Modelling the Electrical Activity of Pancreatic α -cells Based on Experimental Data from Intact Mouse Islets

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Abstract Detailed experimental data from patch clamp experiments on pancreatic α -cells in intact mouse islets are used to model the electrical activity associated with glucagon secretion. Our model incorporates L- and T-type Ca^{2+} currents, delayed rectifying and A-type K^{+} currents, a voltage-gated Na^{+} current, a KATP conductance, and an unspecific leak current. Tolbutamide closes KATP channels in the α -cell, leading to a reduction of the resting conductance from 1.1 nS to 0.4 nS. This causes the α -cell to depolarise from -76 mV to 33 mV. When the basal membrane potential passes the range between -60 and -35 mV, the α -cell generates action potentials. At higher voltages, the α -cell enters a stable depolarised state and the electrical activity ceases. The effects of tolbutamide are simulated by gradually reducing the KATP conductance ($g_{K,ATP}$) from 500 pS to 0 pS. When $g_{K,ATP}$ is between 72 nS and 303 nS, the model generates action potentials in the same voltage range as the α -cell. When $g_{K,ATP}$ is lower than 72 nS, the model enters a stable depolarised state, and firing of action potentials is inhibited due to voltage-dependent inactivation of the Na^{+} and T-type Ca^{2+} currents. This is in accordance with experimental results. Changing the inactivation parameters to those observed in somatostatin-secreting δ -cells abolishes the depolarised inactive state, and leads to β -cell like electrical activity with action potentials generated even after complete closure of the KATP channels.

Key words pancreatic α -cell · ion channel · inactivation · glucagon · membrane potential

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1 Introduction

The primary target of pancreatic islet cell research has always been the β -cell, and the stimulus-secretion coupling in this cell type is relatively well understood. By contrast, the mechanisms by which glucose suppresses glucagon release are still unclear. The main reasons for this are the lack of α -cell lines and the scarcity of α -cells in the islet. Pancreatic α -cells secrete glucagon in response to low blood glucose concentrations. Under hypoglycaemic conditions, glucagon triggers glucose release from the liver and thereby protects the body from energy run-down. It has been shown that α -cells are electrically excitable in the absence of glucose [1–4] and that this is associated with elevated intracellular Ca^{2+} -concentrations [5, 6]. Surprisingly, α -cells possess the same Kir 6.2/Sur1 KATP channels that couple high glucose concentrations to insulin release in the β -cell [7, 8]. Studies on dispersed α -cells indicate that the ATP/ADP ratio remains unaffected by glucose [9]. However, α -cells in intact islets respond with an increase in the ATP/ADP ratio upon stimulation with glucose [10]. Therefore, it is of interest to investigate the response of the α -cell to changes in the KATP conductance. For δ -cells and β -cells, an increase in the glucose concentration leads to an elevation of the intracellular ATP/ADP-ratio, translating into a reduced KATP conductance and, hence, to depolarisation and electrical activity. Ca^{2+} -ions entering the cell during the action potentials then trigger the hormone release in these cells. At first, it may seem puzzling that α -cells, having a similar ion channel set-up as the δ -cells and β -cells, and even responding with an increase in the ATP/ADP ratio when stimulated with glucose, can be regulated oppositely to their pancreatic cousins.

Using patch-clamp techniques, we have recently investigated the properties of the ion-channels in α -cells within intact mouse islets and, based on the obtained data, we have suggested a mechanism for the stimulus-secretion coupling [1, 2]. The ion-channels carrying the action potential are already 50% inactivated at approximately -47 mV. Our investigation showed that α -cells depolarise upon application of tolbutamide and generate action potentials when the basal membrane potential passes the range between -60 and -35 mV. At more positive potentials, the electrical activity ceases and the α -cell becomes permanently depolarised. On this background, we have suggested that glucose stops the electrical activity by closing the KATP-channels. The subsequent strong depolarisation leads to inactivation of the currents generating the action potential.

In the present paper, we propose a mathematical model for the α -cell electrical activity. Like previously published β -cell models [11, 12], our model is based on a Hodgkin–Huxley approach to the cellular electrophysiology. The model contains the membrane capacitance connected in parallel with non-linear conductances representing the most important ionic channels. The voltage and time dependencies of these conductances are formulated on basis of our experimental results. The model is tested in various scenarios, and the results are compared to the corresponding experimental data. Our model reproduces all main features of α -cell electrical activity. Like the α -cell, the model displays hyperpolarisation in the absence of glucose. When adding tolbutamide, simulated by a reduction of the KATP-conductance, the model responds with depolarisation and generation of action potentials in the same voltage range as observed experimentally. When the KATP-channels are almost completely closed, electrical activity ceases and the membrane potential reaches a depolarised

plateau, again as seen experimentally. The simulated voltage clamp currents for L- and T-type Ca^{2+} -channels, voltage-gated Na^{+} -channels and delayed rectifier and transient K^{+} -channels are all within the error bars of the experimental data.

