Minimal modeling of insulin secretion in the perfused rat pancreas: a drug effect case study

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Riz M, Pedersen MG, Toffolo GM, Haschke G, Schneider H, Klabunde T, Margerie D, Cobelli C. Minimal modeling of insulin secretion in the perfused rat pancreas: a drug effect case study. Am J Physiol Endocrinol Metab 306: E627–E634, 2014. First published January 14, 2014; doi:10.1152/ajpendo.00603.2013.—The experimental protocol of the perfused rat pancreas is commonly used to evaluate β-cell function. In this context, mathematical models become useful tools through the determination of indexes that allow the assessment of β-cell function in different experimental groups and the quantification of the effects of antidiabetic drugs, secretagogues, or treatments. However, a minimal model applicable to the isolated perfused rat pancreas has so far been unavailable. In this work, we adapt the C-peptide minimal model applied previously to the intravenous glucose tolerance test to obtain a specific model for the experimental settings of the perfused pancreas. Using the model, it is possible to estimate indexes describing β-cell responsivity for first (ΦD) and second phase (ΦS, Τ) of insulin secretion. The model was initially applied to untreated pancreata and afterward used for the assessment of pharmacologically relevant agents (the gut hormone GLP-1, the potent GLP-1 receptor agonist lixisenatide, and a GPR40/GPR11 agonist, SAR1) to quantify and differentiate their effect on insulin secretion. Model fit was satisfactory, and parameters were estimated with good precision for both untreated and treated pancreata. Model application showed that lixisenatide reaches improvement of β-cell function similarly to GLP-1 (11.7- vs. 13.1-fold increase in ΦD and 2.3- vs. 2.8-fold increase in ΦS) and demonstrated that SAR1 leads to an additional improvement of β-cell function in the presence of postprandial GLP-1 levels.

minimal model; biphasic insulin secretion; perfused pancreas; β-cell function; lixisenatide

IN THE ISOLATED PERFUSED RAT PANCREAS, the biphasic nature of insulin secretion in response to an increase in glucose levels was first observed by Curry et al. (8). Later experiments have shown that in type 2 diabetes the amount of insulin secreted during the first phase in particular is severely reduced (14). It is now generally accepted that deficient insulin secretion plays a crucial role in the pathogenesis of diabetes (19). Thus, reliable methods to investigate and quantify insulin secretion in both clinical and experimental settings are desirable.

Insulin secretion is regulated mainly by glucose through intracellular metabolism of the sugar and is additionally modulated by other factors such as incretin hormones and fatty acids by stimulation via G protein-coupled receptors (GPCRs). In particular, the activation of specific receptors that enhance insulin secretion only in presence of stimulatory glucose levels is therapeutically appealing since they increase insulin levels with minimal risk of hypoglycemia.

The perfused rat pancreas, performed either isolated or in situ, is commonly used for different kinds of investigations whose aim is the evaluation of β-cell function and the effect of various factors on insulin secretion. The most common application of this experimental setting is the analysis of the effect of drugs or other secretagogues (10, 27), in some cases in both normal and diabetic rats (12). Furthermore, it can also be used to assess the effect of fat content in diet (11, 28) or the influence of exercise on insulin secretion and β-cell function (20).

