Systems analysis of GLP-1 receptor signaling in pancreatic β-cells

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Upon elevation of plasma glucose concentration ([glucose]), pancreatic β-cells generate bursts of action potentials to induce cyclic changes in [Ca2+] (55) and regulate pulsatile insulin release (25). This glucose-dependent insulin secretion is synergistically enhanced by incretin hormones, which are released upon meal ingestion from endocrine cells distributed over the intestinal tract (16). The incretin hormones include glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). GLP-1 is more effective than GIP to improve glucose-dependent insulin secretion from endocrine cells in the presence or absence of 3-isobutyl-1-methylxanthine (IBMX). The simulation analysis revealed the presence of two sequential desensitization steps of the GLP1 receptor that occur with fast and very slow reaction rates. The cross talk between glucose- and GLP-1-dependent signal cascades for cAMP synthesis was well reconstructed by integrating the direct regulation of AC and PDE by [Ca2+] into a minimal mathematical model of GLP-1 receptor signal transduction (54, 66) in both -cells and insulinoma cell lines. The model thus developed was validated by reconstructing the dynamic changes in [cAMP] during GLP-1 stimulation in the presence and absence of 3-isobutyl-1-methylxanthine (IBMX) observed under various experimental conditions. The model well-simulated GLP-1-induced [cAMP] elevation and predicted the activities of cAMP effectors PKA and Epac as a function of [cAMP], the key signal underlying the insulinotropic effects (17, 62).

The [cAMP] is determined primarily by the balance between AC and PDE activities, which are regulated by Ca2+-permeable ion channels and transporters as well as Ca2+ release and uptake by the endoplasmic reticulum (ER). The increase in [cAMP] subsequently activates protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac), modulating the activities of multiple ion channels at the plasma membrane (26, 31, 35, 41, 42, 57) and ER (27, 36, 64), which in turn modify the pattern of Ca2+ transients. PKA and Epac also have direct effects on proteins that are involved in exocytosis of insulin vesicles (30), and thus the fine regulation of [cAMP] is critical for the adequate insulinotropic effects of GLP-1. However, since multiple signaling factors are involved in regulating [cAMP], the kinetic aspects of the reaction cascade during GLP-1 stimulation have not yet been determined in pancreatic β-cells.

To overcome this difficulty, we developed a mathematical model of GLP-1 receptor signal transduction. We adopted a strategy of estimating individual reaction rates and model parameters by fitting the theoretical reaction scheme to a variety of key experimental findings published to date (3, 11, 54, 66) in both β-cells and insulinoma cell lines. The model thus developed was validated by reconstructing the dynamic changes in [cAMP] during GLP-1 stimulation in the presence and absence of 3-isobutyl-1-methylxanthine (IBMX) observed under various experimental conditions. The model well-simulated GLP-1-induced [cAMP] elevation and predicted the activities of cAMP effectors PKA and Epac as a function of [cAMP]. The simulation analysis revealed the presence of two transition steps of receptor desensitization that occur with fast and slow kinetics. The molecular basis for synergistic relationship between glucose and GLP-1 signaling in the cAMP synthesis were clarified by calculating the direct regulation of AC and PDE by [Ca2+]. Finally, the robustness of the signaling system in controlling [cAMP] was examined by comparing the AC and PDE activities in the presence or absence of GLP-1 and/or the PDE inhibitor.

Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>[L]</td>
<td>GLP-1 (ligand)</td>
</tr>
<tr>
<td>[R]</td>
<td>free GLP-1receptor</td>
</tr>
<tr>
<td>[Rt]</td>
<td>total GLP-1 receptor</td>
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<tr>
<td>[Rd]</td>
<td>active GLP-1 receptor</td>
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<td>[RD1]</td>
<td>desensitized GLP-1 receptor in state 1</td>
</tr>
<tr>
<td>[RD2]</td>
<td>desensitized GLP-1 receptor in state 2</td>
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<tr>
<td>[LR]</td>
<td>GLP-1 receptor bound with ligand</td>
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1 This article is the topic of an Editorial Focus by Harvey (29a). Address for reprint requests and other correspondence: N. Inagaki, 54 Shogoin, Kawahara-cho, Sakyo-ku, Kyoto-shi, Kyoto, Japan.
A minimal model of the GLP-1 receptor signaling transduction in pancreatic β-cells was constructed. Parameters to define the model, including concentrations, binding constants ($K_i$) of signaling factors, maximum activity ($V_{\text{max}}$) and half-maximal effective concentration ($K_{1/2}$) of substances for activation of enzymes, rate constants, and various magnitude factors of kinetic equations are listed in APPENDIX I. The time-dependent changes in conformations of G protein are calculated by the time-based integration using the Euler method. When assuming instantaneous equilibrium, whereas those marked with blue arrows were calculated by the time-based integration using the Euler method. When calculating the instantaneous equilibrium enclosed with the red rectangle, the constraint of mass conservation was applied to the sum of (R + LR + LRG) (and G and LRG), respectively, at each time step.