2 Formulation of the Model

Hodgkin–Huxley (HH) kinetics is generally regarded as a good approximation to the behaviour of voltage-gated currents in neurons and pancreatic endocrine cells [13]. With this approach, the current I_i , carried by the ion i , is modelled as:

$$\begin{aligned} I_i &= g_i(V - V_i), \\ g_i &= g_i^* m_i^l h_i, \end{aligned} \quad (1)$$

where V is the membrane potential and g_i the channel conductance. The driving force of the current is the difference between the membrane potential and the Nernst (or reversal) potential V_i of the ion that carries the current. The actual conductance for a particular type of channel is given by the open probability $m_i^l h_i$ times the maximal conductance g_i^* . Traditionally, the activation and inactivation functions m_i and h_i are implemented as two independent gates. The open probabilities of these are derived using voltage-dependent transition rates $\alpha(V)$ and $\beta(V)$:

$$\begin{array}{ccc} & \alpha(V) & \\ O & \xleftarrow{\hspace{1cm}} & C \\ & \xrightarrow{\hspace{1cm}} & \\ & \beta(V) & \end{array}$$

$$O^\infty = \frac{\alpha(V)}{\alpha(V) + \beta(V)}, \tau = \frac{1}{\alpha(V) + \beta(V)}, \quad (2)$$

with O and C denoting the open and the closed state, respectively. O^∞ is the equilibrium probability of the gate being in the open state at a given membrane potential, and τ is the time constant associated with the approach to equilibrium. The rate-parameters $\alpha(V)$ and $\beta(V)$ are defined in such a way as to make increasing and decreasing sigmoidal functions fit closely to the voltage dependent steady-states of the activation (m_i^∞) and inactivation (h_i^∞) probabilities. Similarly, the voltage-dependent time constants for activation (τ_i^m) and inactivation (τ_i^h) may be fitted with symmetric, bell-shaped functions. The integer l in Eq. (1) is a parameter that represents the order of the activation process. A possible interpretation of the exponent l is to associate it to the morphology of the channel. We have chosen the value of l to be the integer, $l \in \{1, 2, 3\}$, that gave the best fit to the experimental data. In the formulation of the α -cell model we have fitted sigmoidal threshold functions and bell shaped functions to experimental data for use in the implementation of voltage-gated currents. This description presents a divergence from the traditional HH approach: Eq. (2) shows that in the HH approach, the steady-state activation and inactivation are coupled to the time constants through the functions $\alpha(V)$ and $\beta(V)$. We do not follow this constraint in our implementation. Compared to the time constants obtained through the HH approach, the time constants used in our model

provide considerably better fits to the experiments. We refer to the Appendix A for definitions of the applied sigmoidal and bell shaped functions.

The model is formulated according to the law of charge conservation (Kirchoff's current law):

$$0 = I_{Cap} + I_{Ion} = c_m \frac{dV}{dt} + \sum_i I_i, \quad (3)$$

where I_{Cap} is a capacitive current associated with the recharging of the plasma membrane, and I_{Ion} is the sum of all channel currents. Our model accounts for seven different currents:

$$\begin{aligned} I_{Ca,T} &= g_{Ca,T} m_{Ca,T}^3 h_{Ca,T} (V - V_{Ca}) \\ I_{Ca,L} &= g_{Ca,L} m_{Ca,L}^2 h_{Ca,L} (V - V_{Ca}) \\ I_{Na,V} &= g_{Na,V} m_{Na,V}^3 h_{Na,V} (V - V_{Na}) \\ I_{K,A} &= g_{K,A} m_{K,A}^3 h_{K,A} (V - V_K) \\ I_{K,DR} &= g_{K,DR} m_{K,DR}^4 (V - V_K) \\ I_{K,ATP} &= g_{K,ATP}^* (V - V_K) \\ I_{Leak} &= g_{Leak}^* (V - V_{Leak}) \end{aligned} \quad (4)$$

