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A computational systems analysis of factors regulating α cell glucagon secretion

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Abbreviations: AP, action potential; $[Ca^{2+}]_c$, free cytoplasmic Ca^{2+}; GABA, gamma amino butyric acid; GLP-1, glucagon-like peptide-1; K_{ATP}-channel, ATP-sensitive K^+-channel; K_{Dr}, rapid delayed rectified voltage-gated K^+ channels; K_{GS}, G protein-gated K^+-channel activated by somatostatin; K_v, voltage-gated K^+ channels; $[NADH]_m$, NADH concentration in mitochondria; PM, plasma membrane; V_p, plasma membrane potential; VGCC, voltage-gated Ca^{2+} channel

Glucagon, a peptide hormone secreted from the α-cells of the pancreatic islets, is critical for blood glucose homeostasis. We reviewed the literature and employed a computational systems analysis of intracellular metabolic and electrical regulation of glucagon secretion to better understand these processes. The mathematical model of α-cell metabolic parameters is based on our previous model for pancreatic β-cells. We also formulated an ionic model for action potentials that incorporates Ca^{2+}, K^+, Na^+ and Cl^- currents. Metabolic and ionic models are coupled to the equations describing Ca^{2+} homeostasis and glucagon secretion that depends on activation of specific voltage-gated Ca^{2+} channels. Paracrine and endocrine regulations were analyzed with an emphasis on their effects on a hyperpolarization of membrane potential. This general model simulates and gives insight into the mechanisms of regulation of glucagon secretion under a wide range of experimental conditions. We also reviewed and analyzed dysfunctional mechanisms in α-cells to determine key pharmacological targets for modulating glucagon secretion in type 1 and 2 diabetes.

1. Introduction

Glucagon is the principal blood-glucose-increasing (counter-regulatory) hormone of mammals, with a variety of other effects as well. It is released from α-cells of the pancreatic islets, comprising only a few percent of the total cell mass of an islet. Electrophysiological studies on individual α-cells have revealed that they are electrically excitable. Stimulation of glucagon secretion is secondary to increased action potential (AP) firing and also may be affected by paracrine signals (mediated by factors released from neighboring β- and δ-cells) including insulin, Zn^{2+}, gamma amino butyric acid (GABA) and somatostatin, by endocrine signals such as glucagon-like peptide-1 (GLP-1) or leptin and by autonomic input (for a review, see refs. 1–5). Glucagon plays a critical role in glucose homeostasis, diabetic ketoacidosis and its secretion is dysregulated in hypoglycemia associated with diabetes.[4,6] Thus, it is important to elucidate the signals that trigger glucagon secretion and the transduction of these signals within the α-cell.

Pancreatic α- and β-cells show a completely different behavior depending on the serum glucose level. However, an inhibitory effect of glucose on the α-cell, like its stimulatory effect on the β-cell, requires metabolism of the sugar. Indeed, the α-cells are equipped with many of the proteins involved in the metabolic glucose sensing of the β-cell, including glucokinase[7] and ATP-regulated K^+-channels (K_{ATP}-channels).[2,8] Glucose may also increase the intracellular concentration of ATP in α-cells.[9,10]

Electrical activity in α-cells is difficult to study within an intact islet and so this process is typically studied as isolated purified cells in vitro. Glucose concentrations modulate pancreatic α-cell electrical activity, Ca^{2+} signal and hormone secretion. At low glucose concentrations (3 mM or below) isolated α-cells can be electrically active and AP firing is required for glucagon secretion. Increased glucose concentration paradoxically suppresses electrical activity whereas activity in β-cells in increased.[1,11,12] This electrical activity usually involves a complex interaction between several different ion channels as in pancreatic β-cells. Pancreatic α-cells express voltage-gated Na^+, K^+ and Ca^{2+} channels that regulate AP firing at low glucose level.[1,2,8]

Despite the recent increase in our knowledge of α-cell physiology and biochemistry, we still lack a coherent model of regulation in α-cells. Significant discrepancies exist among studies of glucagon secretion. It is at present unclear how increased glucose metabolism is coupled to the suppression of electrical activity in the α-cell, and the topic is subject to conflicting reports with the possibility of species differences (for review, see refs. 1 and 2). The present work attempts to bridge the gap between electrophysiological recordings, metabolic measurements, Ca^{2+} handling and glucagon secretion.

To achieve a better understanding of α-cell physiology and its role in diabetes we undertook an integrated consideration of data obtained on isolated α-cells, human and rodent islets and in clinical investigations. We used a computational systems biology

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approach with construction of a mathematical model of intracellular processes. We have previously focused on applying this approach to mechanisms of glucose sensing of insulin secretion, Ca^{2+} dynamics, electrophysiology events and exocytosis in pancreatic β-cells.[13-18] Here we focus on pancreatic α-cells based on our computational models for β-cells. This allows also a comparison of the mechanisms of glucose sensing in α– and β-cells. Our aim was the investigation of the properties of rodent and human α–cells with a help of a novel computational model for metabolic processes, electrical activity, Ca^{2+} handling and glucagon secretion.

The mathematical model for α–cells was tested in various scenarios and the results of simulations were compared with the experimental data. The model gives a reasonable fit to important aspects of experimentally measured metabolic and AP properties of α–cells and provides a framework for analyzing the role of metabolic changes and individual ionic currents on glucagon secretion. In additions to a direct effect of glucose, the influence of several paracrine and endocrine mechanisms on AP firing and glucagon release were considered.

Defective regulation of glucagon secretion clearly contributes to type 1 and 2 diabetes in a variety of ways.[6,19,20] For this reason there is significant interest in understanding how α-cells regulate glucagon secretion in humans. We have attempted to analyze these processes and evaluate how particular impairments in the mechanisms of α-cell glucose sensing can lead to glucagon release changes in diabetes. We also discuss examples of potential therapeutic strategies.

2. Components of Glucagon Secretion Regulatory Mechanism and their Mathematical Description

Here we will briefly outline the different parts of the glucagon secretion regulatory mechanisms and describe the equations and parameters utilizing in the simulations of α-cell function. A schematic diagram of the biochemical steps, Ca^{2+} handling, channels and mechanisms of glucagon secretion regulation in α-cell is presented in **Figure 1**. The data used to fit the computational model in our study were taken primarily from isolated rodent α-cells and islets. Since pancreatic α-cells remain incompletely understood, there remain gaps in any completely α-cell specific data set. For the purpose of making a convenient model we have chosen plausible suggestions based on mechanisms of regulation and parameters from β-cells or other cell types to close the gaps pending additional data.

2.1. Intrinsic metabolic regulation. The cellular metabolic mechanisms leading to insulin secretion in pancreatic β-cells are fairly well understood (for reviews, see refs. 2 and 15). Similar machinery may operate in α-cells. Although both cell types possess different glucose transporters, it has been demonstrated that glucose transport is not a limiting factor in the metabolism of this sugar.[21,22] Glucokinase was found in both cell types[7] and may be also the rate-limiting step for glycolysis in α-cells.[23] α-cells are equipped with ATP-sensitive K^+ channels of the same type as those constituting the resting conductance in β-cells (see below in Sec. 3.3.2). It seems that a metabolic mechanism of sensitivity to glucose is similar in α- and β-cells: mitochondrial metabolism generates ATP leading to an increase of the ATP/ADP concentration ratio, leading to closure of K_{ATP} channels. The resulting decrease in K^+ efflux causes PM depolarization which changes the rate of exocytosis of glucagon granules.[2,24]

We have recently developed a mathematical model for regulated glucose sensing in pancreatic β-cells[15,25] and use it here to analyze α-cells. The cytoplasmic part of the model includes equations describing glucokinase, glycolysis, lactate dehydrogenase, NAD(P)H and ATP production and consumption (**Fig. 1**). The mitochondrial part includes production of NADH, which is regulated by pyruvate dehydrogenase. NADH serves as a source of electron transfer in the chain of oxidative reactions to establish a proton motive force, driving the F_1F_0 ATPase. Redox shuttles and mitochondrial Ca^{2+} handling were also modeled. We have performed a computational analysis of β-cell fuel metabolism to quantitatively assess how cytoplasmic ATP/ADP ratio can be controlled by mitochondrial and cytoplasmic processes.[15,25]

Key differences in the rates of energetic processes were found in α-cells vs. β-cells. Increased ATP/ADP ratios and NAD(P)H signals were found in rodent α-cells compared with β-cells at low glucose levels.[23,26] In our previous β-cell model of glucose sensitivity no ATP production was generated without glucose consumption because only glucose served as a source of ATP production during glycolysis and NADH for oxidative phosphorylation. To describe a nonglycolitic source for ATP production in the absence of glucose or in low glucose we introduced a special term (other than pyruvate influx) in the equation for mitochondrial NADH ($[NADH]_m$) dynamics in our model of glucose sensing. Now, the following equation describes the change in $[NADH]_m$ over time:

$$d\,[NADH]_m/dt = (4.6\,J_{PYR} + J_{TNADH} - 0.1\,J_{hres} + k_{ng})/\,V_{mmit} - k_{NADHm}\,[NADH]_m \quad \textbf{(Eqn. 1)}$$

where k_{ng} is the novel coefficient describing a nonglycolitic source of NADH in mitochondria, J_{PYR} is the rate of pyruvate consumption in mitochondria, J_{TNADH} is the flux through the NADH shuttles, J_{hres} is the rate of proton pumping through ETC, 4.6 and 0.1 are the stoichiometric coefficients, V_{mmit} is the relative mitochondrial matrix fraction of the cell volume, k_{NADHm} is the rate constant of NADH consumption in mitochondria (see for detail Eqn. 14 in ref. 15).

Introducing **Equation 1** instead of the corresponding equation for NADP dynamics (Eqn. 14 in the model[15]) we obtained a "complex" model for glucose sensing that can be used for α-cells and β-cells simultaneously even at low glucose levels (see below). This model is distinguished from the previous model[15] only by k_{ng} coefficient in Equation 1 and at $k_{ng} = 0$ these models are identical.

2.2. Channels and plasma membrane potential regulation. Glucagon release, like insulin release, is influenced by physiological α-cell electrical activity that fundamentally resembles the excitation-secretion coupling seen in many secretory cell types (for reviews, see refs. 3 and 27). Like insulin-secreting β-cells, α-cells express different types of ion channels. There are at least four different types of K^+-selective channels: the ATP-sensitive K^+-channel (K_{ATP}-channel), the delayed rectifying K^+-channel

(K_{Dr}-channel), a transient K^+-channel (A-type K^+ channel) and a G protein-gated K^+-channel activated by somatostatin (K_{GS}-channel). Other channels include voltage-gated Na^+-channels, at least four types of voltage-gated Ca^{2+}-channel (VGCC) (T-, N-, P/Q- and L-type) and $GABA_A$ receptor chloride-channels (refs. 1,2,8,28; see also below Sec. 3.3. for details). In the Supplemental Material we describe the properties (mostly similar to that described in other cells) of each type of ion channel and their mathematical descriptions.

A schematic diagram of the α-cell specific channels is presented (**Fig. 1**). The AP in a single α-cell can be described with the following current balance differential equation

$$- C_m (dV_p /dt) = I_{KATP} + I_{KDr} + I_{KA} + I_{KGS} + I_{CaL} + I_{CaNL} + I_{CaT} + I_{PCa} + I_{Na} + I_{Nab} + I_{ClG} \quad \textbf{(Eqn. 2)}$$

where V_p is the plasma membrane potential, t is time, C_m is the whole-cell membrane capacitance, I_{KATP} is the ATP-sensitive K^+ channel current, I_{KDr} is the rapid delayed-rectifier K^+ current, I_{KA} is the high voltage-activated A-type K^+ current, I_{KGS} is the K^+ current through G protein-gated K^+-channel activated by somatostatin, I_{CaL} is the high-voltage-activated L-type Ca^{2+} current, I_{CaNL} is the high-voltage-activated non-L-type Ca^{2+} currents, I_{CaT} is the low-voltage-activated "T-type" Ca^{2+} current, I_{PCa} is the plasma membrane Ca^{2+}-pump current, I_{Na} is the voltage-gated Na^+ current, I_{Nab} is the Na^+ background current, I_{ClG} is the GABA activated Cl^- current.

