICR originating from IP₃R-regulated ER Ca²⁺ stores. Under control conditions in which β-cells were not exposed to a cAMP-elevating agent, the uncaging of Ca²⁺ generated a small increase of [Ca²⁺]_i (Fig. 3A, left arrow) that was not considered to result from ER Ca²⁺ mobilization. However, for β-cells treated with the cAMP-elevating agent forskolin, ER Ca²⁺ mobilization resulting from CICR was observed (Fig. 3A, right arrow), as defined by Kang et al. (25). With this available information in hand, we sought to model the underlying processes of ER Ca²⁺ mobilization.

For the mathematical simulation studies reported here, UV flash photolysis-induced uncaging of Ca²⁺ in the absence of forskolin was simulated as a step-wise intracellular “injection” of Ca²⁺ at a rate of 40 fmol/s into the bulk (blk) cytosolic space for 1 ms while also injecting Ca²⁺ near the release site (nrs) at which IP₃R Ca²⁺ release events occur (left trace of Fig. 3B, injection time indicated by dotted lines; see Eq. 12). For both injection procedures, the increase of [Ca²⁺]_{blk} after Ca²⁺

injection was precisely matched to the increase of $[Ca^{2+}]_{nrs}$ after Ca²⁺ injection (see Eq. 13). For this simulation performed in the absence of forskolin (Fig. 3B, left trace), the $[Ca^{2+}]_{blk}$ quickly rose to $\sim 0.6 \mu M$ during injection of Ca²⁺. However, the response immediately decayed after the offset of injection without generating CICR, as was also the case for the experiment reported by Kang et al. (Fig. 3A, left arrow). To obtain these findings, the $[IP_3]$ was fixed at the basal concentration of $\sim 2 \mu M$, and P_{IP3R} and P_{SERCA} were also set at basal activity levels of 13.65 fl/ms and 0.069 amol/ms, respectively (see Table 2).

To model the sensitizing action of forskolin at the IP₃R, the value for P_{IP3R} was increased, a manipulation that simulates PKA-mediated phosphorylation of the IP₃R (41). Simultaneously, P_{SERCA} was also increased to simulate the higher turnover rate of SERCA that occurs when levels of cAMP are increased (46). CICR was successfully initiated ~ 400 ms after Ca²⁺ injection when P_{IP3R} and P_{SERCA} were increased to 174.72 fl/ms and 0.23 amol/ms, respectively (Fig. 3B, right trace). Properties describing the amplitude and time course of this simulated CICR (peak = 0.5 μM , time-to-peak = 2.2 s, and duration at the 50% amplitude cut-off = 5.6 s) were comparable to that of the Ca²⁺ transient measured by Kang et al. (Fig. 3A, right arrow) for mouse β -cells treated with forskolin (peak = $0.422 \pm 0.063 \mu M$, time-to-peak = 2.98 ± 1.69 s, and duration at the 50% amplitude cut-off = 7.6 ± 1.9 s). P_{IP3R} and P_{SERCA} values of 174.72 fl/ms and 0.23 amol/ms, respectively, provided the best match for simulated and experimental CICR when comparing these properties of Ca²⁺ transient amplitude and duration.

Kang et al. (25) also reported that a membrane permeable caged IP₃ (ci-IP₃/PM) could be used in combination with UV flash photolysis to evaluate how forskolin facilitates IP₃R-mediated Ca²⁺ mobilization in β -cells. In the absence of forskolin, the uncaging of IP₃ produced a small increase of Ca²⁺ (Fig. 3C, left arrow), whereas a Ca²⁺ transient with properties similar to CICR was initiated in β -cells treated with forskolin (Fig. 3C, right arrow). For these β -cells treated with forskolin, experimental properties describing the amplitude and time course of the Ca²⁺ transient triggered by the uncaging of IP₃ (peak = $0.528 \pm 0.096 \mu M$, time-to-peak = 4 ms, and duration at the 50% amplitude cut off = 8.8 ms) matched closely those measured under conditions in which the Ca²⁺ transient was instead triggered by the uncaging of Ca²⁺ (see above).

To simulate experimental findings obtained with caged IP₃, the $[IP_3]$ was increased in a step-wise manner from an initial value of 2 μM to final value of 100 μM . This final value was then maintained for the whole time course of the simulation (Fig. 3D, black horizontal bars). For the sake of simplicity, the rate of degradation of IP₃ was not considered. Continuous elevation of $[IP_3]$ stimulated only a small increase of $[Ca^{2+}]_i$ when values of P_{IP3R} and P_{SERCA} were set at "basal" levels (Fig. 3D, left trace) analogous to experimental findings obtained in the absence of forskolin (Fig. 3C, left arrow). These basal levels for P_{IP3R} and P_{SERCA} corresponded to 13.65 fl/ms and 0.069 amol/ms, respectively.