Of these currents, $I_{Na,V}$, $I_{Ca,T}$, and $I_{K,A}$, are known from experiments [1, 2] to exhibit rapid (i.e., ms) voltage-dependent inactivation, while L-type calcium channels inactivate in a calcium-dependent manner. The inactivation of $I_{Ca,L}$ mimics the calcium-dependent inactivation, but is implemented by means of similar equations as used for conventional voltage-dependent inactivation. The delayed rectifying K^+ current does not inactivate, while $I_{K,ATP}$ and I_{Leak} are passive currents. Hence, these two currents display a constant conductance: $g_{K,ATP} = g_{K,ATP}^*$ and $g_{Leak} = g_{Leak}^*$, respectively. Parameter values are listed in the Appendix A. The model was implemented as a Simulink model and integrated using the Matlab stiff systems solver, ODE15s. Automatic absolute error tolerance and relative error tolerance between 10^{-4} and 10^{-6} were used.

3 Fitting the Gating Functions

3.1 Voltage-gated Na^+ Currents

The black curves in Figure 1a show the average Na^+ current from seven cells evoked by voltage-clamp depolarisations to -10 mV, 0 mV and 10 mV as indicated by the voltage protocol (bottom). The mean standard error (sem) is indicated by the grey shaded area, while the red curve shows the simulated Na^+ current implemented using m^3h kinetics (cf. Eq. (1)). The zero current level is indicated

Figure 1 Simulated and experimentally recorded α -cell Na^+ currents. (a) Simulated and α -cell Na^+ currents in response to voltage pulses between -10 and $+10$ mV (bottom). The Na^+ currents were recorded either in the presence of 20 mM TEA, 10 mM 4-AP and 25 μM nifedipine or they were determined by subtracting currents obtained after addition of TTX from the control currents. The black curve shows the average current from seven α -cells. The sem of these currents is indicated by the grey shaded area. Simulated currents are shown in red. Simulated currents are scaled by a factor $I_{\alpha,0 \text{ mV}}^*/I_{\text{Sim},0 \text{ mV}}^*$, where $I_{\alpha,0 \text{ mV}}^*$ and $I_{\text{Sim},0 \text{ mV}}^*$ are the peak values of the α -cell and simulated currents following a depolarisation to 0 mV, respectively. (b) Voltage dependence of the peak Na^+ current (black: experimental, red: simulated). (c) Steady-state inactivation obtained by applying a pre-pulse to various voltages followed by a test-pulse to -10 mV. Between the two pulses, the cell was held at -70 mV for 1 ms. The currents obtained during the test-pulse are expressed as fraction of the maximum current, I^* . I^* was measured following a pre-pulse more negative than -100 mV. Experimental data are shown in black. Simulated currents with and without a 1 ms interval between the two pulses are shown in green and red, respectively.

by the dotted lines. In order to facilitate the comparison of the time course for the ionic currents, the simulated traces were scaled by a factor corresponding to the ratio between the simulated and α -cell peak currents following a depolarisation to 0 mV. In the experimental Na^+ current shown in Figure 1, we either subtracted the currents obtained after application of the Na^+ channel blocker TTX from the control responses or used currents obtained in the presence of the ion channel blockers TEA, 4-AP and nifedipine, that block the KDR, A and Ca^{2+} currents, respectively. Since 4-AP does not completely block the A-currents, an outward component remains, making the Na^+ current inactivation appear faster than it actually is. Consequently, implementing the Na^+ current with slightly slower inactivation than the apparent experimental currents is likely to be more accurate. The outward current is activated

after the peak Na^+ current has been reached and, therefore, primarily affects the apparent time course of the Na^+ inactivation, but not the peak current itself. The slower K^+ component introduces a skew to the experimental curve, without which the decrease of the current appears to be slower and thus provides a better fit to the model. Hence, we choose a slower time course for the inactivation of the Na^+ currents. The black markers in Figure 1b show the averaged I–V relationship for 10 α -cells, while the red curve shows the simulated Na^+ currents. The experimental data are almost exactly reproduced by the simulation and lie within the error bars at all voltages.

The voltage dependence of the steady-state inactivation of the Na^+ current was determined by the two-pulse protocol defined in [1] and is shown in Figure 1c. The experimental data and a fit to the sigmoidal function (cf. Eq. (5)) are shown in black. The experimental voltage clamp protocol included a 1 ms interval between pre- and test-pulses. In the simulation, intervals of 0 ms and 1 ms were used. The resulting inactivation curves are shown in red and green, respectively. When the two simulated pulses were separated by 1 ms, complete inactivation was not obtained, in contrast to the experimental data. This reflects a faster recovery from inactivation in the simulated currents and it suggests that the HH-like approximation in Eq. (2) is not completely valid in this case. The implications of this finding are discussed below. For all three curves a half maximal inactivation level of -47 mV and a slope parameter of 8 mV were used, cf. Eq. (5).