**Activation of GLP-1 receptor.** Figure 1 shows the reaction scheme of the minimal model of the GLP-1 receptor cascade. Active receptors may form three different conformations: free receptor (R), ligand (L)-bound receptor (LR), and the Gs-bound LR complex (LRG). Binding reactions indicated by black arrows were assumed to be much faster than the rest of reactions associated with conformational changes (22), and thus an instantaneous equilibrium was assumed for the reactions enclosed within the red rectangle. The dissociation constant ($K_d$) for GLP-1 binding to the receptor has been determined through the time-based integration using the Euler method.

**METHODS**

A minimal model of the GLP-1 receptor signaling transduction in pancreatic β-cells was constructed. Parameters to define the model, including concentrations, binding constants ($K_i$) of signaling factors, maximum activity ($V_{\text{max}}$) and half-maximal effective concentration ($K_{1/2}$) of substances for activation of enzymes, rate constants, and various magnitude factors of kinetic equations are listed in APPENDIX I. The time-dependent changes in conformations of G protein are calculated by the time-based integration using the Euler method. When assuming instantaneous equilibrium, whereas those marked with blue arrows were calculated by the time-based integration using the Euler method. When calculating the instantaneous equilibrium enclosed with the red rectangle, the constraint of mass conservation was applied to the sum of (R + LR + LRG) (and G and LRG), respectively, at each time step.

![Fig. 1. Reaction scheme of glucagon-like peptide-1 (GLP-1) receptor activation.](http://ajpcell.physiology.org/)
The G₆αGTP-dependent activation of AC was calculated with a Kₙ₀/₂ determined by Sunahara and colleagues (38). An [ATP] of 3 mM was used in the present study, and the Kₙ₀/₂ of [ATP] defining the substrate dependency of VₐC and VₐC,G were adopted from Dessauer et al. (33). The term for Ca²⁺-dependent regulation of VₐC,G in Eq. 11 was originally developed in Aplysia neurons (69) and was modified to fit the ACVIII activity (21) in the β-cell model by Fridlyand et al. (23). Eq. 11 contains both Ca₆CaM ([Ca₆CaM]+[Ca₄CaM])-mediated activation and Ca²⁺-dependent inhibition. We additionally introduced fₐcₐ, the fraction of Ca₆CaM-dependent VₐC,G. A [Ca²⁺⁻] of 500 nM was assumed under a high-glucose condition and a resting [Ca²⁺⁻] of 100 nM for a lower glucose concentration used in experiments (18, 33, 60). An instantaneous equilibrium was assumed for the binding of Ca²⁺ to CaM using the association and dissociation rate constants given by Yu and colleagues (69). The VₐC,G was determined at 1.8 μM/s from the initial rate of rise (dashed line in Fig. 3A) of [cAMP] evoked by GLP-1 in the presence of high [IBMX] > 250 μM and [glucose] > 20 mM. Based on this estimation, VₐC,G of AC activities (VₐC,G and VₐC,G) and fractions (f) of the Ca²⁺-dependent component of VₐC,G(fₐcₐ,AC) were optimized (see APPENDIX I) by reconstructing experimental findings with the whole reaction scheme.

In β-cells, it has been suggested that several PDE isoforms (1C, 3B, 4B, and 10A) are involved in regulation of insulin secretion (15, 51). However, the fractional contribution of each isoform to cAMP degradation in intact cells has not yet been determined. Sams and Montague (54) observed over 70% of total PDE activity in the supernatant fraction of an homogenate of islets of Langerhans. Their kinetic analysis of the soluble PDEs suggested the presence of at least two fractions with different activities, as indicated by two linear components (dashed and solid black lines) in the Lineweaver-Burk plot (Fig. 2). We reevaluated the experimental results by fitting the data with a sum of two Michaelis-Menten functions (Eq. 12) in the present study.

\[ V_{PD} = V_{max,PD} \cdot \left( \frac{f \cdot [cAMP]}{[cAMP] + K_{m1}} + \frac{(1-f) \cdot [cAMP]}{[cAMP] + K_{m2}} \right) \]

The half-maximal value of Ca₆CaM for the stimulation of PDE (68) was adopted from the PDE model developed for β-cells (23), and fₐc₈,PD reflects the fraction of the Ca₆CaM-dependent component of the enzyme. Given the [cAMP] is determined under various experimental conditions (Table 1), VₐC,G and VₐC,G(PDE) were finely adjusted by reconstructing these experimental findings using 100 or 500 nM [Ca²⁺⁻] according to the [glucose] used in experiments.