To model findings obtained for β -cells treated with forskolin, the values of P_{IP3R} and P_{SERCA} were increased to "activated" levels that corresponded to 174.72 fl/ms and 0.23 amol/ms, respectively. Under these conditions, the Ca²⁺ tran-

sient generated by a simulated step-wise increase of $[IP_3]$ (Fig. 3D, right trace) matched closely that of the Ca²⁺ transient induced by uncaging of IP₃ in β -cells treated with forskolin (Fig. 3C, right arrow). Properties describing the amplitude and time course of the IP₃-induced Ca²⁺ response under activated conditions in this simulation yielded the following values: peak = 0.56 μM , time-to-peak = 3.0 s, and duration at the 50% amplitude cut off = 5.9 s.

When considering these findings obtained in simulation studies that mimic the uncaging of Ca²⁺ (Fig. 3B) or the uncaging of IP₃ (Fig. 3D), and when also considering data in Fig. 1 concerning IP₃R open channel probability, it may be concluded that the present model of IP₃R-mediated Ca²⁺ mobilization faithfully reproduced experimental findings obtained using mouse β -cells (25). Furthermore, these simulation studies indicated that the β -cell IP₃R acts as a molecular coincidence detector in which its gating function is regulated not only by IP₃ and Ca²⁺ but also by cAMP signaling pathways.

Dynamic mechanisms for initiation and termination of Ca²⁺ transients. During the initiation of CICR, a fraction of the ER Ca²⁺ that is released might bind to the CaA site on the IP₃R, and progressively create positive feedback activation of the IP₃R. On the other hand, termination of CICR might result either from simultaneous binding of Ca²⁺ to the receptor's CaI site or from depletion of ER Ca²⁺ content. To reveal the dynamic mechanisms for initiation and termination of CICR, simulation experiments were performed by dissecting these competitive interactions among P_{CaAca} , P_{CaIca} , and $[Ca^{2+}]_{ER}$. First, to investigate how the positive feedback involving Ca²⁺-dependent activation of the IP₃R may be triggered, $[Ca^{2+}]_{nrs}$ and $[Ca^{2+}]_{blk}$ were monitored after injection of Ca²⁺ with increasing flux (as indicated by an arrow) from 40 to ~ 60 fmol/s for 1 ms (Fig. 4Aa). For this simulation, P_{CaIca} and $[Ca^{2+}]_{ER}$ were both fixed at the resting conditions (see Fig. 4, Ab and Ad), and J_{mleak} was excluded to reveal mechanisms of Ca²⁺ mobilization that are intrinsic to ER function.

When Ca²⁺ injection was stimulated with a rate of 40 fmol/s, J_{efflux} ($J_{IP3R} + J_{ERleak}$) and J_{SERCA} increased due to Ca²⁺-dependent activation of IP₃R and SERCA pump, respectively (data now shown). J_{efflux} , however, did not exceed J_{SERCA} and thus $[Ca^{2+}]_{nrs}$ and $[Ca^{2+}]_{blk}$ started to gradually decay to the resting levels (Fig. 4Aa, ①). When the Ca²⁺ injection became strong enough to outrun the threshold (see Fig. 4Aa, ⑤; 54.52 fmol/s), J_{efflux} was elevated over J_{SERCA} (data now shown), further increasing $[Ca^{2+}]_{nrs}$ and $[Ca^{2+}]_{blk}$ to a saturating level (Fig. 4Aa, ⑤–⑦) where J_{efflux} balanced with J_{SERCA} . Involvement of a positive feedback loop for activation of the IP₃R was well revealed by increasing P_{CaAca} (Fig. 4Ad) along with P_o (Fig. 4Ac) for the rising phase of $[Ca^{2+}]_{nrs}$. Note that the rate of the initial rising phase became faster with a stronger Ca²⁺ stimulus, whereas common sigmoidal time courses for self-regenerating responses were observed once positive feedback was triggered.

Next, we investigated whether binding of Ca²⁺ to the receptor's CaI site contributes to the termination of Ca²⁺ transients. To this end, the change in P_{CaIca} was calculated along with P_{CaAca} , whereas $[Ca^{2+}]_{ER}$ remained fixed at the resting conditions for this simulation (Fig. 4, Ba-Bd). When Ca²⁺ injection of 60 fmol/s was applied, similarly to Fig. 4Aa, P_{CaIca} was slowly increased as $[Ca^{2+}]_{nrs}$ was elevated,

compared with the rate of change in P_{CaAca} . This slow change in P_{CaIca} caused a disturbance of the self-regenerating positive feedback activation of IP₃R (see Fig. 4, *Bc* and *Bd*) and an acceleration of the decay of the Ca²⁺ response (Fig. 4*Ba*). Failure to generate the full positive feedback responses was because J_{SERCA} was elevated over J_{efflux} due to a gradual increase in P_{CaIca} (data not shown). Although $[Ca^{2+}]_{ER}$ was constant (Fig. 4*Bb*), the rate of the decay in the Ca²⁺ response was quite similar to that of Ca²⁺ transient in Fig. 3. Thus the slow rate of change in P_{CaIca} was predominantly determined by the rate of decay in the Ca²⁺ response.