In the model, the time constant of inactivation is implemented using a single, voltage-dependent function. This implies that the transitions into and out of the inactivated states take place on the same time scale which is apparently not the case for Na^+ -channels [14]. This becomes evident in the simulation of the membrane potential where a pronounced shoulder is seen during the down-stroke of the action potential (Figures 6b and 8a). This shoulder coincides with an increase of the Na^+ current (Figures 6c and 8b) and disappears when the Na^+ conductance is set to zero during the down-stroke of the action potential. As our model reveals, closer experimental examination of the voltage- and time-dependent properties of the Na^+ current is necessary. In particular, experimental data on the recovery from inactivation are not yet available for the Na^+ current.

3.2 The Two Types of Calcium Channels

In order to clearly identify α -cells by their electrical properties, pipette solutions containing K^+ may be used [1, 2]. The drawback of this procedure is that the relatively small Ca^{2+} currents are easily obscured by non-blockable K^+ currents (see below). To circumvent this problem, Ba^{2+} , which blocks the remaining outward K^+ currents, was used as a charge carrier in these experiments. The disadvantage of this approach is that the Ca^{2+} dependent inactivation of the L-type Ca^{2+} channels cannot be monitored.

The solid lines in Figure 2a show a family of Ba^{2+} currents elicited by voltage pulses (Figure 2a, lower panel) and flowing through the Ca^{2+} channels. Inspection of the figures shows that α -cells contain both a sustained component flowing through L-type channels, and a transient component flowing through T-type Ca^{2+} channels. Of these two Ca^{2+} channels, only the latter exhibits voltage-dependent inactivation. The inactivation parameters for the T-type channel were determined by two-step

Figure 2 Simulated and experimentally recorded Ca^{2+} currents in α -cell. (a) A family of α -cell Ca^{2+} currents (solid lines) elicited by the indicated voltage protocol (bottom). Currents were recorded with Ba^{2+} as charge carrier (for details see [1]). The sum of simulated and L- and T-type Ca^{2+} currents are shown with dotted lines. (b) Current-voltage relationship of the total Ca^{2+} currents (black: experimental, red: simulated). (c) Simulated L-type (top) and T-type (middle) currents elicited by voltage pulses (bottom).

protocol experiments (Figure 6 in [2]), but experimental data for the Ca^{2+} dependent inactivation of the L-type channel were not available for α -cells. A description of the intracellular Ca^{2+} dynamics is not implemented in the model, but it is assumed that the calcium concentration close to the plasma membrane is proportional to the Ca^{2+} influx through the voltage-gated Ca^{2+} channels. The Ca^{2+} -dependent inactivation was approximated by a sigmoidal curve and the parameters were adjusted until inactivation and Ca^{2+} influx matched the experimental data. In the model, the L- and T-type channels are implemented using m^2h and m^3h kinetics, respectively (cf. Eq. (1)).

Figures 2c and d show simulated L- and T-type Ca^{2+} currents. The sum of the two simulated Ca^{2+} currents are superimposed on the experimental traces in Figure 2a. In order to facilitate the comparison of the time courses, the simulated currents have been scaled to the same amplitude as the experimental traces. Again it can be seen that the activation of the simulated Ca^{2+} currents is almost identical to the experimental traces. The discrepancy in the inactivation is due the use of Ba^{2+} as charge carrier, preventing the L-type currents from Ca^{2+} -dependent inactivation. Figure 2b shows the I-V relationship of the total Ca^{2+} current with the simulated values indicated in red. Within the physiological range, the I-V curve used for the model is almost entirely within the error bars of the experimental data.

Figure 3 Transient and sustained outward K^+ currents. (a) K^+ currents were elicited by a voltage-pulse (bottom) to -10 mV before and after addition of TEA. The remaining current after subtraction of the TEA resistant current (green) from the control current (black) is shown in grey. The simulated sustained (blue dotted line) and transient (red dotted line) currents are scaled and superimposed onto the experimental traces. (b) Simulated sustained currents (top) in response to depolarisations between -40 and 0 mV (bottom). (c) Current-voltage relationship of the transient (squares) and sustained (triangles) K^+ currents. Simulated I-V relationships are shown with dotted lines.

