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On the Electrophysiological Component of Pancreatic Alpha-Cell Models

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***Abstract* — Glucagon, the main hormone responsible for increasing blood glucose levels, is secreted by pancreatic alpha-cells in a Ca^{2+} dependent process associated to membrane potential oscillations developed by the dynamic operation of K^+, Na^+ and Ca^{2+} channels. The mechanisms behind membrane potential and Ca^{2+} oscillations in alpha-cells are still under debate, and some new research works have used alpha-cell models to describe electrical activity. In this paper we studied the dynamics of electrical activity of three alpha-cell models using the Lead Potential Analysis method and Bifurcation Diagrams. Our aim is to highlight the differences in their dynamic behavior and therefore, in their response to glucose. Both issues are relevant to understand the stimulus-secretion coupling in alpha-cells and then, the mechanisms behind their dysregulation in Type 2 Diabetes.**

***Clinical Relevance* — A reliable description of the electrophysiological mechanisms in pancreatic alpha-cells is key to understand and treat the dysregulation of these cells in patients with Type 2 Diabetes.**

I. Introduction

Pancreatic α- and β-cells are essential components of the endocrine system in charge of maintaining blood stream glucose concentration around 90 mg/dL (about 5 mM). If glucose level decreases, α-cells release glucagon, a hormone that induces glycogenolysis in liver to prevent hypoglycaemia. In contrast, pancreatic β-cells secrete insulin at high glucose levels, avoiding hyperglycaemia [1]. In type 2 diabetes (T2D), glucose regulation by pancreatic cells is lost. β-cells present insulin secretion dysregulation and α-cells a functional atrophy, which lead to the characteristic hyperglycaemic periods of Diabetes [2]. Thus, precise description of intracellular mechanisms defining α- and β-cells response to glucose is key to reach a wide view of glucose level control as well as of the diabetes disease.

Description of the stimulus-secretion coupling (SSC) in pancreatic β cells has been possible thanks to experimental and modelling research and it is as follows: High glucose concentrations lead to high ATP/ADP ratios inside the cell, which in turn inhibit ATP-dependent K^+ channels. It augments propensity of cells to fire action potentials by increasing membrane potential. Once the threshold of voltage-dependent ion-channels is reached, membrane potential begins to oscillate thanks to the balance between inward and outward currents. This electrical activity provokes intracellular Ca^{2+} oscillations that stimulate insulin secretion [3]. Essential components of this electrical activity in β-cells are both ATP-dependent K^+ channels and voltage-dependent Ca^{2+}-channels. However, these are the main mechanisms of this response to glucose. Many other identified mechanisms like ligand-gated channels and pumps, are necessary to cover the whole repertoire of physiological scenarios under which pancreatic β-cells develop electrical activity. In this regard, accurate electrophysiological characterization together with mathematical models are pivotal to understand the mechanisms affected during diseases such as Diabetes [4].

On the other hand, SSC in pancreatic α-cells is still unresolved. Few modelling studies have been made aiming to elucidate key mechanisms behind the electrical α-cell response to glucose leading to glucagon secretion. Indeed, very recent research works [5]–[7] are based on the models of Diderichsen & Göpel [8], Watts & Sherman [9] and Montefusco & Pedersen [10]. Among them, the description of electrical activity of models [9] and [10] is again based on model [8]. Interestingly, all these studies proposed modifications that lead to relevant qualitative and quantitative differences in electrical activity of α-cells. In this paper, we analyse the contribution of three of these models based on lead potential analysis and bifurcation diagrams in order to highlight their differences and to discuss the physiological implications of each model description.