When the change in $[Ca^{2+}]_{ER}$ was also calculated, a strong Ca²⁺ injection (650 fmol/s) caused a remarkable increase in $[Ca^{2+}]_{ER}$ (Fig. 4*Cb*) and Ca²⁺_{tot} (data not shown) within the minimal cell model from a control level of 11.92 μM and 141.18 amol to 68.15 μM and 797.83 amol, respectively. It also induced a notable increase in $[Ca^{2+}]_{blk}$ and $[Ca^{2+}]_{nrs}$ (Fig. 4*Ca*), whereas these Ca²⁺ levels quickly decayed as P_o of the IP₃R was reduced (Fig. 4*Cc*) along with a large increase in P_{CaIca} (Fig. 4*Cd*). Interestingly, these decreases in intracellular Ca²⁺ responses were followed with a marked sign of Ca²⁺ oscillations.

Taken together, initiation of CICR occurs when Ca²⁺ or IP₃ stimulation activates the IP₃R enough to elevate J_{efflux} ($J_{IP3R} + J_{ERleak}$) over J_{SERCA} (data not shown). Sequential rising and falling phases, followed by an autonomous termination of CICR in an intact system, are predominantly determined by the balance between the time courses of Ca²⁺-dependent activation and inhibition of the IP₃R. This sequential change in Ca²⁺-dependent activation and inhibition of the IP₃R (Fig. 4*Cd*) may also be responsible for the oscillatory behavior of $[Ca^{2+}]_{nrs}$. The mechanisms underlying the behavioral changes in $[Ca^{2+}]_{nrs}$ were further investigated in the following bifurcation analysis.

Bifurcation analysis reveals behavioral characteristics of the model. The simulations described above demonstrated that the IP₃R exhibited fundamental properties of sequential positive and negative feedback regulation due to fast activation and slow inhibition of the channel by Ca²⁺. Such findings strongly suggest that the herein described IP₃R model for CICR has the potential to also predict how oscillations of $[Ca^{2+}]_i$ are generated. Thus bifurcation analysis was performed to test this hypothesis, while also considering intracellular conditions within the minimal cell model that allow oscillations of $[Ca^{2+}]_i$ to take place. Behavioral characteristics of $[Ca^{2+}]_{nrs}$ were investigated by examining steady-state solutions; i.e., EPs and LCs of the model as a function of key model parameters, Ca²⁺_{tot}, P_{IP3R} , P_{SERCA} , α_{CaA} , and α_{CaI} . Figure 5A shows mode changes in $[Ca^{2+}]_{nrs}$ induced by increasing Ca²⁺_{tot}, from 0 to ~3,000 amol, under simulated conditions with a constant $[IP_3]$ (2 μM). When Ca²⁺_{tot} was set at a resting cellular level (141.2 amol, *dotted line a*), only a stable EP appeared. This indicated that $[Ca^{2+}]_{nrs}$ was stabilized on the EP at 0.156 μM (corresponding to the resting $[Ca^{2+}]_{nrs}$, *right a*) even with the activated IP₃R and SERCA pump.

On the other hand, Fig. 5A exhibited a stable LC as Ca²⁺_{tot} was raised above the first bifurcation point of 832.9 amol, suggesting spontaneous oscillations of $[Ca^{2+}]_i$. $[Ca^{2+}]_{nrs}$ oscillations occurring within the range of 0.51~1.14 μM (indicated by the stable LC at the *solid line b*) were predicted when Ca²⁺_{tot} reached 920.3 amol, an estimated total intracellular

Ca²⁺ content that existed during the bursting period in a full-cell model simulation (5). The upper and lower range of the LC (0.51~1.14 μM) matched well to that of $[Ca^{2+}]_{nrs}$ oscillations, *right b*. When Ca²⁺_{tot} increased above 2,522.6 amol, LCs disappeared, indicating termination of the oscillations, and stabilization of $[Ca^{2+}]_{nrs}$, at the corresponding stable EP. Ca²⁺_{tot} was set at 920.3 amol as a reference for the following bifurcation analysis.

Next, we considered how IP₃R and SERCA activity levels participate in the generation of Ca²⁺ oscillations. As Fig. 5B indicates, an increase in P_{IP3R} from a basal (13.65 fl/ms, at the dotted line) to an activated level (174.72 fl/ms, at the solid line) converted $[Ca^{2+}]_{nrs}$ from a stable to an oscillatory pattern. Thus appropriate increases in P_{IP3R} to levels within the range that produced stable limit cycles may, indeed, cause sustained oscillation in $[Ca^{2+}]_{nrs}$. When $[IP_3]$ was elevated, oscillations were predicted to occur at a lower range for P_{IP3R} (data not shown). In a similar manner, a mode change in the behavioral pattern of $[Ca^{2+}]_{nrs}$ was achieved by increasing P_{SERCA} from the resting (0.07 amol/ms, at the dotted line) to enhanced activity level (0.23 amol/ms, at the solid line).