3.3 Voltage-gated Outward K^+ Currents

Figure 3a shows a recording of voltage-gated outward currents under control conditions when Ca^{2+} currents are blocked (black trace) and after addition of TEA to block the K^+ delayed rectifier (KDR) currents (green trace). The Na^+ current is seen as a short downward deflection. The TEA-resistant component is half maximally inactivated at -68 mV (Figure 5c) and partially blocked by 4-AP [2], both being typical features for potassium A-currents [13]. The A-current is implemented in the model using *mh* kinetics (cf. Eq. (1)). This implies that the experimental data are best fitted to a model that assumes a one-step opening process.

The sustained delayed rectifying component of the outward current is not easily isolated from other outward currents due to the lack of specific blockers for the A-current. Instead, we subtracted the current obtained after the addition of TEA from the control current yielding the pure TEA-sensitive KDR component (Figure 3a, grey trace). As for the other currents, the simulated trace was scaled to the experimental data to facilitate comparison of the time courses. The simulated KDR and A-currents are superimposed on the experimental traces with blue and red dotted lines respectively (Figure 3a). KDR is implemented using m^4 kinetics (cf. Eq. (1)). Simulated KDR currents evoked by depolarisations to voltages between -40 and 0 mV from a holding potential of -70 mV are shown in Figure 3b. The KDR current elicited by a depolarisation to 0 mV is fully activated after approximately 70 ms. The I-V curves for the KDR and A-currents are displayed in Figure 3c. The A-current starts to activate already at voltages above -50 mV (Figure 3c, squares), whereas the KDR starts to activate at -20 mV (triangles). The simulated currents used in the model are indicated by the dotted lines. As the other currents, the simulated currents are almost completely within the error bars of the experimental data in the physiological voltage range.

3.4 Ohmic Currents

In addition to the voltage-gated current described in the previous sections, the model contains a KATP current (with conductance $g_{K,ATP}$) and an unspecific leak current. This leak current has a conductance of 0.2 nS and a reversal potential at -20 mV. Although we have no experimental evidence for this current in α -cells we have predicted the existence of a conductance with similar properties in β -cells using a mathematical simulation of β -cell electrical activity (unpublished). This prediction has subsequently been confirmed experimentally in β -cells within intact islets (unpublished). Taking the similarities of the two cell types into account (they both function as glucose sensors and display similar ion channel properties) it is reasonable to include a similar conductance in the α -cell model.

4 Simulation Results

4.1 KATP Conductance Regulates Electrical Activity

Glucagon-secreting α -cells have been shown to express KATP channels [7, 8, 15], but the function of these channels has so far remained obscure. Figure 4 shows a simulation of the membrane potential in response to changes in the KATP conductance compared to a membrane potential recording from an α -cell during application and removal of the KATP channel blocker tolbutamide. Simulating the effect of tolbutamide by decreasing the KATP conductance leads to depolarisation and the subsequent firing of an action potential in the same way as in β -cells or δ -cells. Unlike what one observes in these cells, however, a further decline in the KATP conductance abolishes regenerative electrical activity in α -cells and establishes a depolarised plateau from which no action potentials can be fired. Both the voltage at which the firing of action potentials is initiated and at which the α -cell leaves the firing state and enters the depolarised state (Figure 4, shaded area) are accurately

Figure 4 Closure of KATP-channels inhibits electrical activity in α -cells. (a) membrane potential recorded from an α -cell during application and removal of the KATP-channel blocker tolbutamide. Voltage range from which action potentials are generated is indicated by the shaded area. (b) Simulation of the membrane potential including L- and T-type Ca^{2+} currents, KDR and A-currents, voltage-gated Na^{+} current, an unspecific leak conductance, and KATP channels. The same voltage range as in A is indicated by the shaded area. (c) Changes in the KATP-conductance as a function of time. Tolbutamide is used in the treatment of non-insulin dependent diabetes in order to stimulate the secretion of insulin and strengthen the responsiveness of the insulin-dependent cells.