2.3. Ca^{2+} handling. Ca^{2+} enters into cells through Ca^{2+} channels and is removed by Ca^{2+} pumps and exchangers. Ca^{2+} handling can also include ER and mitochondrial sequestration. Including only fluxes through Ca^{2+} channels and Ca^{2+} pumps on the PM, the equations for free cytoplasmic Ca^{2+} ($[Ca^{2+}]_c$) dynamics can be written:

$$d[Ca^{2+}]_c / dt = f_i (-I_{CaL} - I_{CaNL} - I_{CaT} - 2 I_{Cap}) /(2 F V_i) - k_{sg} [Ca^{2+}]_c \quad \textbf{(Eqn. 3)}$$

where f_i is the fraction of free Ca^{2+} in cytoplasm, F is Faraday's constant, V_i is

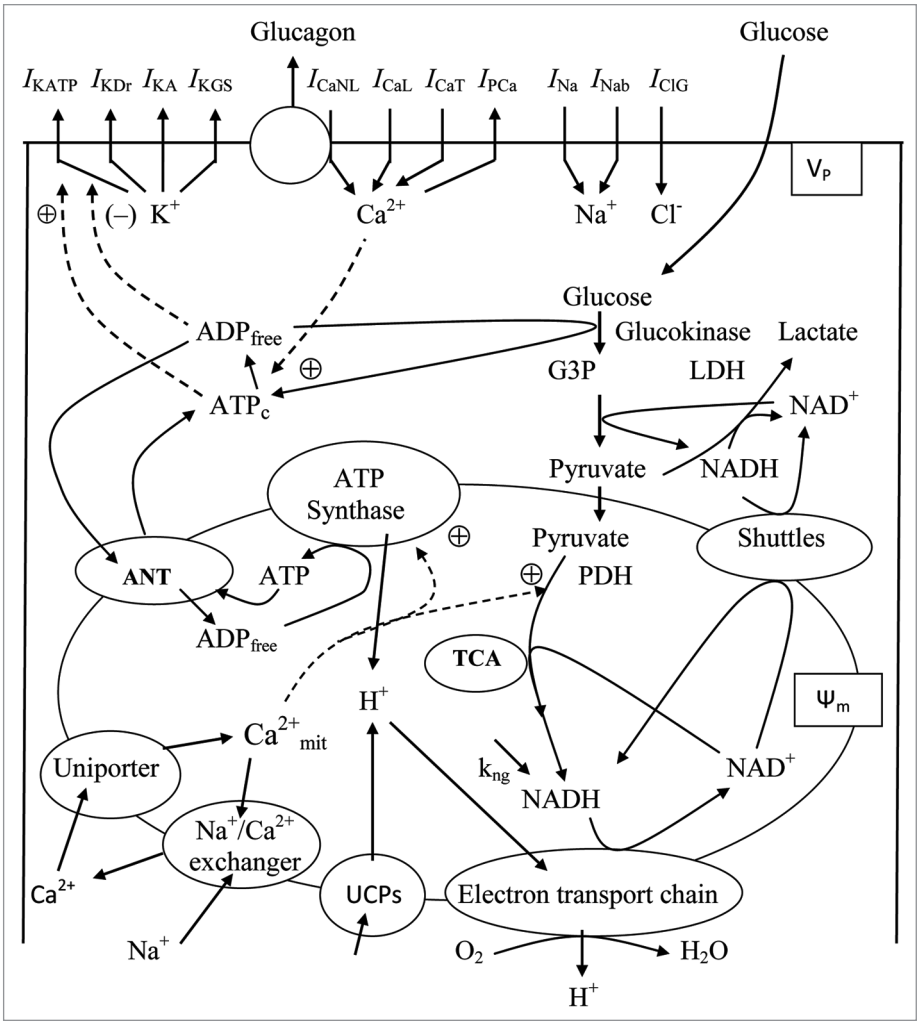

Figure 1. Schematic diagram of the ionic current, metabolic processes, Ca^{2+} fluxes and glucagon secretion mechanisms in pancreatic α-cells. Transmembrane currents are: I_{KATP} is the ATP-sensitive K^+ channel current, I_{KDr} is the rapid delayed-rectifier K^+ current, I_{KA} is the high voltage-activated A-type K^+ current, I_{KGS} is the K^+ current through G protein K^+-channel activated by somatostatin, I_{CaL} and I_{CaNL} and are high-voltage-activated L-type and non L-type Ca^{2+} currents, I_{CaT} is the low-voltage-activated "T-type" Ca^{2+} current, I_{PCa} is the plasma membrane Ca^{2+}-pump current, I_{Na} is the voltage-gated Na^+ current, I_{Nab} is the Na^+ background current, I_{ClG} is the GABA activated Cl^- current. Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase to glucose 6-phosphate, which initiates glycolysis. Lactate dehydrogenase (LDH) converts a portion of pyruvate to lactate. Pyruvate produced by glycolysis enters the mitochondria and is metabolized in the tricarboxylic acid (TCA) cycle, which then yields reducing equivalents in the form of NADH and FADH2. k_{ng} is the coefficient describing a nonglycolitic source of NADH in mitochondria. The transfer of electrons from these reducing equivalents through the mitochondrial electron transport chain is coupled with the pumping of protons from the mitochondrial matrix to the intermembrane space. The resulting transmembrane electrochemical gradient drives the ATP synthesis at ATP-synthase. Part of the protons may leak back through uncoupling proteins (UCPs). The shuttle systems are required for the transfer of reducing equivalents from the cytoplasm to the mitochondrial matrix. ATP is transferred to the cytosol, raising the ATP/ADP ratio. This results in the closure of the ATP sensitive K^+ channels, which in turn leads to depolarization of the cell membrane. In response, the voltage-sensitive Ca^{2+} channels open, promoting calcium entry and triggering exocytosis of glucagon granules. ATP_c and ADP_{free} are the free cytosolic form of ATP and ADP, G3P is the glyceraldehydes 3-phosphate, PDH is the pyruvate dehydrogenase, ANT is the adenine nucleotide translocase, Ψ_m is the mitochondrial membrane potential. Solid lines indicate flux of substrates, and dashed lines indicate regulating effects, where (+) represents activation and (-) repression.

Table 1. Cell parameters

Parameter	Definition	Value	Equation	Reference
C_m	Membrane capacitance	5,000 fF	2	a
V_i	Cytosol volume	0.764 pL	3	b
f_i	Fraction of free Ca^{2+} in cytoplasm	0.01 UI	3	b
F	Faraday constant	96,487.0 C/mol	3	
k_{sg}	Coefficient of the sequestration rate of $[Ca^{2+}]_c$	0.0001 ms^{-1}	3	b
N_a	Amount of α-cells in one islet	200	4	f
V_{pg}	Volume of the vessel	1000 UI	4	f
k_{cg}	Stoichiometric coefficient	1.4 UI	5	f
k_{rg}	Glucagon production in rest conditions	0.0003 UI	5	f
k_{pc}	Perfusion coefficient	0.0005 ms^{-1}	6	f
k_{pg}	Scaling coefficient	2000 UI	6	f

a[44]; b[17]; f, adjusted to fit the experimental values; UI, unitless.

the effective volumes of the cytosolic compartment, and k_{sg} is a coefficient of the sequestration rate of Ca^{2+} at exocytosis.

However, detailed data on Ca^{2+} dynamics during one spike in α-cells is lacking, so we used the coefficients we employed for mouse β–cells electrophysiological model[14] (**Table 1**).

2.4. Role of Ca^{2+} channels in glucagon exocytosis. Capacitance measurements in α-cells have shown that the glucagon granule exocytosis is negligible at membrane potentials more negative than -20 mV but increases steeply at more depolarized voltages.[3,8,29,30] This behavior may reflect a necessity for local calcium entry through high voltage-gated non-L-type Ca^{2+} channels in rodent and human α-cells because the process of release of primed granules in α-cells (as well as in β–cells) may need a local highly increased calcium in specific microdomains (for a review, see refs. 29 and 31). Microdomain Ca^{2+} may be proportional to the Ca^{2+} current through local Ca^{2+} channel,[32] and we model granule exocytosis to a first approximation as the dependence on Ca^{2+} influx through specific non-L-type Ca^{2+} channels.

Here we modeled the electrophysiological events inside one cell. However, in a single β-cell a few insulin granules are released per minute[16,33] and α-cells are likely to have similar characteristics.[5,34] At present it is difficult to model α-cell exocytosis in single cells (see below, Sec. 4.5). Measured rates of glucagon secretion are also very variable and often represent only a comparison of relative rates. However, we found that an evaluation of the relative rates of glucagon secretion can be sufficient for our consideration.

For these reasons, we have adopted the convention of simulating one islet containing many α-cells with which to simulate a relative rate of glucagon secretion. We simulated a hypothetical perfusion experiment, where one islet is localized in a perfusion chamber. In this case the relative glucagon concentration in media surrounding this islet and relative glucagon output from the chamber can be written as

$$d[Glc]_s / dt = f_{gs} N_a / V_{pg} - k_{pc} [Glc]_s \quad \textbf{(Eqn. 4)}$$

where

$$f_{gs} = k_{cg} (-I_{CaNL}/F) + k_{rg} \quad \textbf{(Eqn. 5)}$$

$$G_s = k_{pg} k_{pc} [Glc]_s \quad \textbf{(Eqn. 6)}$$

$[Glc]_s$ is the relative glucagon concentration in medium, N_a is the amount of α-cells in one islet, f_{gs} is the rate of exocytosis in one cell, V_{pg} is the volume of the chamber, k_{cg} is the stoichiometric coefficient, I_{CaNL}/F is the Ca^{2+} influx through specific VGCCs (non-L-type Ca^{2+} channels in this work), k_{rg} is the rate coefficient for relative glucagon production in rest conditions (in hyperpolarized cells), G_s is the relative glucagon secretion rate in the perfusion experiment, k_{pc} is the perfusion coefficient and k_{pg} is the scaling coefficient.

To compare with the experimentally measured rates of glucagon secretion we also propose that α-cells in an islet account for approximately 20% of islet cells, i.e., about 200 α-cells in a mouse islet if an islet has about 1000 cells[33] (coefficient N_a in **Eqn. 4**). Other unknown coefficients in **Equations 4–6** were evaluated according to the proposal that the mean relative glucagon concentration in the medium and the relative secretion rate were about 1 relative unit at low glucose in our hypothetical perfusion experiment (**Table 1**).

2.5. Computational model of regulation of glucagon secretion. In parallel with the metabolic model of glucose sensing, we have constructed a computational model of glucagon secretion in α-cells as the combination of the equations for the regulation of PM potential (**Eqn. 2**), Ca^{2+} handling (**Eqn. 3**), relative glucagon release (**Eqns. 4–6**) and the voltage-dependent gating variables for channels (see **Supp. Material**). The influence of glucose was modeled as the changes in ATP/ADP ratio obtained from the model of glucose sensing that determines K_{ATP} channel opening.

However, precisely detailed mathematical models of electrophysiological, metabolic and exocytotic processes are impossible to construct at the present time (and in the immediate future) due to lack of both adequate experimental data and detailed models of separate processes. We also evaluated the model parameters from the literature, when possible, but they were also found by fitting a set of known experimental data as an important reality test (see Secs. 2.1–2.4 and Supplemental Material).

Table 2. Membrane current parameters

Parameter	Definition	Value	Equation	Reference
E_K	Reversal potential for K^+ current	-75 mV	A1	a
g_{mKATP}	Maximum conductance for I_{KATP}	30,000 pS	-	f
g_{mKDr}	Maximum conductance for I_{DKr}	18,000 pS	A4	f
τ_{dKr}	Time constant for d_{Kr}	18 ms	A5	f
V_{dKr}	Half-activation potential	-15 mV	A6	f
k_{dKr}	Slope of half-activation potential	7 mV	_	f
g_{mKA}	Maximum conductance for I_{KA}	1200 pS	A8	f
τ_{dKA}	Time constant for d_{KA}	0.26 ms	A9	b
V_{dKA}	Half-inactivation potential	-9 mV	A10	f
k_{dKA}	Slope of half-activation potential	8 mV	_	f
τ_{fKA}	Time constant for f_{KA}	12 ms	A11	b
V_{fKA}	Half-inactivation potential	-20 mV	A12	f
k_{fKA}	Slope of half-inactivation potential	4.8 mV	_	f
g_{mKGS}	Maximum conductance for I_{KGS}	0 pS	A13	f
E_{Na}	Reversal potential for Na^+	70 mV	A14	a
g_{mNa}	Maximum conductance for I_{Na}	32000	A14	f
τ_{dNa}	Time constant for d_{Na}	0.15 ms	A15	f
V_{dNa}	Half-activation potential	-30 mV	A16	f
k_{dNa}	Slope of half-activation potential	10 mV	_	f
τ_{f1Na}	Time constant for f_{1Na}	1 ms	A17	f
V_{f1Na}	Half-inactivation potential	-42 mV	A18	f
k_{f1Na}	Slope of half-inactivation potential	6 mV	_	f
τ_{f2Na}	Time constant for f_{2Na}	40 ms	A19	f
V_{f2Na}	Half-inactivation potential	-44 mV	A20	b
k_{f2Na}	Slope of half-in activation potential	6.0 mV	_	f
g_{mNab}	Maximum conductance for I_{Nab}	44 pS	A21	f
E_{Ca}	Reversal potential for Ca^{2+} current	70 mV	A22	f
g_{mCaL}	Conductance for I_{CaL}	100 pS	_	f
V_{dCaL}	Half-activation potential	-20 mV	A24	f
k_{dCaL}	Slope of half-activation potential	6 mV	_	f
τ_{fCaL}	Time constant for f_{1CaL}	6.8 ms	A26	f
V_{fCaL}	Half-inactivation potential	-20 mV	A27	f
k_{fCaL}	Slope of half-inactivation potential	6.5 mV	_	f
g_{mCaNL}	Conductance for I_{CaNL}	300 pS	A28	f
τ_{dCaNL}	Time constant for d_{CaNL}	0.4 ms	A29	f
V_{dNL}	Half-activation potential	-9 mV	A30	f
k_{dN}	Slope of half-activation potential	8 mV	_	f
τ_{fCaNL}	Time constant for f_{CaNL}	18 ms	A31	f
V_{fCaNL}	Half-inactivation potential	-9 mV	A32	f
k_{fCaNL}	Slope of half-inactivation potential	8 mV	_	f
g_{mCaT}	Maximum conductance for I_{CaT}	200 pS	A33	f
τ_{dCaT}	Time constant for d_{CaT}	0.4 ms	A34	f
V_{dCaT}	Half-activation potential	-50 mV	A35	f
k_{dCaT}	Slope of half-activation potential	6 mV	_	f
τ_{fCaT}	Time constant for f_{CaT}	6.8 ms	A36	f
V_{fCaT}	Half-inactivation potential	-74 mV	A37	f

a[14]; b[8]; f, adjusted to fit the experimental values.