Critical rate constants of Ca²⁺-dependent activation and inhibition of the IP₃R for the generation of $[Ca^{2+}]_{nrs}$ oscillations were also examined. As for Fig. 5, A–C, EPs and LCs of $[Ca^{2+}]_{nrs}$ were obtained as a function of varying α_{CaA} (Fig. 5D) and α_{CaI} (Fig. 5E). Accompanying β_{CaA} and β_{CaI} , respectively, were systematically modified so that corresponding dissociation constants (K_{dCaA} and K_{dCaI}) remained constant throughout the analyses. Since α_{CaA} , estimated by the simulation study in Fig. 3 (6 mM/ms, at the solid line in Fig. 5D), was remarkably faster than α_{CaI} (1.5 E-2 mM⁻¹.ms⁻¹, solid line in Fig. 5E), a slight change in α_{CaA} did not cause a significant effect on the oscillatory pattern for $[Ca^{2+}]_{nrs}$, unless it was below the bifurcation point ($\alpha_{CaA} = 0.69$ mM⁻¹.ms⁻¹). A modest rise in α_{CaI} , on the other hand, reduced the amplitude of $[Ca^{2+}]_{nrs}$ oscillations and eventually stabilized $[Ca^{2+}]_{nrs}$ at corresponding stable EPs if α_{CaI} became faster than 0.69 mM⁻¹.ms⁻¹, where a bifurcation occurred. Thus bifurcation analysis revealed the key parameters (Ca_{tot} , P_{IP3R} , P_{SERCA} , α_{CaA} , and α_{CaI}), which allow for IP₃R-mediated oscillatory Ca²⁺ mobilization.

IE analysis of the IP₃R-mediated Ca²⁺ oscillations in pancreatic β-cells. Although the bifurcation analysis successfully described the key parameters summarized above, the dynamic mechanisms describing how Ca²⁺ oscillations are generated at given parameter sets remained elusive. To systematically analyze the quantitative mechanisms underlying IP₃R-mediated Ca²⁺ oscillations, IE analysis of $[Ca^{2+}]_{nrs}$ was performed. The equilibrium $[Ca^{2+}]_{nrs}$ at each time point ($^{IE}[Ca^{2+}]_{nrs}$) was plotted along with $[Ca^{2+}]_{nrs}$ in Fig. 6A. Although $^{IE}[Ca^{2+}]_{nrs}$ always moved in advance of $[Ca^{2+}]_{nrs}$, $[Ca^{2+}]_{nrs}$ apparently overlapped with $^{IE}[Ca^{2+}]_{nrs}$, suggesting that the oscillation occurred nearly at the instantaneous equilibrium. Corresponding time courses of changes in P_{CaAca} and P_{CaIca} during $[Ca^{2+}]_{nrs}$ oscillations in Fig. 6A are shown in Fig. 6B. Note that for these simulations, the cyclic increases (or decreases) in P_{CaIca} were delayed from P_{CaAca} by approximately one-half cycle, exhibiting antiphase changes in the activation and inactivation parameters.

Contributions of P_{CaAca} , P_{CaIca} , and $[Ca^{2+}]_{blk}$ toward the rate of change in $^{IE}[Ca^{2+}]_{nrs}$ ($d^{IE}[Ca^{2+}]_{nrs}/dt$) are indicated in

Fig. 5. Bifurcation analysis demonstrated how model parameters alter oscillatory behavior at $[Ca^{2+}]_{nrs}$. Steady-state solutions, equilibrium points (EPs), and limit cycles (LCs) of $[Ca^{2+}]_{nrs}$ are plotted as functions of various model parameters; Ca^{2+}_{tot} (A), P_{IP3R} (B), P_{SERCA} (C), α_{CaA} (D), and α_{CaI} (E). A LC is represented with two lines: the minimum and maximum values in the oscillation. Parameters in A at the dotted lines (a) are for Ca^{2+}_{tot} expected during the cellular quiescent period (141.2 amol). Ca^{2+}_{tot} at the solid line (b), on the other hand, represents estimated total Ca^{2+} content during glucose-induced continuous bursting in β -cells (920.3 amol). Time-dependent simulation of $[Ca^{2+}]_{nrs}$ using the parameters a and b are shown at right. P_{IP3R} and P_{SERCA} in B and C at dotted lines represent the basal activity levels for the IP₃R and SERCA ($P_{IP3R} = 13.65$ fl/ms, $P_{SERCA} = 0.07$ amol/ms), respectively, whereas those at the solid lines exhibit the activated levels ($P_{IP3R} = 174.72$ fl/ms, $P_{SERCA} = 0.23$ amol/ms), presumably, during agonist-induced elevation of [cAMP]. α_{CaA} and α_{CaI} , at the solid lines in D and E (6 $mM^{-1}\cdot ms^{-1}$ and 1.5 E-2 $mM^{-1}\cdot ms^{-1}$), respectively, are rate constants determined by reproducing Ca^{2+} -induced Ca^{2+} release in Fig. 3.