II. Methods

A. Alpha-cell models

Models under analysis here are those proposed by González-Vélez et al. (GV) [7], Watts & Sherman (WS) [9] and Montefusco & Pedersen (MP) [10]. GV model is used instead of Diderichsen & Göpel [8] model because although electrophysiological parameters are almost as the original, GV model adds an important Ca^{2+} current, the N-type which is associated to glucagon secretion. Ionic currents defining the electrical activity of an α-cell in these three models are:

$$I_{CaL} = \bar{g}_{CaL} \cdot m_{CaL}^2 \cdot h_{CaL} \cdot (V_m - V_{Ca}), \quad (1)$$

$$I_{CaT} = \bar{g}_{CaT} \cdot m_{CaT}^3 \cdot h_{CaT} \cdot (V_m - V_{Ca}), \quad (2)$$

$$I_{CaN} = \bar{g}_{CaN} \cdot m_{CaN}^n \cdot h_{CaT} \cdot (V_m - V_{Ca}), \quad (3)$$

$$I_{Na} = \bar{g}_{Na} \cdot m_{Na}^3 \cdot h_{Na} \cdot (V_m - V_{Na}), \quad (4)$$

$$I_{KA} = \bar{g}_{KA} \cdot m_{KA}^k \cdot h_{KA} \cdot (V_m - V_K), \quad (5)$$

$$I_{KDr} = \bar{g}_{KDr} \cdot m_{KDr}^4 \cdot (V_m - V_K), \quad (6)$$

$$I_{leak} = \bar{g}_{leak} \cdot (V_m - V_{leak}), \quad (7)$$

$$I_{KATP} = g_{KATP} \cdot (V_m - V_K). \quad (8)$$

Equations (1) to (8) are based on Hodgkin-Huxley formalism: g_j is the maximal conductance through *j-type* channels, and m_j and h_j are their activation and inactivation

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variables, respectively; V_m is the membrane potential; and V_i are equilibrium potentials for *i-type* ions (Ca^{2+}, Na^{+}, K^{+} and leak).

The evolution equation for V_m is:

$$V_m{}' = -1/C_m \cdot \Sigma I_j, \quad (9)$$

and those for activation (m_j) and inactivation (h_j) variables are:

$$m_j{}' = \left(m_{ej} - m_j\right)/\tau_{mj}, \;\; h_j{}' = \left(h_{ej} - h_j\right)/\tau_{hj}. \quad (10)$$

In (9), C_m is the cell capacitance, and for all models analysed here it is equal to 5pF. In (10) m_{ej} and h_{ej} are the activation and inactivation variables at equilibrium, while τ_{mj} and τ_{hj} are time constants for activation and inactivation. As in [8], each equilibrium variable is given by a two-parameters sigmoidal function of V_m, and each time constant is given by a four-parameters bell-shaped function of V_m.

The three analysed models define different values for the parameters needed in (1-10); these values can be found in the original publications[7], [9], [10]. Values for some specific parameters are shown in Table I. These parameters are relevant for the purpose of our analysis.

TABLE I. RELEVANT PARAMETER VALUES FOR ANALIZED MODELS.

Param.	Units	Value		
		GV	WS	MP
g_{CaL}	nS	1	0.7	0.85
g_{CaN}	nS	1	0.6	0.35
m_{eCaT}*	mV	-44, 4	-49, 4	-49, 4
h_{eCaT}*	mV	-46, -5	-52, -5	-52, -5
m_{eCaN}*	mV	-4,10.6	-5,10	-1,4
m_{eKDr}*	mV	-18.5,23	-25,23	-25,23
n	-	2	1	1
k	-	3	1	1

* Values in this row are for the two-parameter sigmoidal function.

GV and WS models consider an N-type Ca^{2+} current while MP model includes a P/Q-type Ca^{2+} current in equivalence. In the present work it is named "N-type" but parameters are as in [10]. For the three models, the effect of blood glucose level on electrical activity of α-cell is simulated with changes in the parameter g_{KATP}, which is the effective conductance of ATP-dependent K^{+} channels. An increase in g_{KATP} describes a glucose decrease, while a decrease of g_{KATP} means an extracellular glucose increase.