Table 2. Membrane current parameters (continued)

Parameter	Definition	Value	Equation	Reference
k_{fCaT}	Slope of half-inactivation potential	6 mV	_	f
P_{mCap}	Maximum I_{PCa} current	300 fA	A38	f
K_{Cap}	Half-maximum Ca^{2+} binding constant for I_{PCa}	0.3 μM	_	f
g_{mClG}	Maximum conductance for I_{ClG}	0 pS	A39	f
E_{Cl}	Reversal potential for Cl^-	-60 mV	A39	f

a[14]; b[8]; f, adjusted to fit the experimental values.

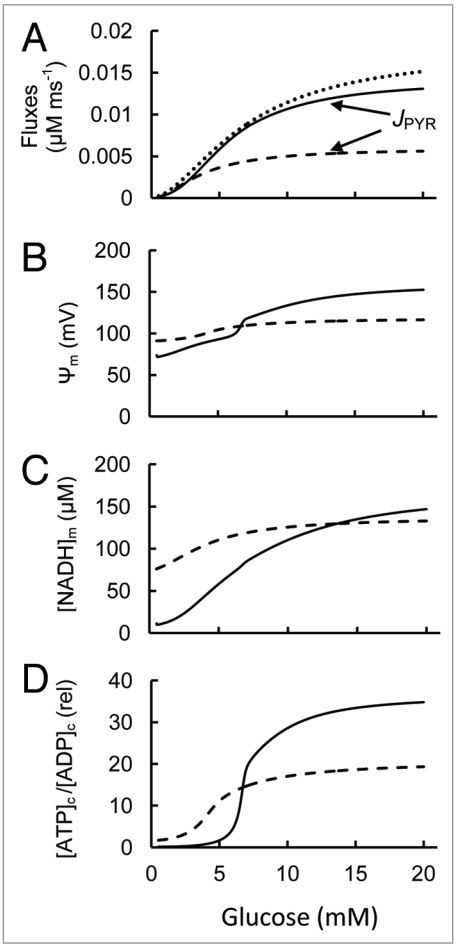

Figure 2. Effect of increasing glucose on α- and β-cells energetics. Extracellular glucose concentration was varied and the steady-state simulations of the model parameters are represented. Simulations were run with the basic set of parameters for β-cells (Tables 2 and 3 from ref. 15) and additionally with the coefficient describing the nonglycolitic source of NADH in mitochondria (k_{ng}, **Eqn. 1**). Corresponding parameters were changed for α-cells: k_{ng} was increased from 0.006 μmol ms^{-1} (for β-cells) to 0.05 μmol ms^{-1}, V_{mLDH} (maximal lactate dehydrogenase activity) was increased from 1.2 μmol ms^{-1} to 12 μmol ms^{-1}, the maximal pyruvate dehydrogenase (PDH) activity (V_{mpdh}) was decreased from 0.3 μmol ms^{-1} to 0.08 μmol ms^{-1}, the regulated leak coefficient (P_{lr}) was increased from 0.0012 μmol ms^{-1} to 0.008 μmol ms^{-1}, the glucokinase activity (V_{glu}) was taken as 0.01 μmol ms^{-1} for both α- and β-cells. (——) represents β- and (- - - -) α-cells. (**A**) (••••) is the rate of the glucokinase reaction (close in α- and β-cells), J_{PYR} is the rate of pyruvate consumption in mitochondria for β- and α-cells. (**B**) Ψ_m is the mitochondrial membrane potential. (**C**) $[NADH]_m$ is mitochondrial NADH. (**D**) $[ATP]_c/[ADP]_c$ is the ATP/ADP ratio in the cytoplasm.

Tables 1 and 2 contain all the information necessary to carry out the simulations presented in this paper and are pointed out in the text as a simulation at basal level. In some cases, when the values of parameters differed from those at basal levels the values assigned are noted in the text or in the corresponding figure legends. To calculate the steady-state cellular parameters, the model was allowed to run up to steady values with no changes in coefficients. The units, except where indicated otherwise, are: time in milliseconds (ms), voltage in millivolts (mV), concentration in micromoles/liter (μM), current in femtoamperes (fA), fluxes in mmol s^{-1}, conductance in picosiemens (pS), capacitance in femtofarads (fF). Numerical integration was performed using standard numerical methods.

These models are available for direct simulation on the website "Virtual Cell" (www.nrcam.uchc.edu) in "MathModel Database" on the "math workspace" in the library "Fridlyand" with the name: "Alpha and beta cells glucose sensing" and "Glucagon secretion" are represented. Visualization and graphical analysis were performed using "Excel."

3. Review, Simulations and Analysis of Experimental Observations of α-cell Intracellular Regulatory Mechanisms

In this section we use computational simulations to critically review and analyze the experimental observations of intracellular regulatory mechanisms, where electrophysiological data were obtained for isolated α-cells. However, because our mathematical model of glucagon secretion has significant limitations (see Sec. 2.5) we will use it mainly to test and explain numerous seemingly mutually incompatible experimental observations and the development of hypotheses concerning the mechanisms of regulation of glucagon secretion, not for simulation of individual experiments.

Here, we define a "low glucose concentration" as approximately 1–2 mM (where glucagon secretion is maximal in isolated α-cells), "medium glucose concentration" is 3–5 mM (where glucagon secretion is minimal) and "high glucose concentration" is above 10 mM (where glucagon secretion begins to increase) (see below in Sec. 3.2).

3.1. Intrinsic metabolic regulation in α-cells. The ratio of lactate dehydrogenase to mitochondrial glycerol phosphate dehydrogenase activity is higher in α- compared with β-cells.[35,36] (Low activity of lactate dehydrogenase is favoring the mitochondrial oxidation of glucose in β-cells[15,35]). In contrast, pyruvate carboxylase, that is required for conversion of pyruvate to oxaloacetate

for repleting the Krebs cycle with intermediates, has significantly higher expression in β-cells.[36] Interestingly in this regard, uncoupling protein 2 is expressed much more highly in α-cells compared with β-cells.[37]

We examined how these differences can affect the metabolic responses of α-and β-cells to glucose, using the corrected model for glucose sensing (**Fig. 2**). To describe characteristic peculiarities of α-cells the activity of pyruvate dehydrogenase was decreased, and the activities of lactate dehydrogenase and uncoupling protein 2 were increased to compare with the β-cell model.[15] The rate of NADH production from nonglucose sources was assumed to be higher in α- compared with β-cells to take into account the increased ATP and NAD(P)H levels in α-cells at low glucose levels (see Sec. 2.1). We have found that in this case the rate of pyruvate consumption in mitochondria (J_{PYR}) was significantly decreased in α-cells (**Fig. 2A**). Mitochondrial membrane potential, ATP/ADP ratio and NADH in mitochondria were higher at low glucose and saturated sharply in α-cells unlike β-cells simulation (**Fig. 2B–D**).

The results of simulations are consistent with experimental data: glycolytic flux, measured as D-[5^{3}H]glucose utilization, was comparable in α- and β-cells. Rates of glucose utilization increased proportionally to the extracellular substrate concentration between 1 and 10 mM substrate and were virtually identical in non-β-cells (mainly α-cells) and β-cells.[22] The ATP/ADP ratio was significantly higher in α-cells compared with β-cells at low glucose levels.[26] For both α- and β-cells, increased glucose concentration leads to an elevation of the intracellular ATP/ADP-ratio.[1,2,38] However, experimental data on glucose metabolism agrees with the observation that ATP/ADP changes in response to glucose are less variable in α-cells compared with β-cells.[9,10,26] The NAD(P)H signal was greater in mouse islet α-cells compared with β-cells at 1 mM glucose, however, the increase of this signal with glucose was less in α-cells.[23] Purified rat non-β cells (approximately 80% glucagon-producing α-cells) exhibit a much lower rate of glucose oxidation (only 30%) in response to glucose than in purified β-cells,[36] corresponding to the decreased rate of pyruvate consumption in mitochondria in our model (**Fig. 2A**). Therefore, our systems analysis indicates that recorded differences in metabolic response between α-cells and β-cells can be explained in part by the variations in expression of the specific enzymes that are responsible for the glucose sensitivity mechanism. Based on this evaluation we can suggest that α-cells might have significantly suppressed mechanism of K_{ATP} channel inhibition with increased ATP/ADP ratio in comparison with β-cells.

3.2. Spontaneous AP firing in α-cells and metabolic mechanisms of regulation of glucagon secretion. Patch-clamp experiments on isolated mouse, rat, guinea pig and human α-cells have show n that, unlike β-cells, they produce spontaneous AP firing in the absence or low glucose levels (see refs. 1, 8, 39 and 40). We were able to simulate AP firing at low glucose levels (at the low [ATP]/[ADP] ratio) using the specified basal set of channels and coefficients (**Fig. 3**). Our model shows that the PM can be depolarized in α–cells up to a threshold for AP firing even with low glucose levels. Simulated AP spikes were in the range between -60 and 10 mV. Note, the model responds with depolarization and generation of AP firing in the same voltage range and frequency as was observed experimentally in mouse and human α-cells (see for example, refs. 8, 11 and 41).

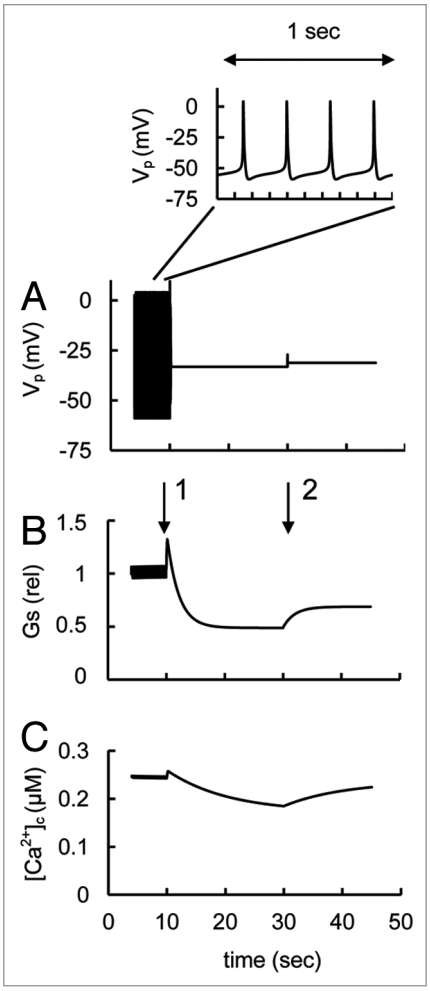

Figure 3. Modeling of spontaneous spikes, glucagon secretion and the changes of intracellular Ca^{2+} concentration in response to a step increase in glucose concentration in α-cells. (**A**) PM voltage (V_p) and corresponding detailed spike picture. (**B**) Relative glucagon secretion rate (Gs). (**C**) Cytoplasmic calcium ($[Ca^{2+}]_c$) transient. Glucose-induced K_{ATP} conductivity changes were simulated by a step increase of the [ATP]/[ADP] ratio at the arrow (1) from a low (ATP/ADP = 2, that corresponds to 1.3 mM glucose in **Fig. 2D**) to an intermediate glucose level (ATP/ADP = 6, that corresponds to 3.6 mM glucose in Figure 2D) and at the arrow (2) from an intermediate to high glucose level (ATP/ADP = 19, that corresponds to 25 mM glucose in **Fig. 2D**). All other parameters were taken from the basic set of parameters (**Tables 1 and 2**). Changes in V_p were simulated using **Equation 1**, $[Ca^{2+}]_c$ using **Equation 3**, and relative glucagon secretion using **Equation 6**.

Isolated mouse and rat pancreatic α-cells were electrically active in the absence of glucose but glucose inhibited electrical activity and suppressed glucagon secretion.[24,40] We have also simulated the increase of [ATP]/[ADP] ratios following a stepwise increase of glucose from low to medium and high levels (arrows 1 and 2 in **Fig. 3B**). Increased [ATP]/[ADP] ratio resulted in the closure of K_{ATP} channels similarly as in the β-cell mathematical

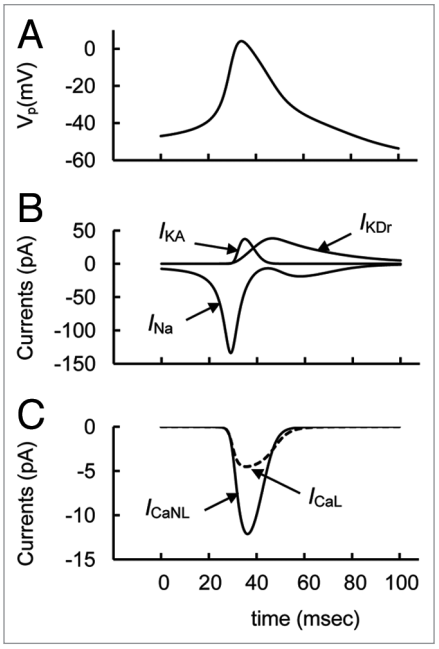

Figure 4. Detailed simulated curves of currents during a single spike from **Figure 3A**. (**A**) Action potential (V_p); (**B**) Principal currents for action potential (I_{Na}, I_{KA} and I_{KDr}) are represented for one characteristic spike, where I_{Na} is main depolarizing and I_{KA} and I_{KDr} are main repolarizing factors. (**C**) Ca^{2+} currents: I_{CaL} and I_{CaNL}.

model (compare refs. 15 and 17) leading to PM depolarization. However, quite the opposite of the case in β-cells, PM depolarization can lead to suppression of existing AP firing in α-cells. This leads to reduced opening of the voltage-gated calcium channels, decreasing Ca^{2+} influx through specific VGCCs (non-L-type in mouse) and suppression of glucagon granule exocytosis with the increase of glucose from low to medium levels. The simulations correspond to the experimental observations discussed above.