predicted by the model. We have recently proposed that the onset and termination of action potential firing is due to the voltage-dependent inactivation parameters of the voltage-gated Na^{+} - and T-type Ca^{2+} channels [2]. This has the surprising consequence that an increase in the K^{+} conductance from low values with subsequent hyperpolarisation enhances the electrical activity. Figure 4b shows a simulation of the α -cell membrane including the currents described above, while panel C in Figure 4 shows the changes in $g_{\text{K,ATP}}$, mimicking the effects of tolbutamide. At $g_{\text{K,ATP}} = 0.5$ nS, the model is in a stable hyperpolarised state at -65 mV. Gradually reducing $g_{\text{K,ATP}}$ depolarises the cell until electrical activity is initiated at $g_{\text{K,ATP}} = 228$ pS. When $g_{\text{K,ATP}}$ is decreased to 72 pS, electrical activity ceases, and the model reaches a stable depolarised state at approximately -20 mV. A subsequent increase of $g_{\text{K,ATP}}$ to 140 pS causes the model to generate action potentials. The model stays in the oscillating state until $g_{\text{K,ATP}}$ reaches 303 pS. Between 303 pS and 390 pS the model enters a labile state at approximately -38 mV. This implies that electrical activity

Figure 5 Comparison of inactivation properties of the transient voltage-gated currents. (a) Voltage dependence of the steady-state inactivation of the Na^+ current in α -cells (black solid curve), δ -cells (black dotted curve) and simulated α -cell Na^+ current (red curve). The same voltage protocol as in Figure 1 was used. (b) Voltage dependence of the steady state inactivation of the T-type Ca^{2+} currents in α -cells (black) and in the simulation (red). The test-pulse to -30 mV was separated from the pre-pulse by 10 ms at -70 mV. (c) Voltage dependence of the steady-state inactivation of the transient K^+ currents in α -cells (black solid line) δ -cells (dotted line) and in the model (red). Same voltage protocol as b except test-pulse to -10 mV. As in Figure 4, the voltage range in which action potentials are generated is indicated by the shaded area.

can occur in our model when $g_{K,ATP}$ is between 303 pS and 72 pS. The labile state is observed only when $g_{K,ATP}$ is ramped from low to high values.

4.2 Inactivation Properties of the Voltage-Gated Channels Account for the Cessation of Electrical Activity at Low K_{ATP} Conductances

Figure 5 summarizes the inactivation properties of the voltage-gated currents forming the action potential in α -cells. As described, the experimental inactivation data were obtained by a standard two-pulse protocol [1], and fitted using sigmoidal threshold functions. The experimental data for the Na^+ (a), T-type Ca^{2+} (b) and transient K^+ currents (c) are shown as solid black lines. The inactivation curves for the simulated currents considered in the model are indicated in red. The dotted lines represent the inactivation curves for the corresponding currents in the δ -cell. The

grey shaded area from -60 mV to -35 mV indicates the voltage range in which electrical activity occurs (cf. Figure 4). It is clear that half maximal inactivation for both T-type Ca^{2+} and Na^{+} currents is reached in the middle of the active voltage range. At voltages above -35 mV, where electrical activity stops, Na^{+} currents are inactivated by approximately 80%. At this voltage, the T-type Ca^{2+} current is inactivated by approximately 90% in the α -cell and by 75% in the model. Thus, depolarisation due to closure of KATP channels leads to inactivation of the currents carrying the upstroke of the action potential and, hence, a depolarised state without action potentials. Considering the A-currents, maximal inactivation is reached at approximately -50 mV. In the depolarised state at -20 mV the remaining outward current is too small to hyperpolarise the cell, which would release the inward currents from inactivation. This implies that the A-current is hardly active when the α -cell fires action potentials from voltages more positive than -50 mV. However, this current might contribute to the shaping of the action potential when the action potentials originate from more negative potentials. This will be discussed further in section 4.4.