B. Relative contribution analysis of ionic currents

In order to evaluate the contribution of each ionic current in the electrical activity generated by the α-cell, we used the Lead Potential Analysis method proposed in [11]. In this method, the relative contribution of each ionic current to the action potential is defined as:

$$r_{cj} = \left(V_L{}' - V_{Lj}{}'\right)/V_L{}', \quad (11)$$

where r_{cj} is the relative contribution of the j current to either depolarization or repolarization, V_L is the so-called lead potential and V_{Lj} is the lead potential when the effect of j current is absent. The lead potential is a dynamic equilibrium potential determining the temporal evolution of the membrane potential since changes in V_L precedes V_m ones (the interested reader is referred to [11]). A positive value of r_{cj} accounts for a mechanism operating in the same direction of V_L', while a negative one operates in the opposite direction. In addition, $\Sigma r_{cj} = 1$ at each time and all r_c are defined as long as $V_L' \neq 0$. Thus, for the action potential generated by each model, the relative contribution of the ionic currents to both depolarization and repolarization was computed.

Numerical integration for solving models as well as relative contribution calculations were performed in Mathematica 12.2.0 for Windows using the "StiffnessSwitching" method with "AccuracyGoal" and "PrecisionGoal" equal to 10 [12].

C. Bifurcation diagrams

Towards the understanding of the α-cells behavior as a dynamic system, we computed the bifurcation diagrams of the membrane potential as a function of g_{KATP}, which is the parameter representing the extracellular glucose level. These diagrams show the steady states (stable or not) reached by the system at each value of the bifurcation parameter. For the models under study, transitions between stable and oscillatory states were analyzed through their bifurcation diagrams which were built using the AUTO package of XPPAUT [13].

III. Results & Discussion

A. Electrical activity in pancreatic alpha-cells

Fig. 1 shows the electrical activity reproduced by the analysed models for three different values of g_{KATP}. The central value, 0.225nS, represents the KATP channel conductance during electrical activity at low glucose [7]. The low value, 0.05nS (in the left), is associated with a higher glucose level, and the conductance value of 0.325nS (in the right), would correspond to a very low glucose level. As can be seen, very different responses are reproduced with the three models when glucose decreases. For low g_{KATP} GV model reaches a stable depolarized state as reported in experimental and theoretical works [8], [14] while WS and MP models show complex and high-frequency stable oscillations, respectively. Notice that none of these oscillations have been reported under normal or high glucose. Indeed, the observed behaviour of pancreatic α-cells under these conditions is either low-frequency oscillations or a stable state, both associated with a reduced glucagon release [15], [16].

For the intermediate g_{KATP}, all models reproduce action potentials firing, which agrees with the expected α-cell response to low glucose condition. However, frequency and amplitude of membrane potential oscillations differ among models: For GV model, amplitude is between -42 and 2mV, and frequency is around 20Hz; for WS model, amplitude is between -50 and 10mV, and frequency around 11Hz; and for MP model, amplitude is between -53 and 15mV, and frequency around 3Hz. All these values agree with experimental observations [17].

B. Contribution of ionic currents in action potentials

The role of each ionic current in depolarization and repolarization phases of action potentials were determined using the Lead Potential Analysis method (Fig. 2). It is worth noticing that duration of depolarization greatly differs among models: for GV model it lasts about 21ms, while for WS and MP models it lasts about 42ms and 74ms, respectively (see Figs. 2A, 2C and 2E).