We will denote this mechanism of glucose action as a metabolic mechanism of regulation of glucagon secretion. According to this mechanism decreased glucagon secretion with increased glucose occurs as a consequence of increased ATP/ADP and block of K_{ATP} channels leading to PM depolarization and suppression of AP firing that exists at low glucose level in α-cells.

In addition to the inhibitory effect of glucose seen at medium (3–5 mM) levels, glucose also exerts a stimulatory effect on glucagon release at higher concentration. For example, glucagon secretion at 20 mM was significantly higher than that observed at 3–5 mM glucose [but it was lower than at low glucose (1–2 mM)] in isolated mouse and human islets.[3,8,42,43] Although the precise mechanism of this stimulatory effect is still obscure, our simulation shows that an additional suppression of K_{ATP} channels with increased glucose up to "high levels" results in PM depolarization and increased glucagon secretion. This may be a result of increasing voltage-dependent activation of non-L-type Ca^{2+} channels even without activation of AP firing (**Fig. 3**).

3.3. Channels as the modulators of PM potential, Ca^{2+} handling and glucagon secretion. We have evaluated here only the role of the most well-studied currents in α-cells. Taking into account this set of channels and currents we can reasonably describe the existing α-cell electrophysiological and Ca^{2+} handling properties together with glucagon secretion data.

3.3.1. Mechanism of AP spikes. A sequence of steps describing the mechanism of AP spikes in α-cells during stimulus-secretion coupling has been proposed.[2,24,44] According to this suggestion, K_{ATP} channels are responsible for changes in the membrane potential. At low glucose concentrations, the negative membrane potential in these cells is situated within a range that allows activation of T-type voltage-dependent Ca^{2+} channels. These channels drive the membrane potential to more positive values, allowing the activation of Na^+ and VGCCs. Repolarization would take place following activation of A- and K_{dr}-type K^+ channel. An increased extracellular glucose level would produce a rise in the ATP/ADP ratio as a consequence of glucose intracellular metabolism, and block K_{ATP} channels. This inhibition would allow the PM to depolarize to potentials that change the entire panoply of currents sustaining the AP, consequently suppressing electrical activity.

Our simulation confirms the possibility for this sequence of steps and allows a detailed analysis to be performed for the role of specific channels. The voltage-dependent currents during one cycle of spontaneous activity in the ionic model have been analyzed (**Fig. 4**). The upstroke phase is clearly initially generated by voltage-dependent Na^+ channels (I_{Na}). When Na^+ enters the cell, the membrane is further depolarized and high-voltage-activated Ca^{2+} channels open and activate transiently. The AP repolarization phase initially includes an activation of fast A-type K^+ channels. After that the rapid delayed rectifier K^+ channels also activate (I_{KDr} current) leading to increased repolarization current. The repolarization phase also includes the effects of the time-dependent inactivation of Na^+ and Ca^{2+} channels. This restores the resting potential and transiently hyperpolarises the α-cell (after-hyperpolarization). The after-hyperpolarization allows complete reactivation of the Na^+-channels and Ca^+-channels so that the next action potential is of full size.

Voltage-dependent Na^+ and K^+ channels (the delayed-rectifier and A-type) together may define the spike-generation mechanism in α-cells. Other currents included in the model were relatively small during a spike generation and had an insignificant influence on AP shape. This simulated pattern corresponds to the experimental observation that the Na^+-current amplitude in α-cells in freshly isolated mouse islets is 5–10-fold larger than combined Ca^+ currents.[31] However, small currents can play an important role in total interspike current and influence on spike frequency and height (see below).

Glucagon secretion rate depends on activation of specific high voltage-gated calcium channels (non-L-type, see discussion above in Sec. 2.4). Simulated Ca^{2+} current through these channels during one spike is represented in **Figure 4C**. (However, the T-type Ca^+ current was relative small and is not shown). A decrease in amplitude or complete inhibition of AP firing will therefore result in small openings of these specific VDCCs and this in turn means that fewer glucagon-containing granules undergo exocytosis.

3.3.2. K_{ATP} channels. Glucose decreases K_{ATP} channel conductance through an increased ATP/ADP ratio that was simulated in our model (**Fig. 3**). However, AP firing in isolated human and rodent α–cells and glucagon release in intact rodent and human islets also can be suppressed both when K_{ATP}-channels are maximally activated by a high concentration of diazoxide (an activator of the K_{ATP}-channel) or when they are inhibited by a high concentration of tolbutamide (a blocker of the K_{ATP}-channel) at low glucose levels.[3,8,24,27,44,45]

We simulated these experimental conditions by changing the maximal conductivity of K_{ATP}-channels and have obtained the results similarly to the experiments described above. That is, AP firing and glucagon secretion was suppressed both under conditions of inhibited and activated K_{ATP}-channels (**Fig. 5**). Note, in our model based on the experimental data, K_{ATP} channels do not open fully at 0 mM glucose and close at high glucose because the ATP/ADP ratio has a sufficiently high level even at low glucose (as discussed above in Sec. 3.1) and the ATP/ADP (ratio) increase cannot fully block the K_{ATP}-channels (see **Supp. Material**, Eqns. A1–A3). For this reason, the inhibitory effect of diazoxide on glucagon secretion can be explained by an additional PM hyperpolarization (as a consequence of increased K_{ATP}-channel conductance) up to a level that is lower than the threshold for AP firing. Abolishing AP firing and the associated decreased glucagon secretion in a simulation of additional K_{ATP}-channel inhibition (that corresponds to the action of tolbutamide) can be explained similarly to the action of increased glucose as a consequence of PM depolarization (see above in Sec. 3.2).

3.3.3. Delayed rectifying K^+-channel. Inhibition of K_{Dr}-channels results in suppression of glucagon secretion at low, but not high, glucose levels in mouse and human isolated islets.[8,46] We simulated these experiments by decreasing K_{Dr} channel conductance that corresponds to the effect of specific blockers of these channels (for example, stromatoxin) (**Fig. 6**). As suggested[8,46] a decrease in maximal K_{Dr} channels conductance suppressed AP firing and decreased glucagon secretion. This occurs because the voltage-gated K_{Dr} channels contribute significantly to the AP downstroke in α-cells (see above in Sec. 3.3.1) and after their inhibition there is no reactivation of the voltage-gated Na^+ and Ca^{2+}-channels. This paradoxically suppressed AP firing even though this leads to PM depolarization. At higher glucose levels AP firing is inhibited and block of K_{Dr} channel did not affect glucagon secretion (simulation not shown).

3.3.4. G protein-gated K^+-channel and somatostatin action. Somatostatin inhibits glucagon secretion in pancreatic α-cells.[39,47-49] Electrophysiological recordings showed that somatostatin activated a low-conductance G protein-coupled K^+-channel in single rat and mouse α-cells, causing hyperpolarization of the PM and inhibition of electrical activity.[39,47,48] We have included this current in our model (I_{KGS}, see **Fig. 1 and Supp. Material**, Eqn. A13). This current acts on the PM potential similarly to the K_{ATP} channel current, because it can be described by similar equation (compare Eqns. A1 and A13 in **Supp. Material**), that is, its activation can lead to PM hyperpolarization. Similarly to activation of K_{ATP} channels this can result in inhibition of AP firing and a decrease in glucagon release. An example of a simulation of somatostatin

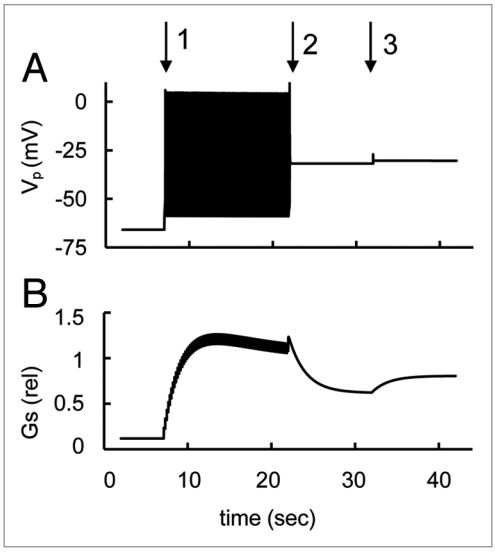

Figure 5. Simulated parameters at activation and block of K_{ATP} channels. (**A**) PM voltage (V_p) and (**B**) glucagon secretion rate transient in response to the changes in K_{ATP} channel conductance at low glucose level (ATP/ADP = 2). Initially the maximal conductance of K_{ATP} channel (g_{mKATP}, Eqn. A1) was increased from basal level up to 60 nS to simulate K_{ATP} channels activation and PM repolarization. Then g_{mKATP} was decreased to basal level (30 nS, **Table 2**) at arrow 1 that leads to AP firing and an increase in relative glucagon secretion. For simulation of K_{ATP} channel block g_{mKATP} was decreased from 30 nS to 10 nS at arrow 2 that leads to PM depolarization, AP firing suppression and decreased relative glucagon release. Then g_{mKATP} was further decreased from 10 nS to 2 nS at arrow 3 leading to additional PM depolarization with an increased glucagon secretion as a consequence of supplementary activation of non-L type Ca^{2+} channels with increased PM potential. Other coefficients and initial conditions were as in **Figure 3**.

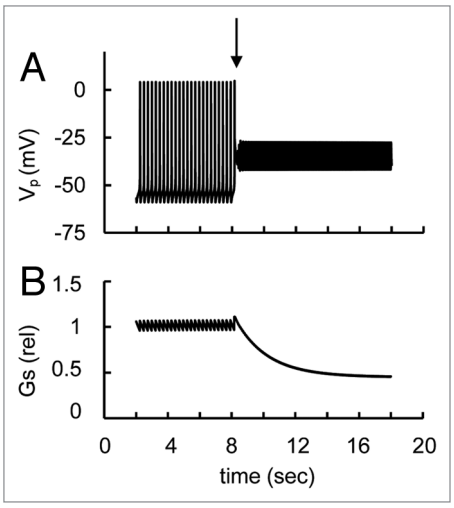

Figure 6. Simulation of block of rapid delayed rectified voltage-gated K^+ (K_{Dr}) channels. (**A**) PM voltage (V_p) and (**B**) glucagon secretion rate transient in response to a decrease of K_{Dr} channel conductance at low glucose level (ATP/ADP = 2). For simulation of block of K_{Dr} channel the maximal conductance (g_{mKr}, Eqn. A4) was decreased from 18 nS (basal level) to 0 nS at arrow leading to PM depolarization, AP firing suppression and a decrease in relative glucagon secretion. Low glucose induced AP firing was simulated initially as in **Figure 3**.

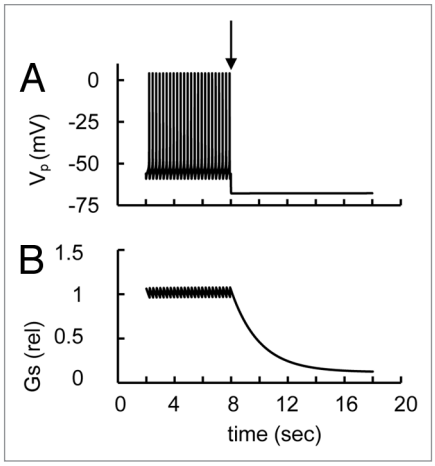

Figure 7. Simulation of activation of G protein-gated K^+-channel activated by somatostatin. (**A**) PM potential (V_p) and (**B**) relative glucagon secretion (Gs). For simulation of G protein-gated K^+-channel activation the maximal conductance (g_{mKGs}, Eqn. A13) was increased from 0 nS (basal level) to 0.5 nS at arrow leading to PM repolarization, AP firing suppression and a decrease in glucagon secretion. Low glucose induced AP firing was simulated initially as in **Figure 3**.