In experimental studies, the rise in [cAMP] evoked by GLP-1 saturates even in the presence of a maximal inhibitory concentration of IBMX, indicating that some fraction of PDE activity still remained, controlling [cAMP]. Ahmad and colleagues (1) showed that ~80% of the soluble PDE activity in a β-cell line (BRIN-BD11 cells) was blocked by [IBMX] > 200 μM, and thus we assumed that the

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Simulation Result</th>
<th>Simulation Data</th>
<th>Simulation Result</th>
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<tr>
<td>cAMP levels, μM</td>
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<tr>
<td>Resting (control)</td>
<td>1.4 mM Glucose</td>
<td>100 nM Ca²⁺⁻</td>
<td>20 mM Glucose</td>
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<tr>
<td>10 nM GLP-1</td>
<td>3.4</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>10 nM GLP-1/IBMX</td>
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<td>12.0</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>38.2</td>
<td>37.0</td>
<td>55.2</td>
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</table>

The cAMP levels were indicated in units of fmol·10⁻³ cells⁻¹ in the experimental work (11), and we converted these units to μM by assuming the cytoplasmic volume of a single β-cell [764 fl; (10)]. Administration of IBMX (250 μM) was simulated by decreasing PDE activity by 80%. See text for definitions of abbreviations.

![Figure 2. Determination of Kₙᵩ values of phosphodiesterase (PDE) based on experimental Lineweaver-Burk plots. Filled circles and fitted (dotted and black) lines are reproduction of experimental data (54) determined in guinea pig islets. The properties of the PDE activity components were reevaluated by fitting the experimental results with Eq. 12 (solid gray line).](http://apjpress.physiology.org/)

The fitting (gray curve in Fig. 2) determined the Kₙᵩ values (Kₙᵩ and Kₙᵩ) and f of the two components. VₐPDE reflects the sum of all PDE activities.

Since Ca₆CaM-sensitive PDE1C plays a functional role in degradation of cAMP in the β-cell lines βTC3 (28) and MIN6 (38), a Ca²⁺-dependent component was added to the PDE model (Eq. 13).

\[ V_{PDE} = V_{max,PD} \cdot \left( \frac{f \cdot [cAMP]}{[cAMP] + K_{m1}} + \frac{(1-f) \cdot [cAMP]}{[cAMP] + K_{m2}} \right) \]

(13)

(1 - fₐc₈,PD) + fₐc₈,PD · ( [Ca₆CaM] + [Ca₄CaM] + 0.000348 )

The half-maximal value of Ca₆CaM for the stimulation of PDE (68) was adopted from the PDE model developed for β-cells (23), and fₐc₈,PD reflects the fraction of the Ca₆CaM-dependent component of the enzyme. Given the [cAMP] is determined under various experimental conditions (Table 1), VₐC,G and VₐC,G(PDE) were finely adjusted by reconstructing these experimental findings using 100 or 500 nM [Ca²⁺⁻] according to the [glucose] used in experiments.

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IBMX-insensitive PDE8 may contribute 20% PDE activity in the presence of a high [IBMX].

PKA and Epac activities. Although pancreatic β-cells most likely express both PKA type I and II (2, 37, 39), the isoform predominantly regulating the insulinotropic effect of GLP-1 has not been investigated. Since $K_d$ of PKA type I [2.9 μM (9)] and the half-maximal [cAMP] for the activation of type II [$K_{i/2} = 2–3$ μM (7)] were very similar, we included one hypothetical type of PKA in the present model, and the activity was calculated with a $K_d$ of 2.9 μM (Hill coefficient, $n_H = 1.4$) determined by Dao et al. (9). Distinct values of $K_{i/2}$ were reported for Epac1 and 2 [30 μM for Epac1 (20) and 20 μM for Epac2 (52, 63)], and thus the active fractions were separately determined.

RESULTS

[cAMP] in pancreatic β-cells under resting conditions and GLP-1 stimulation. The basal level of [cAMP] was 1.6 μM at 100 nM [Ca$^{2+}$] and 1.4 μM at 500 nM [Ca$^{2+}$] in our model simulation (Table 1). These values of [cAMP] are comparable to 3.4 and 3.2 μM measured in rat primary β-cells at 1.4 and 20 mM [glucose], respectively (11). Upon stimulation with 10 nM GLP-1 for 15 min, [cAMP] increased to 4.2 μM at 100 nM [Ca$^{2+}$] and to 5.6 μM at 500 nM [Ca$^{2+}$] in our model. If PDE was inhibited by 80% (corresponding to 250 μM IBMX) in the absence of GLP-1, [cAMP] increased to ~11 μM independently of [Ca$^{2+}$] levels. These results also agreed well with the experimental observations. When stimulated with 10 nM GLP-1 in the presence of IBMX, [cAMP] elevated to 37.0 μM at 100 nM [Ca$^{2+}$] and further to 57.6 μM at 500 nM [Ca$^{2+}$], similarly to the experimental records of 38.2 and 55.2 μM obtained at the low (1.4 mM) and high (20 mM) [glucose], respectively.