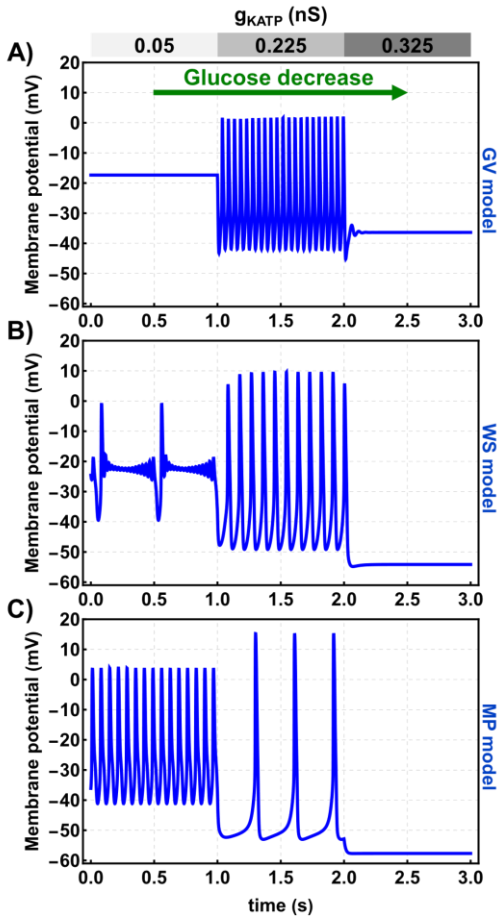

Figure 1. Electrical activity of pancreatic α-cells triggered by three different values of g_{KATP} (effective conductance of ATP-dependent K^+ channels): 0.05, 0.225 and 0.325nS, as indicated on top of the figure. Simulations performed with GV model (A), WS model (B) and MP model (C).

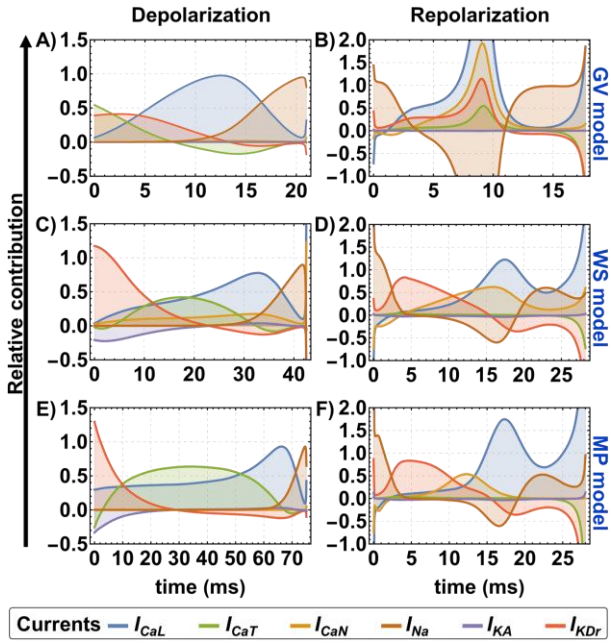

Figure 2. Relative contribution of ionic currents to depolarization and repolarization phases of action potentials quantified for GV model (A and B), WS model (C and D) and MP model (E and F). All action potentials were simulated with g_{KATP} of 0.225nS.

This has an important effect on the oscillatory frequency of action potentials which in turn define the total amount of Ca^{2+} entering the cell leading (or not) to glucagon secretion [18].

Regarding the relative contribution of each ionic current, depolarization in GV model begins with T-type channels activation, it is sustained thanks to L-type channels opening and the maximal depolarizing rate is reached when Na^+ channels are activated. In contrast, for WS and MP models, depolarization begins because of inactivation of Dr-type K^+ channels, it is sustained thanks to a joint action of L- and T-type channels, and as in GV model, the maximal depolarizing rate is reached when Na^+ channels are activated. Participation of T-type channels in the early depolarization as described by GV model leads to the higher frequency discussed above, but also make an important difference in the dynamic features of the whole system, this is discussed in section C.

In all three models, repolarization is initiated by Na^+ channels inactivation (see Figs. 2B, 2D and 2F). Afterwards, for GV model, inactivation of L-type channels dominates repolarization, which slowly progresses due to a second activation of Na^+ channels. For WS and MP models, the KDr-type current defines repolarization and then L-type channels inactivation has the major role. In addition, the N-type current plays a key role in repolarization. At the beginning of repolarization, this Ca^{2+} current operates in opposition to depolarization, which means that it supports the increase of the action potential peak. This effect is greater in WS and MP models and that is why their action potential peaks are higher. Indeed, inhibition of the N-type (or P/Q-) current has been shown to reduce glucagon secretion at low glucose [14]. Notice that even when there are small differences in L- and N-type currents between WS and MP models (see Table I), effects on frequency and amplitude of action potentials are important (see Fig. 1).