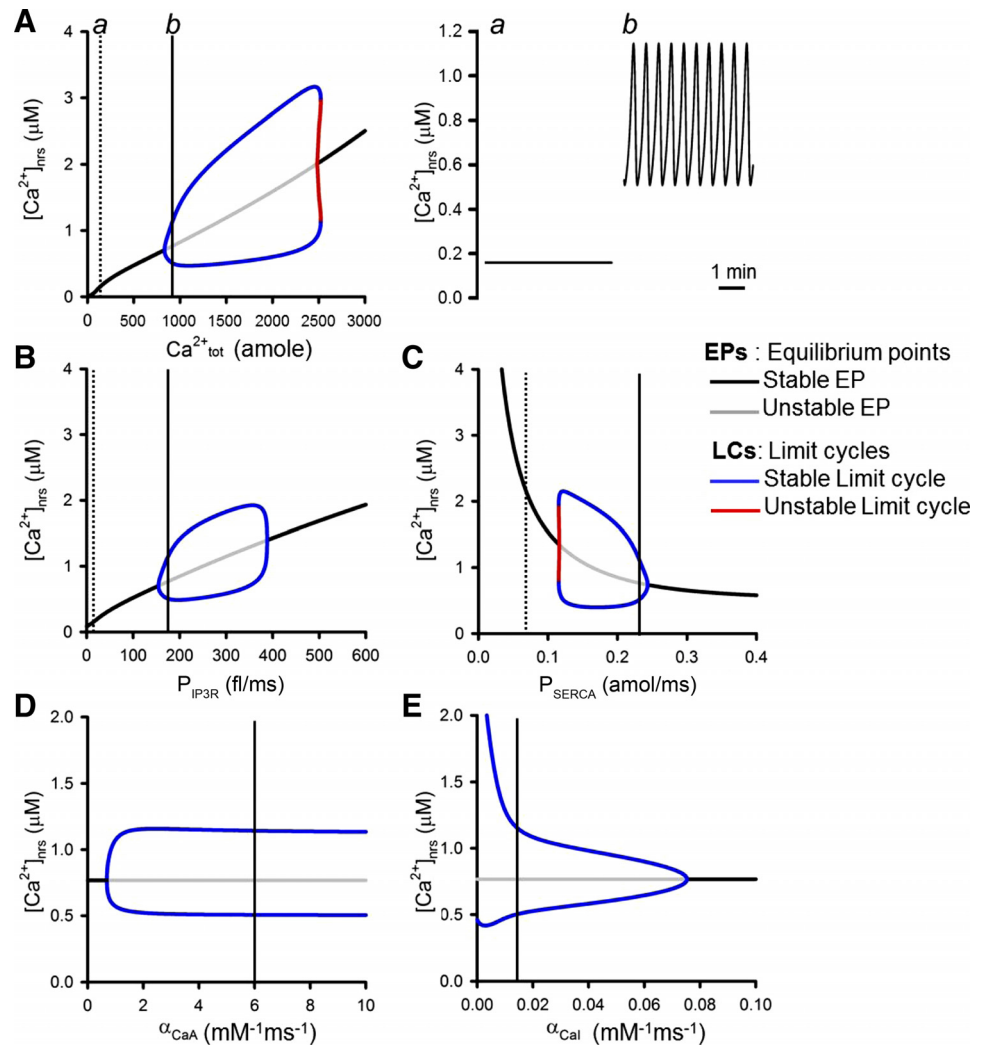


Fig. 6C in a unit of $\mu M/s$ (see Eq. 18) to elucidate the variable component primarily driving the $^{IE}[Ca^{2+}]_{nrs}$ oscillations. For this analysis, at the inflection point of $^{IE}[Ca^{2+}]_{nrs}$, from descending to ascending phases, where the $d^{IE}[Ca^{2+}]_{nrs}/dt$ switched from negative to positive (~ 7.8 s in Fig. 6C), P_{CaIca} was the only variable that provided a positive contribution, specifically, from ~ 2 s before the inflection point (see the expanded trace in Fig. 6D). Thus P_{CaIca} was the key factor to drive the increase of $^{IE}[Ca^{2+}]_{nrs}$. For these simulations, reducing Ca^{2+} -dependent inhibition of the IP₃R during the decay phase of $[Ca^{2+}]_{nrs}$ (see Fig. 6B) conferred an increased P_o for the channel and subsequently drove the increase of $[Ca^{2+}]_{nrs}$. Rate of change in P_{CaIca} thus provided a significant impact on the rate of change in $[Ca^{2+}]_{nrs}$ and therefore the frequency of $[Ca^{2+}]_{nrs}$ oscillations. On the other hand, $[Ca^{2+}]_{blk}$ and P_{CaAca} provided larger positive contributions than P_{CaIca} to $d^{IE}[Ca^{2+}]_{nrs}/dt$ but only after the inflection point of $^{IE}[Ca^{2+}]_{nrs}$. Once $[Ca^{2+}]_{nrs}$ began to increase, $[Ca^{2+}]_{blk}$ also increased, accelerating the rise in $d^{IE}[Ca^{2+}]_{nrs}/dt$ by reducing Ca^{2+} efflux from nrs site (J_{nrsblk} , see Eq. 10).