Ever since the first experiments on the rat pancreas in the 1960s, different models of insulin secretion at the cellular/subcellular level have been developed (2, 6, 15, 22, 24, 29). These models involve the notion of distinct pools to reproduce different patterns in insulin secretion due to various glucose stimuli. The biphasic nature of insulin secretion was suggested to correspond to the release of different granule pools. The first phase of insulin secretion is attributable to granules already residing at the membrane, the so-called readily releasable pool (RRP), which is refilled by the recruitment of granules from a reserve pool, causing the sustained second phase (26). Most of these models are quite detailed and work at the subcellular level. On the other hand, at the macroscopic level, minimal models, which describe the key components of the system and can be viewed as simplified versions of the detailed ones, can be advantageous depending on the aim of the analysis. Previously, we have studied the relation between cellular models and minimal models (21, 23, 25). Minimal models, by postulating a specific glucose-insulin secretion functional relationship, are able to provide indices describing β-cell responsivity to glucose of first- and second-phase secretion. Minimal models describing the glucose/insulin system have been applied successfully for the estimation of indices in a clinical setting (e.g., intravenous and oral glucose tolerance tests) (7). However, a minimal model applicable to the isolated perfused rat pancreas has so far been unavailable. Here, we adapt the C-peptide minimal model that was previously applied to the intravenous glucose tolerance test (33) to obtain a specific model for the experimental settings of the perfused pancreas.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone released from intestinal L cells into the bloodstream after a meal. It acts on the pancreatic β-cell through a Gαs-coupled GPCR and is a potent stimulator of insulin secretion (18). Another potential treatment of type 2 diabetes is the activation of G protein-coupled receptor 40 (GPR40), also known as free fatty acid receptor 1 (FFAR1), a GPCR that is found in pancreatic β-cells (16). Several drug candidates addressing this
receptor are currently in clinical development, with TAK-875 being the most advanced compound, having just entered phase III clinical trials (4). At Sanofi, the compound SAR1 has been identified as a potent and selective GPR40 agonist that is currently in preclinical development. SAR1 clearly demonstrated a glucose-dependent mechanism in increasing the intracellular Ca$^{2+}$ signal and a resulting increase in insulin secretion under elevated glucose concentrations in the perfused rat pancreas (17).

We applied the model for the assessment of pharmacologically relevant agents (GLP-1, the synthetic GLP-1 receptor agonistlixisenatide, and the compound SAR1), with the aim of quantifying and differentiating their effect on insulin secretion.

**MATERIALS AND METHODS**

**Animals and housing.** Animal studies were performed according to German animal protection law as well as according to international animal welfare legislation and rules, and our experiments were approved by the Federal Authority for Animal Research at the Regierungspräsidium Darmstadt. Male Sprague-Dawley rats were purchased at a body weight of 200–220 g from Harlan-Winkelmann (Borchen, Germany). The animals were acclimatized for ≥1 wk and housed under controlled environmental conditions (temperature 22 ± 2°C, humidity 55 ± 10%, 12:12-h dark-light cycle, lights on at 0600) with free access to standard laboratory chow (ssniff R/M-H 1534) and tap water. Animals were housed in Macrolon cages size IV-S (1,400 cm², 2–4 rats/cage) on softwood granulate. For environmental enrichment, all cages were equipped with wood shavings and a wooden gnawing block. All manipulations were performed at the same time in the morning.

**Experimental protocol.** Male fed Sprague-Dawley rats weighting 200–220 g were used throughout the experiments and were anesthetized with pentobarbital sodium (80–100 mg/kg body wt). The pancreas was isolated as described previously (31) and placed into the perfusion chamber, which was constantly gazed with a humidified mixture of O$_2$ and CO$_2$ (ratio 95:5; Carbogen) and was kept at a constant temperature of 37°C. The organ was perfused (perfusion rate: 1.5 ml/min) with a modified solution of Krebs-Henseleit Buffer (KHB; in mM: 118 NaCl, 4.7 KCl, 25 NaHCO$_3$, 1.2 NaH$_2$PO$_4$, 1.25 CaCl$_2$, 1.2 MgSO$_4$) containing 1% bovine serum albumin and glucose as indicated, which was permanently gazed with Carbogen and kept at a temperature of moment 37°C. The solution entered the organ via the aorta abdominalis, circulated through the pancreas, and exited via the portal vein. Insulin was measured from frozen samples using the fluorescent immunoassay (order no. 10-1248-10) from Mercodia according to the manufacturer’s instructions.

This experimental setting was used to test the effect of pharmacologically relevant agents, namely GLP-1, lixisenatide, and SAR1, on insulin secretion. The isolated pancreas was perfused with a buffer (KHB) containing different concentrations of glucose. Initially, the pancreas was perfused with 5.6 mM glucose to ensure that any soluble protease in the pancreas was washed out. To investigate the drug effect, the test compound was added after 6 min at low glucose by switching to a reservoir containing the compound. After 15 min, the concentration of glucose was increased to 16.5 mM by switching to a reservoir with high glucose concentration under the same conditions (testing compound or no drug). The increase to 16.5 mM in glucose concentration induces glucose-stimulated insulin secretion of the isolated organ. The effluent was collected in ice-cooled tubes for the measurement of insulin concentration.