The experimental time course of [cAMP] accumulation induced by GLP-1 (66) was also examined (Fig. 3). Upon stimulation with GLP-1 at 25 mM [glucose] in the presence of IBMX, [cAMP] increased rapidly and slowly declined after reaching peaks within 4 min (filled circles, Fig. 3A). The time course of [cAMP] was well reconstructed by simulation at 20% PDE activity and 500 nM [Ca$^{2+}$] (black curve, Fig. 3A). The velocity of cAMP degradation by PDE gradually increases with increasing [cAMP], and the peak is attained when the production rate of cAMP ($V_{AC,t}$) matched the degradation rate by PDE ($V_{PDE}$ in Eq. 8). Simulations revealed that the time to peak as well as the subsequent slow decline in [cAMP] were also influenced by desensitization of the GLP-1 receptor, predominantly due to the state transition to $R_{D1}$ (Fig. 1). In the absence of IBMX, the balance between AC and PDE activities are attained at a much lower [cAMP], thereby giving a time to peak of <1 min (gray curve, Fig. 3A). The simulation result was also in good agreement with experimental data (open circles, Fig. 3A).

The experimental dose-response relationship obtained by Widmann and colleagues (66) was reconstructed by calculating the [cAMP] accumulation attained over 10 min application of different concentrations of GLP-1 at 20% PDE activity and 500 nM [Ca$^{2+}$] (Figs. 3B and C). At 0.001 and 0.01 nM [GLP-1], [cAMP] increased to a stable saturation level within 2 min (Fig. 3B), whereas at higher [GLP-1], the time to peak was delayed with increasing [GLP-1] and the desensitization became more pronounced. As [GLP-1] increases, the activation of $V_{AC,G}$ (see Eqs. 9–11) became significant when [GLP-1] > 0.1 nM and nearly saturated at [GLP-1] slightly larger than 10 nM. In Fig. 3C, the dose-dependent accumulation of cAMP at the end of 10 min application of different concentrations of GLP-1 are compared between the simulation and experimental...
Ultra-slow desensitization of the GLP-1 receptor. The simulation analysis suggested that the gradual decay of [cAMP] after the peak (Fig. 3, A and B) during GLP-1 stimulation largely reflects desensitization of the ligand-bound receptor (LR and LRG in Fig. 1) to RD1. However, the RD1 kinetics alone failed to reconstruct the very slow inactivation remaining 1 h after washing out agonist as observed by Baggio and colleagues (3). The model including an RD2 state in series with RD1 reconstructed well the desensitization phenomenon in response to the experimental protocol (Fig. 4A). When the 2-h prestimulation protocol was applied (Fig. 4B), the RD1 fraction (blue curve) increased to a maximum of 0.28 at the expense of the active fraction (Ra, red curve) within the initial 10 min, and then both Ra and RD1 slowly declined thereafter due to a continuous transition to RD2 (green curve). During the washout period, the RD1 fraction quickly became insignificant, whereas 85% of RD2 remained even after 1 h washout. The result suggests that the fraction of Ra available for the second application of GLP-1 decreases depending on the preincubation period. Figure 4C shows the [cAMP] response to the experimental protocol with varying pretreatment periods, indicated by different colors. The gray trace was obtained by applying IBMX only at the time point of 180 min. D: [GLP-1] – \( V_{AC,t} \) relations at the end of different stimulation periods of 6 s, 10 s, and 1, 2, 20, 200, 2,000, and 4,000 min, from top to bottom of 8 curves, respectively. The traces of 6 and 10 s stimulation almost overlapped with one another, indicating that the desensitization was invisible with these short periods, whereas traces with 2,000 and 4,000 min stimulation also overlapped, indicating saturation of the desensitization already at ~2,000 min.

To characterize the steady-state desensitization of the GLP-1 receptor, the [GLP-1]-dependent \( V_{AC,t} \) at varying incubation periods (10 s ~ 4,000 min) were computed over the range 0.001–100 nM [GLP-1] (Fig. 4D). With 6- and 10-s applications, \( V_{AC,t} \) nearly overlap one another virtually without any sign of receptor desensitization. When the duration of GLP-1 application was prolonged, the desensitization gradually developed and a steady state was obtained at ~2,000 min application, which gave ~25% of the control \( V_{AC,t} \) at the saturating [GLP-1]. The extent of desensitization was more pronounced when stimulated with higher [GLP-1].

Cross-talk between glucose and GLP-1 signal pathways in determining [cAMP]. Experimental studies in Min6 and INS-1 cells demonstrated that [cAMP] increased in phase with a temporal increase in \([\text{Ca}^{2+}]\) in the presence of GLP-1, whereas the [cAMP] accumulation caused by IBMX alone (Fig. 4C) was subtracted from the [cAMP] responses to the second application of agonist, and the differences were normalized to the control value. The simulation results are compared with the experimental data in INS-1 cells (3). See text for definitions of abbreviations.