4.3 Role of the Individual Currents in Continuous Firing of Action Potentials

Figure 6a displays the time course of an action potential fired from a resting potential of -44 mV. The onset of the fast upstroke, the top of the action potential and the most negative potential after the action potential are indicated by dotted vertical lines. Figure 6b shows a simulation of the membrane potential at $g_{K,ATP} = 190$ pS. Under these conditions, the model continuously fires action potentials and the basal membrane potential does not fall below -40 mV. We refer to these action potentials as ‘depolarisation-induced action potentials.’ In the model, action potentials in this state peak at approximately 0 mV. As described above, continuous firing of action potentials is observed when $g_{K,ATP}$ is between 303 pS and 72 pS. As indicated in Figure 6c, each action potential is initiated by the onset of a T-type Ca^{2+} current that depolarises the cell until the L-type Ca^{2+} channels start to open. This accelerates the depolarisation to the point where the Na^{+} current takes off and produces the full action potential. The T-type Ca^{2+} current activates at voltages more positive than -60 mV, reaching half-maximal activation at -45 mV. Due to the steepness of the T-type activation curve, maximal open probability is already reached at approximately -30 mV (cf Figure 7). Therefore, further depolarisation during the upstroke of the action potential does not increase the T-type Ca^{2+} current. On the other hand, the slow depolarisation before the action potential allows the T-type Ca^{2+} currents to reach a steady state of activation. As a consequence, the function of the T-type current is to provide the basal depolarising conductance between the spikes while it contributes only marginally to the action potential itself. Opening of the L-type channels starts at -40 mV, accounting for most of the second part of the slow depolarisation. Due to the half maximal activation at -25 mV, 20 mV more positive than the value for the T-type currents, the L-type channels also contribute to the fast upstroke of the action potential. The Na^{+} currents are, as the L-type Ca^{2+} current, half-maximally activated at -25 mV, but because of the steepness of the activation curve, they only contribute to the fast upstroke of the action potential. The downstroke of the action potential is primarily due to the inactivation of the Na^{+} current, and simultaneous activation of the KDR channels, the latter being half-maximally

Figure 6 Depolarisation-induced action potentials in the α -cell and the model. (a) Action potential recorded from an α -cell during application of tolbutamide. The vertical lines indicate the onset of the action potential, the top of the action potential and the most negative membrane potential after repolarisation. (b) Simulation of depolarisation-induced action potentials. $g_{K,ATP}$ is set to 300 pS. (c) Development of the individual currents during the firing of the action potentials in panel b.

activated at -18 mV. Interestingly, the Na^+ current is active not only during the upstroke, but also during the down-stroke. The second maximum of the Na^+ current is due to channels leaving the inactivated state and entering the open state, thereby causing a deflection in the membrane potential. Both activation and inactivation of the Na^+ channels operate at a much faster time scale than the other voltage-gated channels. This suggests that the double maximum during the action potentials is unique to the Na^+ current. Inspection of Figure 6c shows that this is indeed the case.

4.4 Noise-induced Firing of Action Potentials

It is of vital importance that the α -cell can generate action potentials at low glucose concentrations. The main function of glucagon is to initiate the release of glucose from the liver, thus protecting the body from dangerously low blood sugar levels.

Figure 7 Voltage dependence of activation of the simulated voltage-gated currents. Steady-state open probability of the activation-gate (m^∞) for individual voltage-gated currents in the model as indicated by the colour coding.

The inset in Figure 8b shows a membrane potential recording from an α -cell in the absence of glucose. The resting potential is clearly more negative than -60 mV which is below the threshold for continuous firing of action potentials. In addition, there are long intervals between the individual action potentials. Due to the high input resistance of the α -cell ($0.92 - 2.6\text{ G}\Omega$) [2]) even small changes in the membrane currents will influence the membrane potential. The black trace in Figure 8a shows an action potential on an extended time scale originating from a more negative membrane potential than the action potential shown in Figure 6a, while the solid red curve shows a simulated action potential. In the model, an increase of the Na^+ conductance by 0.69 nS for 2 ms, corresponding to stochastic opening of approximately 30 Na^+ channels (assuming a single channel conductance of 23 pS [16]), is sufficient to trigger an action potential. Both the upstroke of the action potential and the subsequent plateau of the membrane potential at approximately -40 mV are simulated with good precision. The down-stroke of the action potential, on the other hand, is considerably slower in the model than in the experimental recordings. In addition, during the down-stroke of the action potential in Figures 6 and 8, the Na^+ current reactivates briefly. Clearly, although the properties of the Na^+ currents used in the model differ from those in real α -cells, the time course of the current, the current-voltage relationship of the peak Na^+ current, and the voltage dependence of inactivation are precisely reproduced by the simulated Na^+ current

Figure 8 α -cell and simulated noise-induced action potentials. (a) α -cell action potential originating from a hyperpolarised membrane potential (black) recorded in the absence of tolbutamide and glucose. The red solid line shows a simulated action potential triggered by a 2 ms increase of the Na^+ conductance by 690 pS. Holding the Na^+ conductance at 0 nS when the Na^+ current is maximally inactivated removes the shoulder during the down-stroke (red dotted line). The vertical lines indicate the onset and end of the shoulder caused by reopening of the Na^+ channels. (b) Changes in Na^+ -, K_{ATP} and A-currents during the simulated action potentials in (a). The solid red line shows development of the Na^+ current. The sudden increase of the Na^+ current before the action potential is due to the 2 ms increase of the Na^+ conductance. The dotted line indicates the Na^+ current when the Na^+ conductance was set to 0 nS as indicated by the horizontal lines. (c) KDR, T- and L-type Ca^{2+} and leak currents during the action potentials simulated in (a) as indicated. Note the different current scales. The inset shows the membrane potential of an α -cell recorded in the presence of 10 mM glucose (upper) and after removal of the sugar (lower). The action potentials originate from a membrane potential below -60 mV and are fired irregularly. In the presence of 10 mM glucose the cell is depolarised but does not generate action potentials.