**Mathematical model of insulin secretion in the perfused pancreas.** Pancreatic secretion above basal secretion (SR) is assumed proportional to the amount X (pmol) of readily releasable insulin in the β-cells (33):

$$SR(t) = mX(t). \quad (1)$$

The change in the insulin amount in the RRP X results from the balance between the insulin secretion rate, the provision Y (pmol/min) of insulin refilling the RRP, and recruitment of readily releasable insulin X$_D$ (pmol/min):

$$\frac{dX(t)}{dt} = -mX(t) + Y(t) + X_D(t), \quad X(0) = 0, \quad (2)$$

X$_D$ is responsible for the first phase of secretion and is assumed to be proportional to the rate of increase of glucose via the constant parameter K$_P$:

$$X_D(t) = \begin{cases} K_P \frac{dG(t)}{dt}, & \text{if } \frac{dG(t)}{dt} > 0; \\ 0, & \text{otherwise}. \end{cases} \quad (3)$$

Further explanations for the constraint $dG(t)/dt > 0$ can be found in Refs. 21 and 23.

The provision Y generates the slower second phase and is controlled by glucose according to the equation

$$\frac{dY(t)}{dt} = -\alpha[Y(t) - \beta(G(t) - G_b)], \quad Y(0) = 0, \quad (4)$$

where G$_b$ represents the basal value of glucose.

It is worth noting that the provision is not linearly related to glucose concentration, but it tends, with a time constant of 1/α (min), toward a steady-state value linearly related through the parameter β [pmol/(mmol/l)] to glucose concentration G (mM) above the basal level G$_b$.

A direct measurement of glucose concentration inside the pancreas was not feasible. Therefore, it was modeled using a sigmoidal function trying to mimic the experimental protocol:

$$G(t) = G_b + \frac{G_{\max} - G_b}{1 + e^{-\frac{t}{\theta}}}, \quad (5)$$

where G$_{\max}$ = 16.5 mM represents the maximum glucose stimulus during the experiment, G$_b$ = 5.6 mM represents the glucose level during the first part of the experiment, α controls the steepness of the function, and θ is the time at which the switch from G$_b$ and G$_{\max}$ is centred. Parameters were based on the following considerations. We calculated that the solution at high glucose requires ~0.5 min to reach the aorta abdominalis through the tubing. Additionally, isolated islets show a delay around 1 min before the initiation of insulin secretion by a step increase in extracellular glucose concentration (26, 30). Thus, the function $G(t)$ (Fig. 1A) was chosen to be very steep (n = 6) and centred at θ = 16.5 min, accounting for the delay of 1.5 min due to the sources explained above.

Using a mathematical equation for the glucose concentration allows for an analytic calculation of the glucose derivative without approximations or delays:

$$\frac{dG}{dt} = \frac{n(G_{\max} - G_b)e^{-\frac{t}{\theta}}}{1 + e^{-\frac{t}{\theta}}} - \frac{G_{\max} - G_b}{1 + e^{-\frac{t}{\theta}}}. \quad (6)$$

**Model identification.** All secretory parameters (m, K$_P$, α, and β) are priori uniquely identifiable. The parameters were estimated for each perfused rat pancreas, together with a measurement of their precision, by applying weighted nonlinear least square methods to insulin data using the SAAM II software.

Weights were chosen optimally, i.e., equal to the inverse of the variance of the measurement errors, which were assumed to be independent, Gaussian, and zero mean with unknown constant standard deviation. The variance of the measurement errors was estimated a posteriori.
A Bayesian approach was used when the parameter \( m \) was estimated with insufficient precision to avoid compensatory issues with the anticorrelated parameter \( K_D \). Because the parameter \( m \) in the control group was estimated with good precision without the Bayesian term, its mean value was used as the prior in the treated groups when the parameter \( m \) was estimated with insufficient precision to avoid compensatory issues with the control group was estimated with good precision without the Bayesian term.

### Indices definition and interpretation.

Indices of responsivity to glucose of the first (dynamic) and second (static) phases were also calculated.