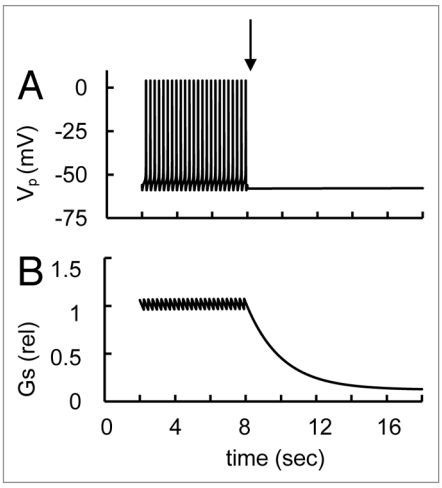

Figure 8. Simulated glucose induced spikes behavior and glucagon secretion at block of Na^+ channel. (**A**) Low glucose induced AP firing (Vp) with the initial simulation as in **Figure 3**. (**B**) relative glucagon secretion rate transients. For simulation Na^+ channel block the maximal conductance gmNa (Eqn. A14) was decreased from 32 nS (basal level, **Table 2**) to 0 nS at arrow that leads to AP firing suppression and a decrease in glucagon secretion.

action is shown in **Figure** 7. This is consistent with the inhibitory effect of somatostatin on glucagon secretion.

3.3.5. Voltage-gated Na^+ channels. Tetrodotoxin, a specific blocker of certain voltage-gated Na^+ channels, strongly inhibited glucagon secretion in mouse and human isolated islets at low but not high glucose levels.[8,24,30,44] We utilized the mathematical model presented here to estimate the impact of Na^+ channels on AP firing and glucagon secretion (**Fig. 8**). Blockade of Na^+ channels induced partial PM repolarization. However, the main effect of Na^+ channel block was a suppression AP firing and consequently glucagon secretion decreased. This occurs because the voltage-gated Na^+-channels are critical for the development of the depolarizing phase of AP in α-cell (see above in Sec. 3.3.1). At high glucose levels AP firing was still inhibited and Na^+ channel block did not change glucagon secretion significantly (not shown).

3.3.6. Na^+ background current. Closure of K_{ATP}-channels alone is not sufficient to cause membrane depolarization. Therefore, β-cells must be equipped with some sort of depolarizing inward current. However, the nature of the depolarizing current remains elusive.[50] For example this current in β-cells might result from forward operation of the Na-Ca exchanger, i.e., this can cause a Na^+ inward current because one Ca^{2+} from the cytoplasm exchanges for three Na^+ from the medium.[51] Another suggestion was activation of cationic (principally Na^+) *Trp*-channels.[52,53] In this article we used a model of some generalized inward Na^+ current (without specifying exactly which one) to simulate this depolarizing current in α-cells (Eqn. 21 in **Supp.Material**). However, clearly additional experiments are required to define the properties of this current and its influence on α-cells in greater detail.

3.3.7. L-type Ca^{2+} channels. Glucagon secretion evoked by low glucose alone is "almost resistant" or only slightly affected by L-type Ca^{2+} channel blockers in isolated mouse islets.[30] Decreased L-type Ca^{2+} channel conductance also did not suppress AP firing and glucagon exocytosis in our model (not shown) because these channels have an insignificant influence on the AP upstroke (see above in Sec. 3.3.1).

However, the L-type Ca^{2+} channel blocker isradipine suppressed the electrical activity induced by low glucose or tolbutamide in isolated α-cells and glucagon secretion in human islets.[8] This raises the question of whether the change in activity of L-type Ca^{2+} channels can lead to appropriate changes in AP firing. We found that suppression of AP firing with L-type Ca^{2+} channel block can be obtained by changing the basal conductivities of some channels in our model. For example, a decrease in conductivity of voltage-gated Na^+ channels and increased conductivity of L-type Ca^{2+} channels in comparision with basal levels in **Table 2** can lead to an increased effect of L-type Ca^{2+} current on spike formation. In such conditions decreased conductivity of voltage-gated L-type Ca^{2+} current can suppress AP firing (**Fig. 9**). Based on this evaluation we can suggest that human α-cells might have increased L-type Ca^{2+} channel activity in comparison with mouse α-cells. Interestingly, small spikes were simulated in this case after block of L-type Ca^{2+} current (as well as in **Fig. 6** at block of K_{Dr} channels) that can also be found after suppression of AP firing in several experimental conditions (see for example[8,54]).

3.3.8. Non-L-type Ca^{2+} channels. Non-L-type Ca^{2+} channels include N- and P/Q-type VGCCs. These channels have been linked to glucagon exocytosis and may be opened at PM potentials that are above that for opening L-type Ca^{2+} channels,[31] and we have modeled this high-voltage activation (**Supp. Material**, Eq. A28 **and Fig. 10**).

An N-type Ca^{2+} channel blocker (ω-conotoxin) inhibited glucagon release elicited by low glucose alone in mouse islets[29,39] and in single rat α-cells.[27] P/Q-type Ca^{2+} channels can serve as

the main component of non-L-type Ca^{2+} channels in triggering exocytosis of glucagon granules in human α-cells.[8] This group found that a P/Q-type Ca^{2+} channel blocker (ω-agatoxin) did not inhibit spontaneous $[Ca^{2+}]_c$ oscillations but suppressed exocytosis in isolated human α-cells and inhibited glucagon secretion in isolated human islets at low glucose levels.[8] Maintenance of spontaneous $[Ca^{2+}]_c$ oscillations suggests that these channels do not have any important effect on AP firing (see below in Sec. 4.4). On the basis of these data we can suggest that block of P/Q-type Ca^{2+} channels in human α- cells leads to suppression of glucagon secretion but AP firing is maintained.

We simulated the action of an non-L-type Ca^{2+} channels blocker by reducing their maximal conductivity (**Fig. 10**). The results of simulation correspond to the suggestion that the non-L-type Ca^{2+} channel blocker can have only weak effects on electrical activity; however it can still significantly suppress glucagon secretion. This is because non-L-type Ca^{2+} channels play a small role in the AP upstroke but they are strongly responsible for glucagon release (see above in Secs. 2.4 and 3.3.1).

3.3.9. Cl^- channels and γ-aminobutyric acid (GABA) action. GABA receptors have been identified in α-cells and glucagon secretion can be inhibited by GABA release from neighboring β-cells in rat.[3,55,56] The existence of Cl^- currents activated by GABA in α-cells was described for cells isolated from guinea pig and rat islets, and application of GABA led to hyperpolarization and termination of AP firing.[28,55] Interestingly, activation of $GABA_A$ receptors induces an inward Cl^- current that inhibits neuronal firing in the CNS as consequence of PM hyperpolarization.[57] We have modeled the GABA-activated inward Cl^- current (**Fig. 1 and Supp. Material**, Eqn. A39). Simulation of an activation of this current (as an increase in its maximal conductance) led to PM hyperpolarization that abolished AP firing and decreased glucagon secretion. For example, the simulated result (at g_{mCIG} = 40 pS and above) was similar to **Figure** 7 (not shown). This mechanism is similar to a PM hyperpolarization and a glucagon secretion decrease by an activation of K_{ATP} channels (see for example left part of **Fig.** 5).

3.4. Ca^{2+} dynamics and glucagon secretion. Increased glucagon secretion at low glucose levels is often associated with elevated $[Ca^{2+}]_c$ in α-cells.[40,58,59] However, a glucose-induced drop in $[Ca^{2+}]_c$ in α-cells likely contributes only partly to the strong glucose-induced inhibition of glucagon secretion in islets.[41] $[Ca^{2+}]_c$ elevation was also found in α-cells of isolated mouse islets simultaneously with decreased glucagon secretion following exposure to increased glucose.[23]

We have analyzed these contradictory experimental evidences using our model. Cytoplasmic Ca^{2+} concentration is a result of the interaction of the characteristic set of Ca^{2+} channels, Ca^{2+} pumps and electrical activity. Ca^{2+} enters cells through all types of open Ca^{2+} channels. On the other hand, glucagon release seems to be regulated mainly by specific high-voltage-activate Ca^{2+} channels (non-L-type, as discussed above in Secs. 2.4 and 3.3.8). This opens up the possibility for separate dynamics of $[Ca^{2+}]_c$ and glucagon secretion, in contrast to β-cells (see ref. 31). Our simulations illustrate these cases. For example, $[Ca^{2+}]_c$ and

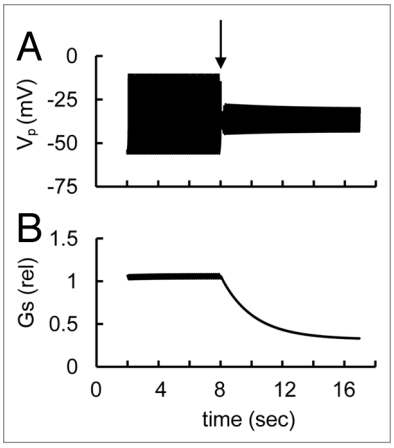

Figure 9. Simulated glucose induced spike pattern and glucagon secretion in response to the L-type Ca^{2+} channel blocker at increased L-type Ca^{2+} channel conductance (to compare with basal level) (Illustration to Sec. 3.3.7). (**A**) PM voltage and (**B**) relative glucagon secretion rate transients. The maximal conductance (g_{mCaL}, **Eqn. A22**) was decreased from 0.4 nS to 0.05 nS at arrow. g_{mNa} (Eqn. A14) was decreased from 32 nS (basal level, **Table 2**) to 22 nS, g_{mKATP} (**Eqn. A1**) was decreased from basal level (30 nS, **Table 2**) to 25 nS and P_{mCap} (**Eqn. A38**) was increased from basal level (300 fA, Table 2) to 500 fA to compensate for an influence of increased L-type Ca^{2+} channel current. These changes lead to a possibility that to AP firing may be blocked in response to the L-type Ca^{2+} channel blocker. Other coefficients and initial conditions were as in **Figure 3**.

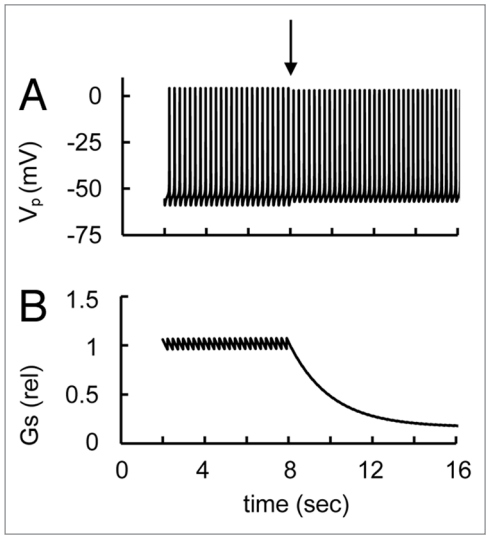

Figure 10. Simulation glucose-induced spikes activity in response to non-L-type Ca^{2+} channels blockers. (**A**) Action potential (V_p) and (**B**) relative glucagon secretion rate (Gs) transients. Block of non-L-type Ca^{2+} channels was simulated by a decrease of their maximal conductance at low glucose level (ATP/ADP = 2). At arrow the g_{mCaNL} (Eqn. A28) was decreased from 0.3 nS (basal level, **Table 2**) to 0.0015 nS. This leads to a decreased glucagon secretion at constant glucose-induced spike activity. Other coefficients were as in **Figure 3**.

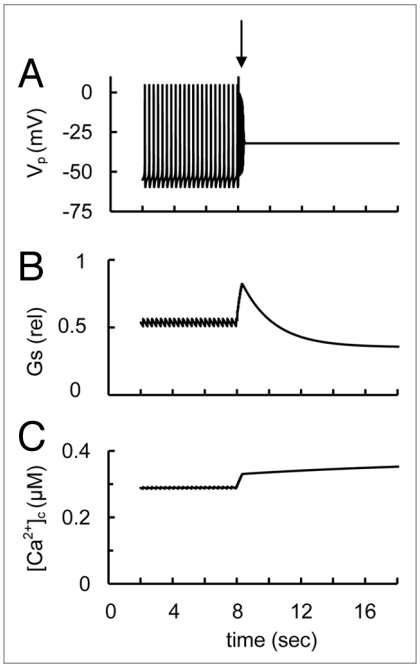

Figure 11. Simulated simultaneous $[Ca^{2+}]_c$ increase with decreased glucagon secretion due to changes in basal conductance of Ca^{2+} channels (illustration to Sec. 3.4). (**A**) Action potential (V_p), (**B**) relative glucagon secretion rate and (C) $[Ca^{2+}]_c$ transients. Non-L-type Ca^{2+} channels conductance (g_{mCaNL}, Eqn. A28) was decreased from basal level (0.3 nS, Table 2) to 0.15 nS. L-type Ca^{2+} channels conductance (g_{mCaL}, Eqn. A22) was increased from basal level (0.1 nS **Table 1**) to 0.3 nS. Glucose-induced changes were simulated as in **Figure 3** by a step increase of the [ATP]/[ADP] ratio from low (ATP/ADP = 2) to the intermediate glucose level (ATP/ADP = 10) at the arrow. All other parameters were taken from the basic set of parameters (**Tables 1 and 2**).