Note that for these simulations, SERCA negatively contributed to the rate of rise in $[Ca^{2+}]_{blk}$ as the concentration of Ca^{2+} increased. However, $[Ca^{2+}]_{blk}$ continued to rise due to J_{efflux} exceeding J_{SERCA} (data not shown). The positive contribution

of P_{CaAca} to the rate of rise in $[Ca^{2+}]_{blk}$ reflected positive feedback regulation involving Ca^{2+} -dependent stimulation of the channel, actively increasing $d^{IE}[Ca^{2+}]_{nrs}/dt$. For this analysis, a contribution of $P_{IP3HIp3}$ to $d^{IE}[Ca^{2+}]_{nrs}/dt$ is not shown in Fig. 6, C–E, since it was negligibly small. Finally, note that the inflection point from ascending to descending phases of $^{IE}[Ca^{2+}]_{nrs}$ (Fig. 6A) was explained by a similar but opposite mechanism to the one described above. The positive rate of change in $^{IE}[Ca^{2+}]_{nrs}$ that switched to negative (~ 28.3 s in Fig. 6C, expanded in the Fig. 6E) was a result of increased P_{CaIca} (see Fig. 6B). Decreasing $[Ca^{2+}]_{blk}$ and P_{CaAca} amplified the decay in $^{IE}[Ca^{2+}]_{nrs}$ after $[Ca^{2+}]_{nrs}$ started to decline. This analysis revealed that the slow rate of change in P_{CaIca} drove and paced Ca^{2+} oscillations.

DISCUSSION

Modeling IP₃R function under the control of GLP-1 in pancreatic β -cells. In the theoretical analysis presented here, cellular mechanisms of IP₃R regulation were investigated to better understand how GLP-1 mobilizes Ca^{2+} , while also stimulating global oscillations of $[Ca^{2+}]_i$. Our analysis took advantage of a new dynamic IP₃R1 model that allowed simulation studies to be performed so that novel aspects of IP₃R