**Dynamic indices**

\[
X_i(t) = X_i(0) + X_i(t),
\]

\[
\frac{dX_i(t)}{dt} = -mX_i(t) + K_D \frac{dG(t)}{dt},
\]

\[
\frac{dX_{id}(t)}{dt} = -mX_{id}(t) + Y(t),
\]

and integrate over time both sides of Eq. 8 between 0 and \( T_{end} \), representing the end of the experiment when \( X_i(t) \) is back to the steady-state condition:

\[
\int_0^{T_{end}} \frac{dX_i(t)}{dt} dt = \int_0^{T_{end}} \left(-mX_i(t) + K_D \frac{dG(t)}{dt}\right) dt.
\]

Alternatively,

\[
\int_0^{T_{end}} dX_i(t) = \int_0^{T_{end}} mX_i(t) dt + K_D \int_0^{T_{end}} dG(t),
\]

which gives

\[
X_i(T_{end}) - X_i(0) = \int_0^{T_{end}} mX_i(t) dt + K_D (G(T_{end}) - G(0)).
\]

Since at \( T_{end} \) the system is back to the steady-state condition, \( X_i(0) = X_i(T_{end}) = 0 \). We can obtain the quantity of insulin secreted during the first phase from Eq. 12:

\[
\int_0^{T_{end}} SR_i(t) dt = \int_0^{T_{end}} mX_i(t) dt = K_D (G_{max} - G_S).
\]

Finally, normalizing by the glucose increment \( \Delta G = G_{max} - G_S \), we get

\[
\Phi_D = \int_0^{T_{end}} SR_i(t) dt / \Delta G = K_D.
\]

The dynamic index is a measurement of the stimulatory effect of the increased rate of glucose on the secretion of readily releasable insulin.

The static sensitivity \([\Phi_S (10^9 \text{min}/\text{I})]\) is defined as the ratio between the provision and the glucose concentration (above the basal level) at the steady state. Starting from Eq. 4, it is possible to calculate the provision at the steady state:

\[
Y_{ss} = \beta (G_{ss} - G_b).
\]

Hence, normalizing by the glucose step, and since \( G_{ss} = G_{max} \), we obtain the following:

\[
\Phi_S = Y_{ss} / \Delta G = \beta.
\]

The model also allows us to quantify the delay time of the second phase \( T \) (min). It is

\[
T = \frac{1}{\alpha},
\]

simply the time constant with which the provision reaches its steady-state value, and it is related to the time required for new granules to become ready for release.

### Statistical methods.

Indices of \( \beta \)-cell function were compared between groups with two-sided \( t \)-tests using Microsoft Excel, and \( P \) values were Bonferroni corrected to adjust for multiple comparisons. Linear statistical modeling (using \( r \)) was used to assess whether SAR1 (0.1 \( \mu \)M) had an effect in the presence of physiological GLP-1 levels (10 pM) by including an interaction term in the linear models of the form

\[
Z_j = \beta_0 + \beta_1 \text{GLP-1}_j + \beta_2 (\text{GLP-1} \cdot \text{SAR1})_j + e_j,
\]

where \( j \) identifies the experiment, \( Z \) is one of the estimated \( \beta \)-cell indices (\( \Phi_D \), \( \Phi_S \), \( T \)), \( \beta_0 \) is the estimate of the index in the control group, \( \beta_1 \) indicates an additional effect of 10 pM GLP-1, and \( \beta_2 \) estimates how much SAR1 further modifies the index. GLP1j is a
factor indicating whether the pancreas was exposed to GLP1, and (GLP1 SAR1), indicates whether both GLP-1 and SAR1 were present. Two-sided t-tests were used to evaluate whether the regression coefficients $\beta_1$ and $\beta_2$ were significantly different from zero for each $\beta$-cell index.

RESULTS

Mean insulin secretion profiles of control and treated groups are shown in Fig. 1 along with their standard errors. After a silent phase in which secretion is at basal values, each profile exhibits the typical biphasic insulin secretion pattern in response to the glucose step. In some groups the addition of the compound alone perturbs the basal secretion. This effect was measured by the area under the curve (between minutes 6 and 15) above basal (before minute 6) of insulin secretion in the prestimulus state. If it was >9 pmol, corresponding to an average flux of 1 pmol/min above basal, the perturbation was considered significant. In the SAR1 and GLP1 + SAR1 groups, the compound significantly perturbed more than half of the individual pancreases in the basal state.

Individual secretion parameters for each group are summarized in Table 1 together with their precision. The ability of the model to fit the data is shown in Fig. 1, B and C. Precise estimates were obtained with the model for all pancreases in each group.