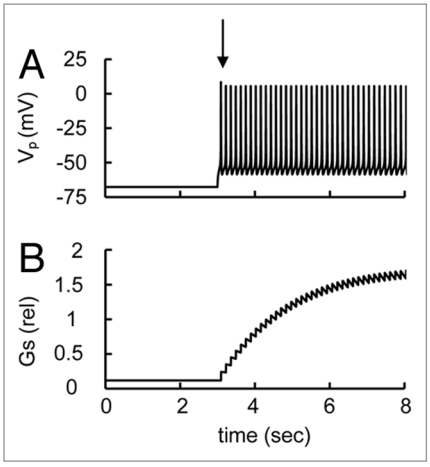

Figure 12. Simulated glucose induced spike pattern and glucagon secretion at increased conductance of K_{ATP} channels (Illustration to Sec. 3.5). (**A**) Action potential (V_p) and (**B**) relative glucagon secretion (Gs) rate transients. Maximal K_{ATP} channels conductance (g_{mKATP} in Eq. A1) was increased from 30 nS (basal level) to 75 nS. Glucose-changes were simulated (as in **Fig. 3**) by a step increase of the [ATP]/[ADP] ratio at the arrow from a low (ATP/ADP = 2) to an intermediate glucose level (ATP/ADP = 10, that corresponds to 4.7 mM glucose in **Fig. 2D**). All other parameters were taken from the basic set of parameters (**Tables 1 and 2**). AP firing was induced only at high glucose levels.

glucagon secretion have changed in one direction in the simulation of increased glucose using a chosen basal set of parameters (**Fig. 3**). However, the simulation in **Figure 11** shows that PM depolarization following increased glucose can lead simultaneously to increased $[Ca^{2+}]_c$ and decreased glucagon secretion when the conductivity for L-type channel was increased and the conductivity a non L-type Ca^{2+} channel was decreased (in comparison with the basal set in **Table 2**).

This result shows that when Ca^{2+} enters cells mainly through Ca^{+} channels which also promote glucagon release (non-L-type) the $[Ca^{2+}]_c$ and glucagon secretion should change in the same direction with increased glucose (as in **Fig. 3**), because the fraction of Ca^{2+} that penetrates into cell through L-type Ca^{+} channels during spikes is small. However, glucagon secretion can be decreased as a consequence of suppression of AP firing and closing non-L-type Ca^{+} channels, but $[Ca^{2+}]_c$ can increase following an increase of Ca^{2+} influx through L-type Ca^{2+} channels following further PM depolarization, if the fractional Ca^{2+} entering via L-type Ca^{+} channels is increased. These examples show that Ca^{2+} dynamics may depend on the relationship between Ca^{+} channels that have different voltage-dependent characteristics.

However, $[Ca^{2+}]_c$ and glucagon secretion should always decrease simultaneously with PM hyperpolarization that blocks all high voltage-gated Ca^{2+} channels (simulations not shown). This conclusion is consistent with the experimental data that opening of K_{ATP} channels with diazoxide that leads to PM repolarization lowered $[Ca^{2+}]_c$ in isolated mouse α-cells.[24,41]

3.5. Experiments with activation of AP firing and glucagon secretion at high glucose levels. Isolated, purified α-cells are removed from any potential paracrine regulation from neighboring β- and δ-cells and only intrinsic glucose regulation can take place. Two different patterns were found in the investigations of isolated rodent α-cells. Isolated rat pancreatic α-cells were electrically active in the absence of glucose but glucose (20 mM) inhibited electrical activity.[40] Glucose reduced also AP firing leading to PM depolarization and an inhibition of glucagon secretion in isolated normal mouse α-cells.[24] Obviously, this pattern corresponds well to the metabolic mechanism of regulation of glucagon secretion (as discussed above in Sec. 3.2 and **Fig. 3**).

Other investigators found that glucose stimulated rather than inhibited glucagon release in isolated rat α-cells.[27,54,60,61] Recent measurements of glucagon secretion from pure populations of flow-sorted mouse α-cells also have shown that contrary to its effect on intact islets, glucose stimulated glucagon secretion.[23] Indeed, the PM was repolarized and AP firing was suppressed at low glucose but AP firing appeared and glucagon secretion increased with increased glucose in isolated rat α-cells.[54] Depolarization from a hyperpolarized level and an appearance of AP firing were also observed in response to glucose in dispersed mouse α-cells[11,44]

We were able to simulate this pattern using our model. For example, we found that AP firing is absent at low glucose levels but can be stimulated with increased glucose (**Fig. 12**) if the maximal conductance of K_{ATP} channels was increased in

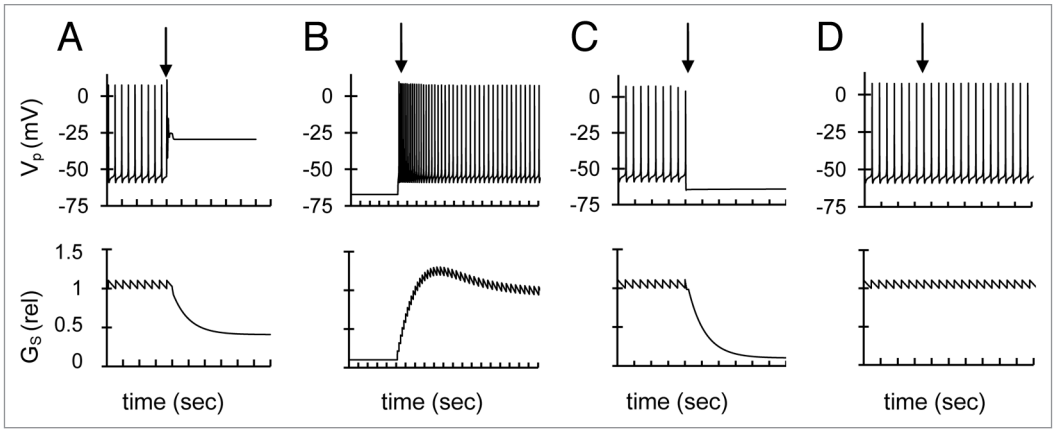

Figure 13. Schematic diagram of metabolic and paracrine mechanisms at electrophisiologycal regulation of glucagon secretion. (**A**) Metabolic mechanism of regulation. This mechanism suggests that glucose metabolism leads to K_{ATP} channel inhibition at high glucose, which depolarizes the α-cell, inhibiting action potential firing and glucagon release (as in **Fig. 3**). Glucose-changes were simulated (as in **Fig. 3**) by a step increase of the [ATP]/[ADP] ratio at the arrow. V_p is PM potential, Gs is the relative rate of glucagon secretion. (**B**) Metabolic mechanism with additional PM hyperpolarization. Additional PM hyperpolarization (for example by an increase of K_{ATP} channels conductivity, see Sec. 3.5 and **Fig. 12**) leads to suppression of AP firing and glucagon release at low glucose level. However, AP firing activates with increase of glucose as a consequence of K_{ATP} channel inhibition. Glucose-changes were simulated (as in **Fig. 3**) by a step increase of the [ATP]/[ADP] ratio at the arrow. (**C**) Paracrine interaction in regulation of islet glucagon release. At low glucose level, where paracrine influence is insignificant, intrinsic α-cell mechanisms of channel interaction lead to AP firing and increased glucagon release. Increased glucose level causes insulin, zinc and GABA release from β-cells and somatostatin from δ-cells. This leads to membrane hyperpolarization, block of AP firing and inhibition of glucagon release (Sec. 4.2). Paracrine action leading to PM repolarisation was simulated as in **Figure 7** at the arrow. (**D**) Glucagon secretion with suppressed both metabolic and paracrine regulatory mechanisms. Intrinsic α-cell mechanisms of channel interaction lead to AP firing and increased glucagon release independently on glucose concentration if both metabolic and paracrine regulatory mechanisms are suppressed or can compensate for each other. Glucose-change is represented by arrow.

comparison with the basal level (that we used for simulation in **Fig. 3**), even though ATP/ADP ratio changes were the same as describing a simulation of α-cell glucose sensing in **Figure 3**. This can be attributed to the additional hyperpolarization of PM with increased I_{KATP} leading to suppression of AP firing and glucagon secretion at low glucose. [We have considered above the similar suppression of AP firing and glucagon release at the increase of maximal conductance of K_{ATP} channels at action of activators (left part of **Fig. 5**)]. It is noteworthy that any influence on ion currents leading to additional PM hyperpolarization at low glucose levels (other than voltage-gated currents) can lead to a similar effect. For example, the corresponding somatostatin activation of the G protein-gated K^+ channel or GABA activation of Cl^- current (that were suggested as zero for basal set of coefficients) can lead to the pattern in our model that is similar to **Figure 12** (not shown).

These data and our previous analysis show that increased glucagon secretion with increased glucose from a low to high level can take place if AP firing was suppressed at low glucose but can be induced by PM depolarization with increased glucose for example as a consequence of closing K_{ATP} channels (see **Fig. 12** and Sec. 3.5). We suggest that the metabolic mechanism of regulation of glucagon secretion can act in both cases. This leads to PM depolarization with an increase of glucose leading to different patterns that can depend on initial PM repolarization with an associated increase in threshold potential in the second case that blocks AP firing at low glucose. Such differences in PM polarization in isolated α-cells are difficult to explain, but may be due in part to different methods of α-cells isolation leading to different conductivities of channels. However, these differences require further investigations.

4. Computational Systems Analysis of the Mechanisms of Glucagon Secretion in Pancreatic α-cells

We analyzed the mechanism of metabolic regulation of glucagon secretion in Sec. 3. In this section we will analyze other mechanisms for regulation of AP firing and glucagon secretion that were described in literature. Schematic diagrams for these discussed mechanisms are presented in **Figure 13**.

4.1. Regulation of glucagon secretion through an effect on electrical activity. Numerous experimental studies and our simulations show that glucagon secretion can be regulated by electrical activity in α-cells if the opening of specific VGCCs is required for glucagon secretion (see above in Sec. 3). The ATP-sensitive K^+ channel is considered to be the primary channel that can regulate PM potential. Experiments in which increasing concentrations of tolbutamide and diazoxide were used to titrate K_{ATP}-channels activity in α-cells in intact mouse, rat and human islets, indicate that an increase of glucagon secretion only occurs within a narrow window of K_{ATP}-channel activity where AP firing occurs.[3,8,30,41] We were able to simulate these results by changing K_{ATP}-channel activity (**Fig. 5**). However, our simulation also shows that PM depolarization can increase glucagon release even without activation of AP firing simply by opening additional specific VGCCs (right part of **Fig. 5**).

In general, these experiments and our simulations suggested that glucagon release may depend on membrane potential in a complex way that can lead to opposite results for the dependence on an initial PM potential. For example, PM depolarization can lead to AP firing and increased glucagon secretion if α-cells were hyperpolarized previously below threshold for AP firing and a depolarization activates this AP firing, or if there is an increased PM potential from medium to high level even though AP firing is absent (see **Fig. 5**). On the other hand, a depolarization can block AP firing and decrease glucagon secretion if AP firing was pre-existent (**Figs. 3 and 13A**). There are many different mechanisms that can influence electrical activity and consequently glucagon secretion and we have attempted to analyze several of them.

4.2. Electrophysiological basis of paracrine regulation of glucagon secretion. Rodents form a characteristic α-cell mantle surrounding the core of β-cells, however, α- and β-cells show a more mixed distribution and the α-cells are frequently juxtaposed to the β-cells within human islets.[62,63] Products released by β-cells can enter the immediate proximity of the α-cells. Thus, the α-cells can be exposed to high interstitial concentration of β- and δ-cells secretory products whenever they are secreted. This interplay between neighboring islet cells is a kind of "paracrine interaction" that may control glucagon release in α-cells. In this regard, in addition to a glucose, several paracrine inhibitors of glucagon secretion from non α-cells (i.e., insulin, zinc ions or GABA from β-cells or somatostatin from δ-cells) have been proposed (for a review see refs.[1,3,4,64]).

Modulation of ion channel activity would permit paracrine signals to have a rapid and precise effect on glucagon secretion.[65] We analyzed this proposal using our computational systems analysis approach. For example, our analysis showed that somatostatin and GABA can strongly inhibit glucagon secretion in pancreatic α-cells activating the corresponding channels that cause hyperpolarization of the PM, suppression of electrical activity and decreased glucagon secretion (see above in Secs. 3.3.4 and 3.3.9).

Insulin also has been proposed as an intraislet paracrine factor from neighboring β-cells that can suppress glucagon secretion in rodent and human islets.[1,3,4,8,64,66-68] Consistent with this role, the insulin receptor and the insulin–receptor downstream signaling molecules are highly expressed in pancreatic α-cells (see for Rev.[69]). Recent investigations have provided some insight into the mechanism of insulin action. Insulin transiently inhibited electrical activity and glucagon secretion in isolated rat α-cells, most probably through a signaling pathway that triggered membrane hyperpolarization.[54] However, insulin may not modulate the specific channels as for GABA or somatostatin. Insulin can activate K_{ATP}-channels in mouse α-cells, an effect that may be mediated via the phosphatidylinositol 3-kinase signaling pathway[70] leading to hyperpolarization. Interestingly, insulin can also act by modulating Akt, a critical downstream effector of the phosphatidylinositol 3-kinase, leading to a translocation of $GABA_A$ receptors to the cellular membrane to allow its ligand, GABA, also leading to membrane hyperpolarization.[71] We have analyzed the mechanisms of inhibition of glucagon secretion by PM hyperpolarisation by activation of K_{ATP}- and GABA-dependent Cl^- channels (see above in left part of **Fig. 5** and Secs. 3.3.2 and 3.3.9).