<table>
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<th>Preincubation Period With 100 nM GLP-1</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
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<tr>
<td>% Reduction</td>
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<td>26</td>
<td>40</td>
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<tr>
<td>Experimental results</td>
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The [cAMP] accumulation caused by IBMX alone (Fig. 4C) was subtracted from the [cAMP] responses to the second application of agonist, and the differences were normalized to the control value. The simulation results are compared with the experimental data in INS-1 cells (3). See text for definitions of abbreviations.
the relation between [cAMP] and [Ca\(^{2+}\)] was out of phase without agonist (19, 23, 38). The simulation in Fig. 5 examines mechanisms of [Ca\(^{2+}\)]-dependent regulation of [cAMP] in the absence or presence of GLP-1. In the absence of GLP-1, the total cAMP production rates \(V_{AC,1} (V_{AC} + V_{AC,G})\) matched the \(V_{PDE}\) at a resting [cAMP] of 1.6 \(\mu\)M (black curve in Fig. 5A) at 100 nM [Ca\(^{2+}\)]. When [Ca\(^{2+}\)] was increased to 500 nM (grey curve), [cAMP] slightly decreased to 1.4 \(\mu\)M due to the facilitation of PDE activity by [Ca\(^{2+}\)] \(V_{Cd_PDE}\) (grey curve in Fig. 5C). After 30 min simulation with 100 nM [GLP-1], [cAMP] increased to 4.1 \(\mu\)M (Fig. 5B) due to enhanced \(V_{AC,G}\) at 100 nM [Ca\(^{2+}\)]. Increasing [Ca\(^{2+}\)] to 500 nM further elevated [cAMP] in a reversible manner up to 5.4 \(\mu\)M because the activation of \(AC_G\) by [Ca\(^{2+}\)] \(V_{Cd_AC}\) (black curve in Fig. 5D) was relatively larger than that of PDE \(V_{Cd_PDE}\). These simulation results revealed the mechanisms underlying the in-phase or out-of-phase patterns of [cAMP] fluctuations in response to the cyclic changes in [Ca\(^{2+}\)] (19, 23, 38). To get a deeper insight into the physiological significance of [Ca\(^{2+}\)] in determining [cAMP], the changes in [cAMP] in response to increasing [Ca\(^{2+}\)] from 0.05 to 5.0 \(\mu\)M were simulated at varying [GLP-1]. The depression of [cAMP] response induced by increasing [Ca\(^{2+}\)] at a lower [GLP-1] was inverted at \(\sim 0.054 \text{ nM} \) [GLP-1], and the enhancement of [cAMP] synthesis by [Ca\(^{2+}\)] was augmented further with increasing [GLP-1], saturating at \(\sim 2 \text{ \mu M} \) [Ca\(^{2+}\)] (Fig. 5E). Note that with higher [Ca\(^{2+}\)] (>5 \(\mu\)M), cAMP response to GLP-1 starts to decrease, reflecting the [Ca\(^{2+}\)]-dependent inactivation (see Eq. 11).

Activation of PKA and Epac by GLP-1. Time courses of [cAMP] responses to GLP-1 were measured using a PKA-based biosensor in INS-1 cells (19). The fluorescent signals were detected using evanescent wave microscopy reflecting [cAMP] in the submembrane space. However, since the signals were not calibrated, extent of PKA activation by the GLP-1 stimulus was not obtained from the experimental results. We thus attempted to predict PKA activity using the GLP-1 receptor signal cascade model developed in the present study (Fig. 6).

Toward this end, it was essential to examine the localization of cAMP under the surface membrane compared with the bulk [cAMP], since the PKA activities may vary within the cytosol due to an uneven distribution of cAMP. In pancreatic \(\beta\)-cells, highly localized cAMP microdomain may possibly be present.

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**Fig. 5.** [cAMP] responses to changes in [Ca\(^{2+}\)]. A and B: [Ca\(^{2+}\)] (gray lines) was varied from 100 to 500 nM with a time constant of 30 s in the absence (A) or presence (B) of 100 nM GLP-1. [cAMP] (black lines) responds to the corresponding [Ca\(^{2+}\)]. C and D: [Ca\(^{2+}\)]-dependent AC\(_G\) \(V_{Cd_AC}\) (black line) and PDE \(V_{Cd_PDE}\) (grey line) activities under the conditions examined in A and B, respectively. E: [GLP-1]-[cAMP] response curves at varying [Ca\(^{2+}\)] (0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 \(\mu\)M). Dotted gray line indicates [cAMP] at [GLP-1] = 0.054 nM, where decreasing [cAMP] responses to increasing [Ca\(^{2+}\)] at a lower [GLP-1] is converted to increasing [cAMP].
at submembrane space, since production of cAMP by ACs is limited at the surface membrane (29, 38), while PDE-mediated degradation occurs diffusely within the cytosol (4, 54). Indeed, cAMP microdomain has been observed beneath the surface membrane in other cell types, such as cardiac myocytes (70) and HEK cells (61).