A significant difference was found in presence of 10 nM GLP-1 compared with control for all of the responsivity indices (dynamic $\Phi_D$, static $\Phi_S$, time delay $T$); 10 nM lixisenatide had a significant effect on all three indices relative to the control, whereas SAR1, at a concentration of 1 $\mu$M, exhibits a significant difference compared with the control for the dynamic index $\Phi_D$ and the time delay $T$ but not for the static index $\Phi_S$ ($P = 0.90$) (Fig. 2).

Physiological levels of GLP-1 (10 pM) had a significant effect on all three indices compared with control (Fig. 3). SAR1, when administered in presence of 10 pM GLP-1, was found to have a significant additional effect on both dynamic and static $\beta$-cell responsivity indices. In contrast, no further significant decrease in the time delay was detectable (Fig. 3).

DISCUSSION

In this article, we present a mathematical model of insulin secretion for the isolated perfused rat pancreas. This type of experiment is used in different kinds of investigations aimed at evaluating the effect on $\beta$-cell functionality of several factors, such as drugs and secretagogues, but also diet composition and exercise. Hence, mathematical models that are able to characterize insulin secretion phases and quantify $\beta$-cell function may be useful tools for the analysis of the perfused rat pancreas experiment.

The C-peptide minimal modeling approach has been applied successfully in humans to a variety of glucose stimuli, e.g., intravenous glucose tolerance test (IVGTT) (33), oral glucose tolerance test (OGTT) (3), and up- and downgraded infusion (32). Since the change from low to high glucose concentration is very fast, as during an IVGTT, we start from the C-peptide minimal model used previously in this experimental setting and built a new version of the model suitable for the perfused pancreas protocol. The model is simpler in the sense that there is no need to include the C-peptide dynamics, since the insulin secretion profile is measured directly. The model assumes that glucose can stimulate pancreatic insulin secretion via both a dynamic control proportional to its rate of change and a static control related to its concentration. The dynamic control is connected to the model assumption of the presence in the $\beta$-cell of a pool of readily releasable insulin, which can be promptly secreted when glucose increases above its basal value and whose recruitment is proportional via the parameter $K_D$ to the glucose increase rate. From Eq. 10, is easy to verify that the product of $K_D$ and the total increase in glucose concentration in the rising portion of the glucose function measures the total amount of releasable insulin stored in the $\beta$-cell before the experiment, which corresponds to the variable $X_0$ in the IVGTT model (C-peptide total amount). The static control is related to an insulin secretion provision process, which accounts for a slower and delayed component of secretion.

The time course of insulin secretion in response to a glucose stimulus is biphasic, and each phase can be described by a $\beta$-cell responsivity index. A dynamic responsivity index ($\Phi_D$; describing the first phase) reflects the ability of the $\beta$-cell to respond to the rate of an increase in glucose levels, whereas the static responsivity index ($\Phi_S$; describing the second phase), together with the time constant $T$, depicts the response to above-basal blood glucose.

Drug effect case study. We applied the model for the assessment of pharmacologically relevant agents, namely the gut hormone GLP-1, a potent synthetic GLP-1 receptor agonist (lixisenatide), and a GPR40 agonist (SAR1). This particular case study becomes meaningful considering that drug development has the need to assess and quantify the action of antidiabetic drugs in earlier preclinical phases, when the experimental setting of the perfused rat pancreas is often used.

Our data show that GLP-1 amplifies both the static and dynamic $\beta$-cell responsivity in a dose-dependent fashion. At the higher dose applied (10 nM), we found a 13.1-fold increase in $\Phi_D$ and a 2.8-fold increase in $\Phi_S$ relative to the control. Our results show that lixisenatide at the same concentration (10 nM) of GLP-1 reaches an improvement of $\beta$-cell function that is similar to GLP-1 (improvement of 11.7- and 2.3-fold in $\Phi_D$ and $\Phi_S$, respectively). Since type 2 diabetes is characterized by a loss of the early phase of insulin secretion (14), the treatment may benefit especially from the pronounced enhancement of the first phase, as reflected by the dynamic responsivity index.

Compared with the control experiment, 1 $\mu$M SAR1 showed a 5.3-fold improvement in $\Phi_D$, whereas no effect on $\Phi_S$ could be detected. However, it should be noted that the delay constant $T$ was reduced from 25 to 6 min. Thus, 1 $\mu$M SAR1 has an effect on the second phase, but this is apparent only in its faster onset and not in the magnitude. Other studies using rat (35) or mouse (13) islets found that GPR40 agonists stimulated the magnitude of the second phase. The perfused rat pancreas in control conditions presents a larger second phase compared with the isolate islet data (Fig. 1) (8). The magnitude of a small second phase, as seen in the isolated islets, might be readily increased by GPR40 agonists. This may explain the discrepancy between our results and those in Refs. 13 and 35.