Zinc is present in and co-crystallizes with insulin in β-cell granules and it is secreted from β-cells on exposure to high glucose, and thus could have an inhibitory effect on rat and mouse glucagon secretion (for a review, see refs. 54, 64 and 72). Zn^{2+} may reversibly activate K_{ATP}-channels concordantly reducing electrical activity and glucagon secretion in isolated rat α-cells.[54] Similarly, a decrease in Zn^{2+} may lead to closing K_{ATP} channels in isolated mouse islets.[72] The mechanism of inhibition of glucagon secretion as a consequence of an activation of K_{ATP}-channels and PM repolarization was analyzed above (see Sec. 3.3.2 and left part of **Fig. 5**).

Based on the preceding analysis we can explain the manner in which paracrine mechanisms are able to inhibit glucagon secretion with increased glucose. Negative PM potential can exist in α-cells at low glucose level within a range that allows AP firing leading to glucagon release. Increased glucose leads to increased pancreatic insulin, GABA and zinc secretion from β-cells and somatostatin from δ-cells. These paracrine factors should lead to α-cell repolarization as a consequence of activation of corresponding channels. Decreased glucagon secretion occurs when α-cells are hyperpolarized lower than a threshold level for AP firing (**Fig. 13C**).

4.3. Hormonal regulation. Several hormonal regulators were also investigated. Leptin receptor isoforms are expressed in mouse and human α-cells.[73] Application of leptin hyperpolarized the membrane potential in glucagon-secreting alphaTC1–9 cells and suppressed the electrical activity induced by low glucose. These effects were accompanied by decreased glucagon release from α TC1–9 cells and mouse islets.[73] According to these data and based on our analysis decreased glucagon secretion after the application of leptin can be explained by PM repolarization and suppress of AP firing as was suggested for paracrine mechanisms (see Sec. 4.2 and **Fig. 13C**). The action of leptin leading to PM hyperpolarization may be mediated by opening K_{ATP} channels in the mouse pancreatic β-cells.[74,75] A similar process of K_{ATP} channels activation by leptin may also occur in the pancreatic α-cells (see also[73]).

The incretin hormone GLP-1 is a powerful suppressor of glucagon secretion. The mechanism of suppression of glucagon secretion seems to include direct action of GLP-1 on α-cells and an indirect one through activation of β- and δ-cells.[39,76,77] Interestingly, GLP-1 can inhibit glucagon secretion without much of an effect on α-cell electrical activity in isolated mouse islets, and it was suggested that GLP-1 can selectively inhibit non-L-type Ca^{2+} channels in mouse which are responsible for glucagon release.[39] According to our simulation an inhibition of non-L-type Ca^{2+} channel can indeed lead to inhibition of glucagon secretion without suppression of AP firing (see above, **Fig. 10** and Sec. 3.3.8).

GLP-1 receptor mRNA was not detected in isolated rat α-cells nor did GLP-1 stimulate glucagon release in these cells.[54] It was proposed that the mechanism of action of GLP-1 might involve inhibition of the rat α-cells by somatostatin secreted from

neighboring somatostatin-producing δ-cells that can be activated by GLP-1.[77] We considered this mechanism of somatostatin action above (see **Fig.** 7). GLP-1 can also activate insulin secretion in β-cells,[78,79] and insulin can inhibit glucagon secretion (see above in Sec. 4.2). However, the molecular details of GLP-1 action on α-cells remain elusive. For example, GLP-1 might also directly effects regulation of glucagon granule exocytosis (as it occurs in β-cells, see refs. 16 and 79). At present these mechanisms have not been investigated in α-cells and we were not able to analyze them using our computational approach.

4.4. $[Ca^{2+}]_c$ handling in α-cells. A rise in the cytoplasmic Ca^{2+} is the important physiological trigger for fusion of the insulin-containing granules in β-cells (for a review see refs. 16 and 80). However, global $[Ca^{2+}]_c$ may not directly determine glucagon release in α-cells even though a calcium influx through VGCCs located in the plasma membrane can play a decisive role in the fusion process for exocytosis of granules both in α- and β-cells (as discussed above in Secs. 2.4 and 3.4). We found that the difference in the voltage dependence of corresponding Ca^{2+} channels can explain contradictory data about cytoplasmic Ca^{2+} and glucagon secretion dynamics (see above in Sec. 3.4).

Isolated α-cells in intact mouse and human islets exposed to low glucose (or in the absence of glucose) exhibited spontaneous $[Ca^{2+}]_c$ oscillations with period up to several minutes (for a review see refs.[8,9,23,39,41,59]). Spontaneous $[Ca^{2+}]_c$ oscillations in isolated mouse α-cells result from synchronous spiking electrical activity.[41] The mechanisms that generate and terminate AP spikes are responsible for the periodical AP firing (bursting) in β-cells (see ref. 18). The periodical AP firing in β-cells provides an effective mechanism for rapid changes in extracellular Ca^{2+} influx through VDCCs that leads to cytosolic $[Ca^{2+}]_c$ oscillations.[14,81,82] For these reasons, we also suggest that periodic AP firing (bursting) can also result in $[Ca^{2+}]_c$ oscillations in α-cells. Then, an occurrence of $[Ca^{2+}]_c$ oscillations reflects the existence of periodic AP firing. However, our analysis has shown that block of AP firing can occur both during PM repolarization and depolarization in α-cells (see discussion above in Sec. 3.3), and it may be difficult to recognize the direction of PM potential change (i.e., repolarization or depolarization) if only the block of $[Ca^{2+}]_c$ oscillations is detected.

Subsequent generation and termination of AP firing may be determined by small cyclic changes in some voltage-gated and/or voltage independent gating variable currents in β-cells.[14,18,83] We have recently performed a detailed analysis of possible mechanisms of oscillatory processes in β-cells.[18] In light of this analysis, the mechanisms of $[Ca^{2+}]_c$ oscillations and periodical hormonal secretion in the isolated α-cells compared with β-cells could be more closely examined in the future.

4.5. Regulation of exocytosis of glucagon–containing granules. Glucagon is secreted by exocytosis of large dense-core vesicles. Regulation of glucagon–containing granule secretion in α-cells is both complex and multifactorial and not limited to the effect of electrical activity.[1,5,34,84] Regulation of insulin–containing granule secretion in β-cells is also very complex, but the hierarchy and interplay between the different levels of control are understood well enough to form a broad outline (for a review see refs. 79 and 80). Mathematical modeling of the regulating pathway and its influence on insulin granule secretion in β-cells was discussed.[16,85] Similarly, several proposals have been made for the influence of paracrine and endocrine factors on second messengers pathways (such as cAMP) and on glucagon–containing granule secretion,[1,86,87] and a kinetic model for glucagon secretion that describes Ca^{2+} triggered exocytosis in α-cells was recently developed.[59] However, in contrast to β-cells, our knowledge of the molecular regulation of exocytosis of glucagon-containing granules remains fragmentary. For this reason, we were not able to apply directly our computational approach to these mechanisms of regulation of glucagon release.

4.6. α-cells modeling approach. There are a large number of studies on computational modeling of β-cell function (for review see refs. 14, 15, 18 and 88). However, we have found only two computational theoretical models in the literature that consider intracellular regulatory electrophysiological mechanisms in α-cells. In one, a Hodgkin-Huxley-type ionic model for action potentials in α-cells was developed.[89] Simulation of AP firing was obtained in this model and it was shown that block of K_{ATP} channels can lead to a suppression of AP firing. However, no attempts were made for a simulation or analysis of metabolite changes, paracrine regulation, $[Ca^{2+}]_c$ handling and glucagon secretion. Ion channels involved in the electrical activity in mouse α-cells were modeled using Monte Carlo algorithms to fit steady-state channel currents.[90] However, simulation of AP firing or glucagon secretion was not considered.

4.7. Mechanisms of regulation of glucagon secretion in non-human mammals. There is a significant difference in the mechanisms of metabolic and paracrine inhibition of glucagon secretion following increased glucose. According to our analysis both of these mechanisms of inhibition are based on a suppression of AP firing. However, PM depolarization should be initiated as an effect of the metabolic mechanism, but PM repolarization could play a decisive role for the paracrine and endocrine factors. This raises the question as to what mechanisms act in islets?

In this light, knowledge of both electrical activity and glucagon secretion is required to establish the mechanism for stimulus-secretion coupling in α-cell. However, electrical activity of α-cells was not measured in many studies performed on isolated islets so that analysis is restricted to only a few papers.

4.7.1. Isolated rodent islets. Glucose inhibited glucagon secretion in isolated mouse and rat islets.[3,27,39,76] However, we were able to find only one article where AP firing was measured directly in α-cells inside rodent islets. PM depolarization and AP firing were found in α-cells in mouse and rat isolated islets after a change of glucose concentration from 11.1 to 2.8 mM and hyperpolarization and suppression of AP firing after return to 11.1 mm glucose.[12] Interestingly, other investigators[91] followed changes in membrane potential of individual cell types in whole mouse islets using a fluorescent membrane potential sensitive probe. They demonstrated that an increase in glucose concentration from 3 to 10 mM is paralleled by repolarization of α-cells and depolarization of β-cells. Our consideration suggests that the registration

of PM repolarization with high glucose levels corresponds to the action of the paracrine mechanism of regulation (see **Fig. 13C** and Sec. 4.2) (in compliance with suggestion in ref. 12).

Paracrine concept is difficult to reconcile for every condition, since several experiments have shown that the inhibition of glucagon secretion in isolated mouse islets already occurred at low glucose concentrations that have no effect on insulin or somatostatin secretion.[3] In this case the metabolic mechanism of regulation of glucagon secretion can explain these observations. However, AP firing was not measured directly in α-cells inside islets and this makes an explanation of these results problematic.

4.7.2. Diabetic animal models. Alloxan and streptozotozin treated rodent, dog and pig islets have been studied as islet injury or /β cell depletion models. When glucose was infused without insulin glucagon did not decrease but rose up to 50% in a diabetic dog. However, when large amounts of insulin was infused together with glucose, a prompt decline in glucagon (4-fold) was observed.[92] Postprandial insulin-driven suppression of glucagon secretion was also lost in the pigs treated with alloxan.[93] Similarly, glucagon secretion did not increase during hypoglycemia in streptozotocin treated rats, but it could be increased if the intrapancreatic insulin was switched off during hypoglycaemia.[94] A decisive role for endogenous insulin for the glucose-induced suppression of mouse glucagon secretion was found for streptozotozin treated mice.[68] Obviously, a metabolic mechanism could not explain the suppression of glucagon secretion in these cases but a paracrine mechanism may do so.

Importantly, our analysis of experimental observations obtained in vivo leads to the conclusion that paracrine mechanisms acting through PM repolarization may play a decisive role in suppression of glucagon secretion following increased glucose in non-human mammals.

Other proposals have also been made concerning mechanisms of regulation of glucagon release (such as direct effects on granule exocytosis, see above in Sec. 4.5) besides mechanisms acting through changes of PM potential. However, these proposals require further investigations and at present they are beyond the scope of our computational systems analysis.

4.8. Comparison of the mechanisms of regulation in α-and β-cells. It may seem puzzling that α-cells, having a similar metabolic pathways and ion channel set as the β-cells (at least in human, see refs. 8 and 95) can be regulated oppositely to β-cells. It was proposed that differences in ion channel expression contribute to the opposing regulation of insulin and glucagon secretion following changing levels of glucose.[2] Our computational analysis indicates how the variations in the conductivities of the channels can lead to a switch from characteristic α-cell AP firing at low glucose levels to AP firing only at increased glucose. We have shown in silico that an activation of the relatively strong hyperpolarizating currents in α-cells can perform such a shift (see above in Sec. 3.5). For example, the increase in K_{ATP} channel conductance is enough to transform AP firing from α- to β-cell type behavior (**Fig. 12**). Interestingly, a relatively higher density of K_{ATP} channels in β- cells than α-cells was reported.[11,44,96,97] According to our analysis this can be sufficient to explain the differences in glucose dependent electrophysiological properties α- and β-cells. However, any influence on ion currents resulting in additional PM hyperpolarization at low glucose levels may lead to a similar effect (see Sec. 3.5.). For this reason, it is also possible that β-cells have decreased activity of Na^+ background current or some other depolarizing factors in comparison with α-cells that also can lead to a corresponding shift in AP firing in region of increased glucose.

Another important difference is the possibility that over-all cytoplasmic calcium concentration may not play a decisive role in an activation of glucagon release in α-cells (as compared with microdomain sequestered calcium entry) whereas insulin secretion depends significantly on global cytoplasmic calcium (see discussion above in Sec. 3.4).

PM depolarization as well as a hyperpolarization can lead to block of AP firing and associated decrease of glucagon secretion in α-cells (see above in Secs. 3.3.2 and 4.1). This is a significant distinction from β-cells where only hyperpolarization leads to a decrease of insulin secretion in physiological conditions.

The molecular characterization of a metabolic glucose sensor is most worked out in pancreatic β-cells, with important roles for glucokinase and mitochondrial oxidative fluxes in the regulation of ATP-sensitive K^+ channels. We suggest that the metabolic mechanism of PM depolarization by K_{ATP} channel inhibition with increased ATP/ADP ratio is significantly suppressed in α-cells (see above in Sec. 3.1). Specific components of this metabolic mechanism which are well expressed in β-cells (such as a low activity of lactate dehydrogenase and uncoupling protein 2 and increased activity of pyruvate dehydrogenase) are compromised in α-cells (see above in Secs. 2.1 and 3.1). In this case the metabolic mechanism can serve to maintain a sufficient ATP/ADP ratio at low glucose levels that leads to regulation of the PM potential within narrow limits where AP firing can occur. However, the increase in ATP/ADP ratio following a further increase in glucose is supressed in α-cells (see discussion above in Sec. 3.1). An important component of metabolic glucose sensing such as K_{ATP} channels may play an important role in regulation of α-cell function through a change in PM potential. For example, a repolarization of PM through a paracrine mechanism action may occur through opening of K_{ATP} channels (see Sec. 4.2).