To simulate the distribution of $[\text{cAMP}]$, intracellular diffusion of cAMP was calculated (Fig. 7). For simplicity, one-dimensional diffusion was assumed over a distance of 4 μm (estimated from Ref. 48) from the surface membrane toward the nucleus as indicated in Fig. 7A. The diffusion path ($x$) was separated into 200 compartments, and $[\text{cAMP}]$ in each compartment at time $t$ [$C(x,t)$] was calculated using the following equation:

$$\frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2}$$

(14)

With boundary condition of

$$\frac{\partial C}{\partial x} \bigg|_{x=0.4} = 0$$

where the diffusion coefficient ($D$) was 0.3 μm$^2$/ms based on experimental measurements of 0.27 μm$^2$/ms (6) and 0.33 μm$^2$/ms (32). The cAMP production by AC stimulated by a [GLP-1] of 10 nM was assumed only in the first compartment (29, 38), whereas cAMP degradation by PDE was calculated in all compartments (4, 54). A quasi-steady-state concentration gradient of $[\text{cAMP}]$ was established within 100 ms after the onset of AC activation, and the difference in $[\text{cAMP}]$ over the diffusion path of 4 μm was only about 0.05 μM. Theoretically, but unrealistically, a much larger gradient (4 μM) was developed only when $V_{\text{PDE}}$ was increased by 100 times. The distributions of $[\text{cAMP}]$ relative to $[\text{cAMP}]$ in the submembrane space simulated using the control and high $V_{\text{PDE}}$ are shown in Fig. 7, B and C, respectively. This finding agrees with simulation results demonstrated by Oliveira et al. (47) (see DISCUSSION for more detail). The flat distribution in Fig. 7B may justify the use of average $[\text{cAMP}]$ in estimation of active fractions of cAMP effectors that are distributed throughout the cytosol of pancreatic β-cells.

With the use of the GLP-1 receptor signal transduction model, the dose-dependent activities of PKA at the end of 30 min application of various concentrations (0.001–100 nM) of GLP-1 under high [Ca$^{2+}$] condition were simulated (Fig. 6A). The $[\text{cAMP}]$-dependent PKA activation was calculated using a
**C799**

**SYSTEMS ANALYSIS ON THE BALANCE BETWEEN THE PRODUCTION AND DEGRADATION OF cAMP.** The level of [cAMP] is determined by the balance between AC and PDE activities (Eq. 8). Depression of PDE activity led to a decrease in the [cAMP] level. In both experiments and computer simulations, quasi-steady-state [cAMP] levels were obtained when the PDE activity was depressed by a saturating dose (>200 μM) of IBMX. This is because balancing V_{AC,1} with V_{PDE} was established with the IBMX-insensitive PDE fraction (~20% of total PDE activities) under this condition. To examine the robustness of the [cAMP] homeostasis in our model, we defined the V_{AC,1} level and the steady-state level of [cAMP] by the intersection of V_{AC,1} with the [cAMP]-PDE curve in Fig. 8. When the V_{AC,1} was maximized at 500 μM [Ca^{2+}] using the saturating IBMX concentration of 100 nM [GLP-1], the equilibrium [cAMP] was obtained at ~115.7 μM with 20% V_{PDE} (grey sigmoidal curve) as indicated by the arrow a. This analysis determines that depression of PDE activity will cause collapse of the [cAMP] homeostasis when V_{AC,1} was maximized. Under physiological conditions, the steady state [cAMP] was maintained at 7.9 μM (arrow b) with intact V_{PDE} (black sigmoidal curve) even when AC was fully activated. The lower limit of [cAMP] in the absence of GLP-1 stimulation, on the other hand, is determined by the activity of the G protein-insensitive component of AC, and the [cAMP] was balanced at 1.4 μM (arrow c). It should be noted that this range of [cAMP] change (1.4–7.9 μM) well fits the dynamic range of the PKA activation (K_{1/2} = ~3 μM). This analysis revealed that the intact PDE is highly capable of balancing V_{AC,1}, and thus we conclude that the system of [cAMP] regulation is quite robust in pancreatic β-cells. The delayed desensitization of the GLP-1 receptor (Fig. 4D) may further strengthen the robustness of the system.