Under physiological conditions, in the postprandial state, incretin levels increase and will have an effect on the insulin secretion from the pancreatic islets. This enhancement is not reflected in the basic control experiment, where only the glucose but not the incretin concentration is increased. To
Table 1. Individual secretion parameters

<table>
<thead>
<tr>
<th></th>
<th>$\Phi_D$ (10^{-9} l)</th>
<th>$\Phi_s$ (10^{-9} l/min)</th>
<th>$T$ (min)</th>
<th>$m$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 1</td>
<td>0.753 (11)</td>
<td>1.072 (10)</td>
<td>23.652 (15)</td>
<td>0.605 (18)</td>
</tr>
<tr>
<td>Rat 2</td>
<td>0.809 (11)</td>
<td>1.101 (10)</td>
<td>20.547 (16)</td>
<td>0.892 (18)</td>
</tr>
<tr>
<td>Rat 3</td>
<td>1.034 (15)</td>
<td>1.694 (6)</td>
<td>16.273 (13)</td>
<td>0.713 (32)</td>
</tr>
<tr>
<td>Rat 4</td>
<td>0.536 (16)</td>
<td>1.566 (10)</td>
<td>26.103 (16)</td>
<td>0.818 (28)</td>
</tr>
<tr>
<td>Rat 5</td>
<td>0.701 (14)</td>
<td>1.584 (13)</td>
<td>30.111 (20)</td>
<td>0.636 (30)</td>
</tr>
<tr>
<td>Rat 6</td>
<td>0.408 (18)</td>
<td>3.044 (29)</td>
<td>62.383 (34)</td>
<td>1.368 (29)</td>
</tr>
<tr>
<td>Rat 7</td>
<td>0.584 (19)</td>
<td>0.767 (5)</td>
<td>12.129 (14)</td>
<td>0.536 (38)</td>
</tr>
<tr>
<td>Rat 8</td>
<td>0.854 (14)</td>
<td>1.187 (13)</td>
<td>12.809 (23)</td>
<td>0.698 (26)</td>
</tr>
<tr>
<td>Rat 9</td>
<td>1.033 (15)</td>
<td>1.585 (6)</td>
<td>19.724 (11)</td>
<td>0.720 (23)</td>
</tr>
<tr>
<td>Rat 10</td>
<td>0.730 (13)</td>
<td>2.944 (9)</td>
<td>19.080 (10)</td>
<td>0.757 (24)</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td>0.677 (15)</td>
<td>1.529 (11)</td>
<td>24.911 (18)</td>
<td>0.796 (28)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.195 (4)</td>
<td>0.589 (7)</td>
<td>13.922 (7)</td>
<td>0.232 (7)</td>
</tr>
<tr>
<td><strong>GLP-1 (10 nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 1</td>
<td>5.609 (9)</td>
<td>3.419 (4)</td>
<td>8.757 (11)</td>
<td>0.587 (13)</td>
</tr>
<tr>
<td>Rat 2</td>
<td>9.735 (8)</td>
<td>4.220 (3)</td>
<td>4.767 (12)</td>
<td>0.758 (14)</td>
</tr>
<tr>
<td>Rat 3</td>
<td>10.482 (9)</td>
<td>4.405 (3)</td>
<td>5.617 (10)</td>
<td>0.509 (15)</td>
</tr>
<tr>
<td>Rat 4</td>
<td>10.253 (8)</td>
<td>4.140 (3)</td>
<td>3.750 (14)</td>
<td>0.856 (13)</td>
</tr>
<tr>
<td>Rat 5</td>
<td>6.488 (8)</td>
<td>4.260 (4)</td>
<td>7.933 (12)</td>
<td>0.858 (10)</td>
</tr>
<tr>
<td>Rat 6</td>
<td>10.664 (6)</td>
<td>5.244 (3)</td>
<td>4.968 (10)</td>
<td>0.911 (9)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>8.872 (8)</td>
<td>4.281 (3)</td>
<td>5.965 (11)</td>
<td>0.747 (12)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>2.226 (1)</td>
<td>0.585 (1)</td>
<td>1.956 (2)</td>
<td>0.163 (2)</td>
</tr>
<tr>
<td><strong>Lixisenatide (10 nM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 1</td>
<td>3.445 (8)</td>
<td>1.267 (3)</td>
<td>3.324 (14)</td>
<td>0.900 (13)</td>
</tr>
<tr>
<td>Rat 2</td>
<td>2.574 (9)</td>
<td>1.594 (8)</td>
<td>6.578 (17)</td>