We suggest that such α-cell behavior is not surprising, if an aim of this system is a suppression of glucagon secretion following increased glucose. Indeed PM depolarization is the mechanism leading to insulin secretion in β–cells. For this reason we suggest that PM repolarization should be responsible for decreased glucagon secretion that according to our analysis may be a consequence of paracrine mechanisms. Block of a metabolic mechanism can open the way for this kind of mechanism for PM repolarization (see above in Sec. 4.2).

We hypothesize that the suppression of a glucose sensitive mechanism in the α-cell can also lead to different fates of α- and β-cells during the developing of type 2 diabetes mellitus. The existence of specific mechanisms for β-cell glucose sensitivity itself, and particularly the increase of mitochondrial membrane potential with increased glucose, may underlie the increased sensitivity of these cells to injury (see for details, ref. 25). According

to this hypothesis the absence of a significant increase of mitochondrial potential with glucose, as simulated in our model (**Fig. 2B**), may prevent α-cells from metabolic injury, keeping them intact when β-cells can be damaged.

5. Human α-cell Function and Diabetes

The growing intense focus on the role of the α-cell in diabetes (for a review see refs. 6, 19 and 20) has increased the importance of understanding how electrical activity induced by metabolism of sugar and other agonists is established and how it is altered in various pathological states.

5.1. Human isolated α-cells and islets. Human islets contain a substantially higher proportion of α-cells than rodent islets, normal α-cell mass in the whole human pancreas amounting to ~40% of β-cell mass.[98] Comprehensive electrophysiological characterization of the ion channels was made recently for isolated human α-cells.[8] The same set of channels exist in mouse and human α-cells (see refs. 8 and 31).

Regenerative electrical activity was observed in the majority of isolated human α-cells analyzed at low glucose levels. Tolbutamide (100–400 μmol/l) depolarized the cells, and was associated with a reduction of AP peak voltage.[8] These results can be explained by some role of K_{ATP} channels as a factor that regulates PM potential similarly to isolated mouse α-cells (see Sec. 3.3.2). However, electrical activity of α-cells inside human islets was not measured.

Intracellular Ca^{2+} oscillations in α-cells inside isolated human islets were registered at low glucose levels and these oscillations were suppressed with increased glucose.[99] This pattern can correspond to existence of AP firing at low glucose and its suppression with increased glucose level (see discussion above in Sec. 4.3).

Increased glucose concentration inhibited significantly glucagon secretion at 6 mM in comparison with 1 mM, and insulin inhibited glucagon secretion at low glucose level (1mM) in isolated human islets.[8] Insulin secretion increased also sharply in isolated human islets (other than in isolated mouse islets at increased glucose level from 1 to 5 mM.[3,8] Inhibition of $GABA_A$ receptor increased glucagon secretion significantly at high glucose levels, however this inhibition did not effect insulin secretion in isolated human islets.[56] These data can be interpreted as evidence of suppression of glucagon secretion by insulin and GABA at elevated glucose. β-cell-mediated paracrine signaling may predominate over intrinsic signals in the regulation of glucagon secretion in human and there are numerous indirect data and speculations that intrinsic insulin can be major paracrine factor for α-cells in human islets.[4,6,64]

Distinct paracrine factors may play a different role in rodent and human islets. For example, it was found that Zn^{2+} stimulated rather than inhibited glucagon secretion in isolated human islets.[8] Zn^{2+} did not play a significant role in the regulation of glucagon release in type 1 diabetic humans where only a few β-cells exist.[100]

Based on these limited date and our previous consideration we can suggest that α-cells in normal human islets (as well as in non-human mammals) have AP firing at low glucose levels with an associated increase in glucagon secretion. AP firing and glucagon secretion are suppressed by paracrine factors (possible by insulin) with increased glucose and a metabolic regulatory mechanism may not play a significant role. However, further studies are necessary to investigate this important question.

5.2. Abnormal α-cells function and diabetes. Normally, the postprandial increase in plasma glucose leads to increased insulin secretion and the moderate suppression of glucagon release. On the other hand, the physiological defense against falling plasma glucose includes decreased pancreatic insulin, Zn^{2+} and GABA secretion from the β-cell and somatostatin from δ-cells, as well as an increased glucagon secretion that prevent a sharp decrease of glucose concentration in blood.[6,20]

These defense mechanisms are compromised in type 1 and type 2 diabetes where β-cell function is significantly inhibited. Glucose sensing in glucagon release becomes defective. Diabetes is associated with aberrant secretion of glucagon at high plasma glucose levels (normally inhibited at such levels).[6,19,101,102] In patients with type 2 diabetes it has been shown that the increased glucagon concentration postprandially contributes to hyperglycemia because hyperglucagonemia results in increased hepatic glucose production. This adds an endogenous source of glucose to the exogenous glucose from the meal.[103,104] However, mean fasting plasma glucagon concentration may not differ significantly between nondiabetic and type 2 (often and type 1) diabetic subjects.[6,102,105]

Although the precise mechanisms of relative hyperglucagonemia with increased plasma glucose level in the diabetic state is still obscure, this behavior can be caused by loss of the decrement in intraislet paracrinic factors from β-cells (insulin, GABA and/or zinc) that normally results in suppressing of glucagon secretion at high glucose level (as discussed above in Sec. 4.7.2).

AP firing is required to maintain a significant rate of glucagon secretion (see discussion above in Secs. 3.2 and 5.1). Maintenance of AP firing and the associated high rate of glucagon release at elevated glucose level can readily be obtained in our model by blocking paracrine mechanisms of glucose sensing (**Fig. 13D**). It is also possible that the metabolic mechanism leading to PM depolarization under conditions of low paracrine activity, that usually leads to hyperpolarization, can compensate for each other at high glucose levels to continue AP firing.

Interestingly, elevated plasma glucagon levels are associated in type 1 diabetes with severe diabetic ketoacidosis.[102,106] While mechanisms underlying hyperglucagonemia in these cases are still obscure this may be due to the combination of low insulin concentrations and β-cell contents in the islet. For example, insulin can regulate gene expression in α-cells through several transcription factors (see ref. 69).

In islets isolated from donors with type-2 diabetes insulin release was increased (however significantly less than for non-diabetic donors) and glucagon release was decreased (however this decrease was less than for nondiabetic donors) at transitions from low (1 mM) to high (16.7 mM) glucose level.[56] These data correspond to an expected decrease in paracrine inhibition glucagon release with decreased activity of β-cells.

However, glucagon release from islets isolated from donors with type-2 diabetes was reduced at low glucose and that glucose significantly stimulated rather than inhibited secretion in these islets in work.[3] Based on our foregoing analysis we can explain this observation. For example, at suppressed paracrine mechanisms this behavior resembles the second case for isolated rodent α-cells (see **Fig. 13B** and Sec. 3.5) and can be explained in the same manner, that is the initial PM potential could be lower than the threshold for AP firing in these islets and AP firing is then activated when glucose is increased leading to PM depolarization. However, further studies, including electrophysiological recordings with secretion measurements, are necessary to determine the mechanisms of regulation of glucagon secretion in diabetic human islets.

5.3. Potential therapeutic approaches. New therapeutic approaches targeting glucagon secretion may prove useful in the treatment of diabetes.[19,20] According to our analysis the paracrine mechanism acting through PM repolarization may play a decisive role in a suppression of glucagon secretion following increased glucose in human as well as in non-human mammals. For this reason we can suggest that agents inducing PM hyperpolarization with increased glucose may be useful as a therapeutic approach. For example, somatostatin infusion inducing PM repolarization (see above in Sec. 4.2) was used to suppress hyperglucagonemia in type 1 diabetes. However, several significant side effects of somatostatin and its long acting analogs preclude its chronic use (see refs. 20 and 107).

Leptin can also hyperpolarize the PM in α-cells (see above in Sec. 4.3). For this reason, the inhibitory effect of leptin on the α-cells may be a plausible therapeutic strategy for suppression of glucagon secretion in Type 1 diabetes.[20,108] However, leptin could also suppress insulin secretion[74] so this strategy may have complex effects on both glucagon and insulin secretion if β-cells maintain activity (see also refs. 20,73).

Amylin (IAPP, or islet-ssociated polypeptide) is a β-cell hormone that is co-secreted with insulin. Clinical studies, in which a commercially available amylin analog (pramlintide) was used, have shown that amylin can decrease circulating glucagon levels.[20,109] The mechanism of amylin analog action, as well as other paracrine factors, may lead to PM hyperpolarization in α-cells. However, further studies, including electrophysiological investigations, are necessary.

Interestingly, K_{ATP} channels openers (e.g., diazoxide) can hyperpolarize the α-cell PM, prevent AP firing and blocking glucagon secretion (as discussed above in Secs. 3.3.2 and 4.1). However, K_{ATP} channel activation will also suppress insulin secretion. Application of a K_{ATP} channel opener before or with meals may decrease both glucagon secretion and hepatic glucose production in Type 1 and advanced type 2 diabetes, where insulin secretion is insignificant. We suggest that K_{ATP} channels openers can also be potential therapeutic agents in treatment of diabetes. However, this has not been directly addressed in clinical studies.

Finding specific agents that lead to PM hyperpolarization only in α-cells rather than β-cells may be helpful for treatment of type 2 diabetes where intact β-cells remain active. This can include specific activators of K^+ and Cl^- outward currents or blockers of inward Na^+ current in α-cells. Paracrine factors may also lead to α-cell repolarization as a consequence of activation of hyperpolarizing ion channels (see discussion above in Sec. 4.2) and an activation of specific receptors can increase the associated currents even though the amount of paracrine factors from β-cells may be greatly reduced in diabetes.

PM depolarization in α-cells can suppress AP firing and glucagon secretion (see above in Sec. 3.2). Sulphonylurea drugs (like tolbutamide and glibenclamide) block K_{ATP}-channels leading to depolarization. However, sulphonylurea therapy has in fact been reported to stimulate glucagon secretion in type-1 diabetes[105,110,111] and in contrast to inhibit glucagon secretion in both healthy[66,105] and type-2 diabetic subjects.[112] Our model suggests that the effect of sulphonylureas depends on initial PM potential conditions (see Sec. 4.1) and, in principle, can even increase glucagon release if AP firing is initially suppressed and PM depolarization leads to initiation of APs as shown in **Figure 13B**. Increased PM depolarization from a medium level (in medium glucose) even at suppressed AP firing can also increase glucagon secretion (see discussion above in Sec. 3.2 and right part of **Fig. 3**). This may explain the contradictory data on the effect of sulphonylureas on glucagon secretion.

The incretin hormone GLP-1 is a powerful suppressor of glucagon secretion (see above in Sec. 4.3). GLP-1 based therapies target both insulin and glucagon secretion, and inhibition of glucagon accounts for a large part of the glucose-lowering effect in type 2 diabetic patients.[19,77] There is reason to believe that indirect reciprocal β-cell-mediated paracrine signaling (partially the GLP-1 mediated increase in insulin secretion) may predominate in the suppression of glucagon secretion in type 2 diabetic patients.[105] Interestingly, the use of a GLP-1 analog, liraglutide, may help in controlling hyperglycemia with a concomitant reduction in insulin dose in type 1 patients[113] indicating a potential action of GLP-1 analogs directly on α-cells. However, the mechanisms behind the possible direct inhibitory effect of GLP-1 on glucagon granule exocytosis have not been elucidated and at present additional studies are needed to apply the computational approach to this effect.

Conclusion

Here we have reviewed intrinsic glucose-sensing, metabolic, paracrine and endocrine mechanisms that control glucagon secretion in α-cells and proposed a computational model for these processes. This model was developed to gain further insights into coupling of metabolic processes, PM potential, $[Ca^{2+}]_c$ handling and glucagon release in α-cells, that remains incompletely understood. We have shown seemingly contradictory experimental data in this field may be synthesized into a coherent mathematical model. In this model the metabolic features and major ionic currents can be fitted to recent experimental data and expression experiments. The key feature of the model is that it can be used to explore mechanisms underlying AP spikes that regulate glucagon secretion. The model reproduces a number of experimental observations ranging from

metabolic changes and voltage-clamp current traces to Ca^{2+} and glucagon secretion dynamics. This model can be used as the groundwork for *in silico* examination of the regulation of glucagon secretion through a change in the metabolic processes, channels function and $[Ca^{2+}]_c$ dynamics. Understanding the cellular and molecular regulation of glucagon release can lead to refinement of current therapies and to the discovery of new targets that improve treatment of both major types of diabetes and also give additional insight in several types of monogenic diabetes. However, further studies supported by improved mathematical models are necessary to understand the stimulus-secretion coupling in the α-cell.

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Supplemental Materials

For the appendix and references 114–125, please see the supplemental materials, which may be found here: www.landesbioscience.com/journals/islets/article/22193

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