**DISCUSSION**

In the present study, a minimal model of GLP-1 receptor signal transduction was developed mostly based on experimental data reported in β-cells or related cell lines. The model seeks to reconstruct the experimental findings of dynamic changes in [cAMP] during agonist stimulation in the absence or presence of IBMX at two representative levels of [Ca^{2+}] (Fig. 3). The model predicted the activity of cAMP effectors PKA and Epac during GLP-1 stimulation (Fig. 6). The simulation also demonstrated that the GLP-1 receptor desensitization kinetics applied to the model successfully explained the fast and very slow inactivation steps, which have significant effects on the decay kinetics of [cAMP] during continuous GLP-1 stimulation (Fig. 3). The cross talk between glucose- and GLP-1-dependent signal cascades in synergistic synthesis of cAMP was well reconstructed by incorporating the GLP-1 receptor signaling cascad model into appropriate whole β-cell models (24, 44) in the future.
limited at the submembrane space, while PDE-mediated degradation occurs diffusely within the cytosol. Our finding is, however, different from the localized CAMP microdomain observed in other cell types [cardiac myocytes (70) and HEK293 cells (61)]. The theoretical study by Oliveira et al. (47) demonstrated a large [CAMP] gradient (−5 μM) in HEK293 cells upon stimulation with PGE1. They concluded that the PDE4D activity enhanced by PKA-mediated phosphorylation was necessary and sufficient for generating the CAMP microdomain observed by Terrin et al. (61), and no physical barrier was required against the CAMP diffusion. If compared with our simulation, the CAMP diffusion constant used in their study was essentially the same magnitude as in our calculations. Interestingly, our diffusion model also generated a similar [CAMP] gradient of −4 μM (Fig. 7C) when simulated using the $V_{\text{PDE}}$ equivalent to that used in the Oliveira et al. (47). However, their $V_{\text{PDE}}$ was much higher (~100 fold) than that determined by the model fitting to the published experimental data in the present study. If we adopt the higher PDE activity, the model failed to reconstruct experimental records of [CAMP] changes during GLP-1 stimulation. Our simulation results strongly suggest that the distribution of CAMP is homogeneous in pancreatic β-cells because of relatively low PDE activities. These results, however, do not necessarily exclude the possibility of a functional coupling among AC, PKA, and/or Epac and effector proteins by AKAP near the membrane, which has been suggested in the heart and brain (12, 46). On the other hand, the rapid diffusion of [CAMP] could possibly be an essential factor in the GLP-1 receptor signaling for a proper modulation of insulin release, since PKA and Epac, the target proteins of CAMP, are widely distributed throughout the intracellular space (12, 40, 49). For activation of all these enzymes, the rapid access of CAMP might be critical to fulfill the basic needs of subsequent modulatory actions on ion channels on the surface and ER membrane as well as exocytotic machinery in concert within the entire intracellular space.

### Sensitivity of the model to varying parameters.

Although [R] was estimated in a previous study (66), [G] has not yet been determined in pancreatic β-cells. In the present study, [G] was referred by Post et al. (50), who suggested the expression of Gαi protein in large excess relative to β-adrenergic receptor in cardiac myocytes and hypothesized that this stoichiometry of [G] to [R] will be applicable to other G protein-coupled hormone receptor systems. We found that the EC50 of [GLP-1]-dependent [CAMP] accumulation is dependent on the agonist-induced [LRG] complex ([LRG] = [G1-LR]/Kd), and thereby the EC50 can be adjusted by modifying either [G] or Kd of [G]-[LR] binding. Since both parameters have not been investigated in pancreatic β-cells, [GLP-1]-dependent [CAMP] accumulation was reconstructed by determining an appropriate Kd in the present study under the assumption that [G] is expressed to a similar extent as in the cardiac tissue (50). The fraction of $[R_{\text{a}}]$ is one of signaling factors that have the strong influence on AC activities and thus [CAMP]. Under physiological conditions, $[R_{\text{a}}]$ will largely fluctuate because of extensive desensitization (75% at the maximum, see Fig. 4D), which is expected to proceed during several hours of the meal digestion.

The maximum AC activity ($V_{\text{max,AC}} = V_{\text{max,AC}} + V_{\text{max,AC,Ca}^{2+}}$) is much larger than the $V_{\text{max}}$ of PDE in our model. The lower AC activity under physiological conditions is mostly due to the low sensitivity of AC to GαiGTP in addition to the low [GαiGTP] production, even with a saturating [GLP-1] (see Eq. 11). Other modulatory factors of the AC activity, such as the dependencies on the substrate ATP and the Ca2+-dependent inactivation, are nearly saturated with the physiological level of [ATP] and [Ca2+]. Although Ca2+CaM-dependent activation of VAC,Ca is pronounced with increasing [GLP-1], [CAMP] production becomes partially compensated by the parallel activation of PDE. More specifically, [CAMP] level balances at 7.61 μM under the stimulation with 10 nM GLP-1 at 500 nM [Ca2+] without desensitization processes, whereas it increased to 8.61 μM when the component of Ca2+CaM-dependent activation of $V_{\text{PDE}}$ was excluded from the model. The PDE component showing the high CAMP sensitivity ($K_{d_{\text{P}}}$) also plays a subtle role in regulation [CAMP], whereas the low CAMP sensitivity ($K_{m_{\text{P}}}$) component virtually fulfills the physiological role in CAMP hydrolysis in the present model.

### Limitations.

The kinetics of the simple sequential transition of desensitized receptors from R0 to R2 was modeled to describe the time course of the delayed recovery from slow inactivation (Fig. 4). Indeed, the reaction scheme for receptor desensitization (Fig. 1) well simulated both of the fast and very slow desensitization observed in experimental studies (see RESULTS). Up to date, it is clear that the phosphorylation of the GLP-1 receptor is the key desensitization step, whereas Widmann and colleagues (67) have shown that neither PKA nor PKC are involved in the process. The involvement of β-Arrestin2 and GRK5 was suggested by Jorgensen et al. (34), whereas it is still highly controversial since a different group demonstrated the desensitization was independent of β-Arrestin2 (59). The model scheme will need to be improved when the molecular mechanisms are established in future experimental studies. It may also be examined whether the activation of PDE through phosphorylation by PKA is responsible for a small fraction of the spontaneous decay of [CAMP], although the present study attributed the decay only to desensitization.