<td>0.906 (10)</td>
</tr>
<tr>
<td>Rat 3</td>
<td>3.193 (6)</td>
<td>1.601 (4)</td>
<td>9.112 (9)</td>
<td>0.832 (9)</td>
</tr>
<tr>
<td>Rat 4</td>
<td>2.617 (3)</td>
<td>1.888 (4)</td>
<td>7.547 (13)</td>
<td>0.846 (12)</td>
</tr>
<tr>
<td>Rat 5</td>
<td>5.536 (5)</td>
<td>1.692 (2)</td>
<td>5.374 (9)</td>
<td>0.614 (7)</td>
</tr>
<tr>
<td>Rat 6</td>
<td>3.419 (10)</td>
<td>1.538 (5)</td>
<td>4.312 (20)</td>
<td>0.815 (12)</td>
</tr>
<tr>
<td>Rat 7</td>
<td>3.929 (11)</td>
<td>2.004 (8)</td>
<td>6.572 (23)</td>
<td>0.899 (18)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>3.561 (8)</td>
<td>1.555 (5)</td>
<td>6.131 (15)</td>
<td>0.830 (12)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.976 (2)</td>
<td>0.272 (2)</td>
<td>1.964 (5)</td>
<td>0.102 (3)</td>
</tr>
<tr>
<td><strong>SAR1 (1 μM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 1</td>
<td>5.584 (8)</td>
<td>3.301 (2)</td>
<td>5.799 (8)</td>
<td>0.669 (14)</td>
</tr>
<tr>
<td>Rat 2</td>
<td>7.247 (10)</td>
<td>2.433 (7)</td>
<td>13.534 (14)</td>
<td>0.507 (14)</td>
</tr>
<tr>
<td>Rat 3</td>
<td>8.069 (7)</td>
<td>3.146 (4)</td>
<td>7.578 (13)</td>
<td>0.623 (14)</td>
</tr>
<tr>
<td>Rat 4</td>
<td>4.219 (10)</td>
<td>3.537 (8)</td>
<td>13.233 (17)</td>
<td>0.800 (11)</td>
</tr>
<tr>
<td>Rat 5</td>
<td>4.297 (9)</td>
<td>1.771 (4)</td>
<td>9.797 (10)</td>
<td>0.577 (14)</td>
</tr>
<tr>
<td>Rat 6</td>
<td>4.447 (12)</td>
<td>2.886 (6)</td>
<td>11.545 (14)</td>
<td>0.511 (19)</td>
</tr>
<tr>
<td>Rat 7</td>
<td>0.992 (13)</td>
<td>1.007 (8)</td>
<td>13.702 (16)</td>
<td>0.710 (14)</td>
</tr>
<tr>
<td>Rat 8</td>
<td>1.336 (11)</td>
<td>1.635 (7)</td>
<td>13.998 (13)</td>
<td>0.708 (13)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>4.163 (10)</td>
<td>2.355 (6)</td>
<td>11.042 (14)</td>
<td>0.616 (15)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>2.528 (2)</td>
<td>0.845 (2)</td>
<td>3.332 (2)</td>
<td>0.113 (2)</td>
</tr>
<tr>
<td><strong>GLP1 (10 pM) + SAR1 (0.1 μM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 1</td>
<td>5.584 (8)</td>
<td>3.301 (2)</td>
<td>5.799 (8)</td>
<td>0.669 (14)</td>
</tr>
<tr>
<td>Rat 2</td>
<td>7.588 (7)</td>
<td>4.674 (4)</td>
<td>9.173 (11)</td>
<td>0.878 (9)</td>
</tr>
<tr>
<td>Rat 3</td>
<td>6.533 (7)</td>
<td>2.745 (4)</td>
<td>6.555 (14)</td>
<td>0.953 (8)</td>
</tr>
<tr>
<td>Rat 4</td>
<td>4.566 (6)</td>
<td>2.419 (3)</td>
<td>6.585 (10)</td>
<td>0.869 (8)</td>
</tr>
<tr>
<td>Rat 5</td>
<td>5.217 (5)</td>
<td>2.539 (3)</td>
<td>7.817 (10)</td>
<td>0.909 (8)</td>
</tr>
<tr>
<td>Rat 6</td>
<td>5.032 (9)</td>
<td>3.415 (3)</td>
<td>6.873 (11)</td>
<td>0.729 (12)</td>
</tr>
<tr>
<td>Rat 7</td>
<td>5.217 (9)</td>
<td>3.314 (3)</td>
<td>5.103 (12)</td>
<td>0.856 (11)</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>5.677 (7)</td>
<td>3.201 (3)</td>
<td>6.843 (11)</td>
<td>0.837 (10)</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>1.038 (2)</td>
<td>0.763 (1)</td>
<td>1.332 (2)</td>
<td>0.101 (2)</td>
</tr>
</tbody>
</table>