C. Steady states for different glucose levels

To study the whole electrophysiological behavior of the three α-cell models under different glucose levels, we obtained the plots of the membrane potential (electrical activity) as a function of the g_{KATP} value (glucose level) in the form of bifurcation diagrams. In the diagrams, black continuous lines represent stable steady states and black dashed lines account for unstable ones. Blue continuous lines are for stable oscillations while red dotted lines are regions of unstable oscillations.

Fig. 3A shows the electrical response using GV description. For low values of g_{KATP} (below 0.07nS), the system is in a stable depolarized state with Vm around -17mV, while for values between 0.07 and 0.3nS, stable oscillations appear (bursts of action potentials). This behaviour represents the activation of the α-cell by hypoglycaemia, and, as observed in experiments, this kind of electrical activity is linked to glucagon secretion periods [19]. Above 0.3nS a stable polarized state can be established to either -35mV or -60mV. Notice that when g_{KATP} crosses the value of 0.25nS, a Hopf bifurcation marks the transition from the stable state to a stable oscillatory state that lasts up to 0.11nS (second Hopf bifurcation).

Fig. 3B shows the electrical activity as a function of g_{KATP} obtained with WS and MP models. Some differences can be

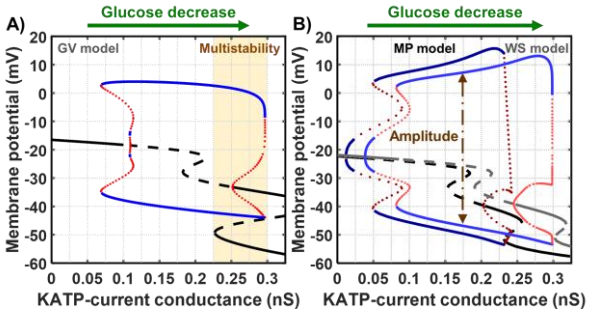

Figure 3. Bifurcation diagrams of membrane potential (V_m) when g_{KATP} changes based on the pancreatic α-cell models proposed by (A) González-Vélez et al. [7], and by (B) Watts & Sherman [9] (B) and Montefusco & Pedersen [10]. In (A), there is a region of multistability, marked in yellow.

observed when compared to the diagram in Fig. 3A. Among them, the increase of action potentials amplitude as glucose decreases (direction of green arrow) is an important result of these two models since this behaviour is linked to a higher Ca^{2+} entry through voltage-dependent Ca^{2+}-channels leading to an increase in glucagon secretion [20]. This is a future improvement needed in GV model.

An interesting feature of GV model is that it shows multistability (coexistence of various stable states) while WS and MP models do not. This occurs for g_{KATP} values between 0.22 and 0.3nS (see Fig. 3A, yellow area), and the loss of this feature in WS and MP models is due to the shift of KDr- and T-type currents to more negative potentials (see Table I). Multistability is physiologically relevant because, in many cell types, this feature accounts for different origins of the input stimulation to the system [21], [22]. Moreover, for values above 0.3nS, the system displays bistability indicating that the α-cell could reach a basal membrane potential (about -60mV) or a depolarized state (about -40mV), depending on how fast glucose decreases. It is highly important to develop further studies of this behaviour and its relation with abnormal glucagon secretion during hypoglycaemic periods. It is worth noticing that effects of high glucose on α-cells electrical activity should require an adaptation of GV model to consider paracrine effects such as glucagon inhibition by insulin and somatostatin [1], [2].

IV. Conclusions

In this paper, three electrophysiological models of pancreatic α-cells were studied through dynamic simulations, lead potential analysis, and bifurcation diagrams. All these theoretical tools allowed us to evaluate the contribution of ionic currents to action potentials, as well as the presence of bi- and multi-stability, dynamic features that have been reported in various cell types such as neurons, but still not in pancreatic α-cells. In particular, theoretical analysis allowed us to study the electrical activity displayed by pancreatic α-cells in response to glucose towards the understanding of subcellular dysregulation during hypoglycaemic periods in patients with Type 2 Diabetes.

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