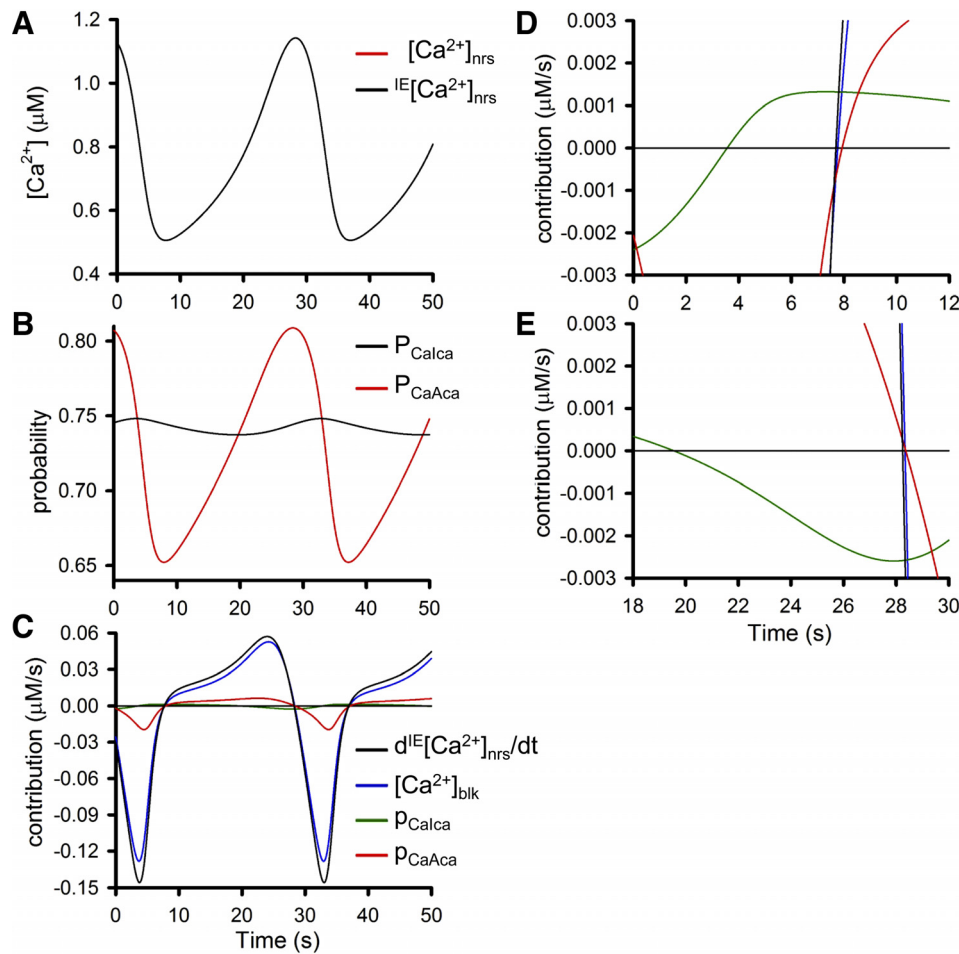


Fig. 6. Instantaneous equilibrium (IE) analysis for the quantitative mechanisms underlying IP₃R-mediated Ca²⁺ oscillations in β -cells. The time course in 1 full cycle of the $[Ca^{2+}]_{nrs}$ oscillation was plotted with $IE[Ca^{2+}]_{nrs}$ in A, and the corresponding rate of change in $IE[Ca^{2+}]_{nrs}$ along with the contributions of variables; $[Ca^{2+}]_{blk}$, P_{CaAca} , and P_{CaIca} toward $IE[Ca^{2+}]_{nrs}/dt$, are shown in B with different colors. The analysis from 0 to ~ 12 and 18 to ~ 30 s in E were expanded in C and D, respectively.

regulation were revealed. The model reproduced steady-state activities of the IP₃R1 that occur as a consequence of the receptor's allosteric regulation by $[Ca^{2+}]$ and $[IP_3]$ (Fig. 1). Using a minimal cell model in which GLP-1-stimulated cAMP production induced an increase of IP₃R and SERCA activities, we successfully reconstructed not only the Ca²⁺- and IP₃-induced Ca²⁺ transients (Fig. 3, B and D) but also oscillatory Ca²⁺ responses initiated by GLP-1. Ca²⁺ transients and Ca²⁺ oscillations could be induced when IP₃R and SERCA activities were elevated from basal levels ($P_{IP3R} = 13.65$ fl/ms and $P_{SERCA} = 0.069$ amol/ms) to elevated levels ($P_{IP3R} = 174.72$ fl/ms and $P_{SERCA} = 0.23$ amol/ms) that are predicted to exist when β -cells are stimulated with GLP-1.

Simulation studies presented here (Fig. 4) suggest that the time courses of the Ca²⁺ transients are dictated by sequential positive and negative feedback regulation in which there is faster activation and slower inhibition of the IP₃R by Ca²⁺. Initiation of the Ca²⁺ transient (CICR) occurs due to positive feedback involving a Ca²⁺-dependent increase in P_{CaAca} when Ca²⁺ or IP₃ activate the IP₃R enough so that Ca²⁺ liberation ($J_{IP3R} + J_{ERleak}$) exceeds its removal from the cytosol ($J_{SERCA} + J_{mleak}$). The peak of the Ca²⁺ response is achieved when the J_{IP3R} starts to decline primarily due to an increase in P_{CaIca} , so that J_{efflux} drops below $J_{excretion}$. The rate of change in P_{CaIca} provides a remarkable contribution to the rate of decay in the transient response and to ultimate termination of CICR. Bifurcation analysis also revealed that sequential posi-

tive and negative feedback regulation of the IP₃R by Ca²⁺ was a crucial property to induce Ca²⁺ oscillations. IE analysis simultaneously disclosed that the key factor to drive and pace Ca²⁺ oscillations was the slower rate of change in P_{CaIca} causing gradual development of Ca²⁺-dependent inhibition of the IP₃R followed by recovery from inhibition (Fig. 6).

Experimental findings compared with stimulation-based studies. Bifurcation analysis presented in Fig. 5, A–C, further clarified that the generation of oscillatory Ca²⁺ events was explained by an elevation of Ca^{2+}_{tot} , as well as IP₃R and SERCA activation that reached levels exhibiting stable limit cycles. In true physiological conditions, the increase of Ca^{2+}_{tot} occurs during the bursting period when concentrations of glucose rise to stimulatory levels. Simultaneously, IP₃R and SERCA activity is increased by GLP-1, as discussed above. Although these fundamental mechanisms underlie rhythmic IP₃R-mediated Ca²⁺ mobilization, it must be pointed out that only a few reports exist in which oscillatory Ca²⁺ release was found to occur in β -cells (18, 31–33). The difficulty to demonstrate oscillatory Ca²⁺ events might be attributed to the involvement of multiple cellular factors that must occur in a temporally coincident manner. In other words, any failure in the system that causes an insufficient increase in either IP₃R or SERCA activity levels, or Ca^{2+}_{tot} , will lead to imperfect or unsuccessful generation of IP₃R-mediated rhythmic Ca²⁺ mobilization.