The active fractions of PKA as well as Epac1 and 2 were calculated by a use of biochemically determined $K_{d}$ or $K_{1/2}$, half-maximal [CAMP] for the activation of these enzymes. However, especially for PKA activation, there has been obvious disagreement in published values of $K_{1/2}$, which vary over a nanomolar to micromolar range. It seems that the experimental $K_{1/2}$ is highly related to the concentration of enzyme used in biochemical investigations (7). $K_{1/2}$ was ~3 μM for more physiological concentration of the PKA isozone II (400 nM), whereas it was significantly reduced to ~50 nM when tested on 1 nM holoenzyme. Indeed, other investigators (14) used low holoenzyme concentrations of 20~30 nM and obtained a $K_{1/2}$ of 98 nM and 540 nM for PKAI and PKAII, respectively. In the present study, PKA activities were calculated with $K_{1/2}$ of ~3 μM, yet, it may need to be reevaluated when more accurate experimental measurements of $K_{1/2}$ values as well as concentrations of PKA isozone become available.

CAMP is distributed within a β-cell in forms of free CAMP and PKA or Epac-bound form (cAMP-PKA and cAMP-Epac). In the present study, however, [cAMP] was calculated neglecting [cAMP-PKA] and [cAMP-Epac]. If the total amount of CAMP is comparable to PKA and/or Epac, it will be necessary to consider [PKA] and/or [Epac] in calculating the concentration of unbound CAMP ([cAMP]total = [cAMP] + [cAMP-PKA] + [cAMP-Epac]). Similarly, the mass conservation should also be consid-
ered for $[G_{0}\text{GTP}]$ ($[G_{0}\text{GTP}_{\text{total}}] = [G_{0}\text{GTP}] + [G_{0}\text{GTP-AC}]$) when [AC] is available. Since the amounts of PKA, Epac, and AC are not determined in β-cells, we excluded these conservation equations from the present model.

APPENDIX I

A) Parameters Determined in Published Experimental Studies

1) Parameters determined based on GLP-1 signaling system in pancreatic β-cell and a β-cell line

- Total amount of receptor, $[R_0]$ = 0.00434 μM (Ref. 66)
- Binding between [L] and [R], $K_d = 0.004$ μM (Refs. 43, 65)

2) Parameters determined by biochemical investigations

- Total amount of Gs, $[G_s]$ = 2.83 μM (Ref. 50)
- $G_s\text{CaM}$-dependent AC activity $K_{1/2} = 0.4$ μM (Ref. 58)
- ATP-dependent AC activity $K_m = 1.03$ mM (Ref. 13)
- ATP-dependent AC activity $K_m = 0.315$ mM (Ref. 13)
- Ca, CaM-dependent AC activity $K_{1/2} = 0.348$ μM (Ref. 21)
- Ca$^{2+}$-dependent AC inhibition $K_{1/2} = 75$ μM (Ref. 21)
- Ca, CaM-dependent PDE activity $K_{1/2} = 0.348$ μM (Ref. 68)
- cAMP-dependent PKA activity $K_d = 2.9$ μM, $V_{max}$ = 1.4 (Ref. 9)
- cAMP-dependent Epac1 activity $K_{1/2} = 30$ μM (Ref. 20)
- cAMP-dependent Epac2 activity $K_{1/2} = 20$ μM (Refs. 52, 63)

The kinetics for the activation and deactivation of Gs protein $k_5 = 16$ s$^{-1}$, $k_6 = 1$ s$^{-1}$, $k_7 = 1$, 200, 000 mM/s (Refs. 5, 56)

B) Parameters Determined in the Present Study by Fitting Specific Experimental Records or Measurements in References

Desensitization rate constants

- $k_1 = 0.0025$ s$^{-1}$, $k_2 = 0.005833$ s$^{-1}$ (Fig. 3)
- $k_3 = 0.0002833$ s$^{-1}$, $k_4 = 0.00005$ s$^{-1}$ (Fig. 4 and Table 3)

AC and PDE activities (Figs. 3 and 6 and Table 2)

- $V_{AC,G}V_{max,AC} = 0.0006173$ mM/s
- $V_{AC,G}V_{max,AC,G} = 0.01738$ mM/s
- $f_{C,AC} = 0.6$

C) Parameters Determined in the Present Study by Fitting the Overall Model Scheme to Experimental Records or Measurements In References

- Binding between [G] and [LR], $K_d = 0.372$ μM (Ref. 66)

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DISCLOSURES

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

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