Data in parentheses are precision of parameter estimates, expressed as %coefficient of variation. $\Phi_D$, dynamic responsivity index; $\Phi_s$, static responsivity index; $T$, time delay.
approximate the physiological situation, we added 10 pM GLP-1 to the perfusate during the experiment. This GLP-1 concentration is approximately the one found postprandially in the rat (1). Already under this condition, a substantial increase in \( \Phi_D \) compared with the incretin-free experiment is observed (6.2-fold increase in \( \Phi_D \) and 1.5-fold increase in \( \Phi_S \), whereas \( T \) was reduced to 11 min).

In our previous work (5), where glycemic profiles in an OGTT were matched in the same human subjects by means of an intravenous glucose infusion, we found an increase of 58% for the static index due to incretin effect, which is similar to the 54% increase with 10 pM GLP-1 in the perfused rat pancreas found here. The changes to \( \Phi_D \) are not directly comparable due to the different patterns of the glucose profiles during an OGTT and in the perfused pancreas experiment.

What effect could we expect if we were to add SAR1 on top of 10 pM GLP-1? Using the 10 pM GLP-1 experiment as a
control, we see a 1.4-fold increase in static and a 1.4-fold increase in dynamic responsivity by SAR1. It is interesting to note that SAR1 (1 μM) is not able to improve static β-cell responsivity in the absence of a physiological GLP-1 receptor stimulation, whereas even 0.1 μM SAR1 has an effect on ΨS in the presence of 10 pM GLP-1. This could indicate a synergistic effect of the Gαs-coupled GLP-1 receptor and the Gαq-coupled GPR40 on the second phase of insulin secretion, although care must be taken because of the different SAR1 concentrations used. Overall, this analysis demonstrates that, even in the presence of postprandial GLP-1 levels, SAR1 leads to an additional improvement of β-cell function in the isolated perfused pancreas.

The work presented in this article illustrates how the mathematical model developed here is able to assess the ability of a compound to improve β-cell function in an ex vivo experiment, as it is used in the early phases of drug development. The parameter estimates obtained here represent valuable information to rank and classify compounds in development (e.g., by the ability of acting predominantly on 1 type of β-cell responsivity). Moreover, these values (or their relative changes) can be transferred to a mathematical model of the human glucose insulin system [e.g., the meal simulation model in (9)] that also contains parameters for static and dynamic β-cell responsivities. Here, the effect of the new compound on insulin secretion and postprandial glucose lowering can be simulated, thereby translating results from an animal experiment into the human situation (certainly taking into account the uncertainty arising from the species difference). Including the pharmacodynamics of the compound into the model could then make the simulations even more relevant for the assessment of a potential drug. Such a process would then give valuable guidance for the design of first clinical trials.

In summary, we have presented a minimal model that is able to quantify β-cell function in the perfused rat pancreas. The model should be of general interest for studies on the effects of a variety of interventions aimed at treating diabetes.

ACKNOWLEDGMENTS
G. Haschke is now affiliated with the Max Planck Institute of Biophysics, Frankfurt, Germany.

DISCLOSURES
During this work, D. Margerie, G. Haschke, H.-C. Schneider, and T. Klabunde were employees of Sanofi Deutschland, which develops SAR1 and markets lixisenatide.

AUTHOR CONTRIBUTIONS

REFERENCES