Importantly, our analysis verifies that IP₃R-mediated Ca²⁺ oscillations can be generated when the cytosolic [IP₃] is held constant. This finding is consistent with prior experimental results (1, 34, 44). However, since IP₃ is the primary agonist for the IP₃R, it is evident that [IP₃] is an essential contributing factor to determine the activity level of the IP₃R so that it may be modulated by cAMP and [Ca²⁺]_i. It is particularly interesting to note that β-cells express a Ca²⁺-sensitive phospholipase C (PLC) that might participate in the generation of Ca²⁺ oscillations. It catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce IP₃ (42), and in fact Ca²⁺-dependent feedback activation of PLC by Ca²⁺ mobilized from IP₃R-regulated Ca²⁺ stores is likely to occur (43). Such a feedback process might impact the shape of Ca²⁺ oscillations, as suggested by De Young and Keizer (10).

Modeling vol_{nrs} reveals insights concerning the importance of Ca²⁺ microdomains. For the simulations reported here, it was assumed that relative to the bulk cytosolic Ca²⁺ concentration ([Ca²⁺]_{blk}), individual microdomains (“nrs” in Fig. 2) of elevated Ca²⁺ concentration ([Ca²⁺]_{nrs}) existed at Ca²⁺ release sites where IP₃ receptors were located. Therefore, the resultant steep gradient of [Ca²⁺] between IP₃Rs and the bulk space was mathematically represented by incorporation of Ca²⁺ microdomains into the present model. Since there was no reference that suggests the specific size of Ca²⁺ microdomains in β-cells, vol_{nrs} was assumed to be ~1% of the size of vol_{blk} in this study (vol_{nrs} = 7.73 fl). With this assumption, simulations were successfully performed. However, arbitrarily decreasing vol_{nrs} in concert with increasing P_{nrsblk} (a parameter that determines the flux of Ca²⁺ from nrs to the blk space) also generated Ca²⁺ transients and oscillations, the time courses and amplitudes of which were similar to those simulated with control parameters (cf. Figs. 3 and 5Ab). Thus an interplay between vol_{nrs} and P_{nrsblk} led to an inability to precisely determine the exact values for these two parameters. Although exact values for vol_{nrs} and P_{nrsblk} were not estimated, our assumption of small microdomains of high [Ca²⁺] occurring at point sources of Ca²⁺ release (“nrs”) was shown to be a valid. Omission of “nrs” from the model led to a situation in which the simulations failed to reconstruct transient and oscillatory Ca²⁺ responses that were previously observed under experimental conditions (i.e., the peak as well as the interval separating the oscillatory Ca²⁺ release events were severely increased, data not shown).

Conclusion. For nearly 30 yr it has been appreciated that the insulin secretagogue action of the GLP-1 is a glucose-dependent phenomenon (28). More specifically, GLP-1 potentiates glucose-stimulated insulin secretion only when levels of glucose are elevated to concentrations that are themselves capable of initiating insulin exocytosis. This glucose-dependent action of GLP-1 is due, at least in part, to synergistic activation of the IP₃R by glucose metabolism and the GLP-1/cAMP signaling pathways. When considering the action of glucose, it is established that oxidative glucose metabolism in β-cells generates cytosolic ATP, a nucleotide that increases IP₃R Ca²⁺ sensitivity by producing a left-shift of the IP₃R open probability (P_o) as a function of the Ca²⁺ concentration relationship (15). Glucose metabolism also promotes Ca²⁺ influx, and the ensuing increase of [Ca²⁺]_i allows Ca²⁺ to act as a coagonist with IP₃ so that it may activate the IP₃R. When considering the action of GLP-1, evidence exists for the participation of PKA,

a protein kinase that phosphorylates IP₃ receptors so that they open more efficiently in response to Ca²⁺ (41). GLP-1 also activates the cAMP sensor Epac2 to stimulate a novel PLCε that promotes protein kinase C (PKC) activation and additional Ca²⁺ mobilization (13). Thus coadministration of glucose and GLP-1 to β-cells leads to cyclic activation of the IP₃R with resultant oscillations of [Ca]_i that stimulate insulin exocytosis. This constitutes a novel mechanism of CICR, and as demonstrated by the mathematical simulations reported here, IP₃ receptors are considered to be molecular coincidence detectors dually activated by IP₃ and Ca²⁺ in a context-specific manner. Elevated glucose metabolism is an essential contributing factor, whereas GLP-1/cAMP renders IP₃ receptors fully competent to release Ca²⁺ in response to both IP₃ and Ca²⁺. Although the simulations presented here are clearly relevant to mouse β-cells, our minimal model will eventually need to be expanded to include the participation of other types of intracellular Ca²⁺ release channels. For example, evidence exists that in human and rat β-cells, RYR2-mediated CICR can contribute to processes of ER Ca²⁺ mobilization (7, 20).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.T. and A.N. conception and design of research; Y.T. and A.N. performed experiments; Y.T., T.S., and G.G.H. analyzed data; Y.T., T.S., and G.G.H. interpreted results of experiments; Y.T. and T.S. prepared figures; Y.T. and G.G.H. drafted manuscript; Y.T., T.S., G.G.H., and A.N. edited and revised manuscript; Y.T., T.S., G.G.H., and A.N. approved final version of manuscript.

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