(cf. Figure 1). As expected, eliminating the Na^+ current after the first maximum during the action potential (Figure 8b, dotted line) removes the shoulder during the down-stroke and increases the duration of the depolarised phase after the action potential. The resulting simulated action potential shown in Figure 8a (dotted curve) resembles more accurately the shape of the α -cell action potential. Unlike what we observed during the depolarisation-induced action potentials in Figure 6, the A-current is activated and actually contributes to the down-stroke of the action potential (Figure 8b).

In the previous sections we showed that action potentials can be produced in two ways: First, the depolarisation-induced action potentials: setting $g_{\text{K,ATP}}$ to values between 72 pS and 303 pS leads to repetitive firing of action potentials. Second, the noise-induced action potentials: due to the high input resistance of the α -cell, action

potentials can be triggered by noise even when $g_{K,ATP}$ is higher than 303 pS where the membrane potential is otherwise hyperpolarised and spontaneous firing of action potentials does not take place. Although the A-current is inactivated when the α -cell is firing depolarisation-induced action potentials (Figures 5, 6), this current might play a role during noise-induced action potentials.

5 Adjustments of the α -cell Model to Represent Pancreatic δ -cells

Somatostatin-secreting δ -cells, which are also present in the pancreatic islets, have an array of ion channels very similar to those of the α -cell. The δ -cells express KATP channels, voltage-gated Ca^{2+} channels, Na^+ channels, KDR channels and a transient K^+ current [1]. Similarly to action potentials fired in α -cells, the δ -cell action potentials are primarily carried by the voltage-gated Na^+ currents. However, the glucose response of δ -cells resembles that of the β -cell in being electrically active at high glucose concentrations and silent at low. Whereas the voltage dependence of the activation of the voltage-gated channels is similar in α -cells and δ -cells, the voltage dependence of the inactivation differs for the Na^+ and transient K^+ currents [1]. In order to validate our hypothesis concerning the regulation of electrical activity in α -cells, we converted our α -cell model into a δ -cell model by adjusting, primarily, the inactivation parameters. The differences between the α -cell and δ -cell models are summarised in Table I. Since δ -cells do not express transient Ca^{2+} channels, the T-type channels were removed from the model. Figure 9a shows the membrane potential recorded from a δ -cell in the absence (left) and presence (right) of 10 mM glucose. In the absence of glucose, the δ -cell is hyperpolarised to approximately -80 mV. Addition of 10 mM glucose depolarises the cell by closing the KATP channels, leading to continuous firing of action potentials originating from -40 mV. Adjusting the α -cell model parameters to δ -cell values removes the depolarised plateau at low KATP conductances. Instead, the model responds with depolarisation upon reduction of the KATP conductance until electrical activity is initiated when $g_{K,ATP}$ reaches approximately 92 pS. Action potentials are generated even after complete closure of the KATP channels (Figure 9b).

This concept opens the door for another mode of regulation: electrical activity might not only be linked to glucose via the KATP-channels and the resting potential.

Table I Comparison of α -cell and β -cell parameters.

	α -cell	β -cell
m_{Na,V^∞}	sig (V, -25 mV, 4 mV)	sig (V, 17.6 mV, 5.5 mV)
h_{Na,V^∞}	sig (V, -47 mV, -8 mV)	sig (V, -28 mV, -7 mV)
V_{Na}	110 mV 70 mV	
g_{Ca,T^*}	1 nS	0 nS
m_{K,A^∞}	sig (V, 0 mV, 10 mV)	sig (V, 16 mV, 15 mV)
h_{K,A^∞}	sig (V, -68 mV, -4 mV)	sig (V, -34 mV, -4 mV)
g_{K,A^*}	20 nS	6 nS
g_{Leak^*}	0.2 nS	0.6 nS

Figure 9 Shifting the inactivation parameters of the α -cell model to more positive voltages results in δ -cell-like KATP responsiveness (a) Membrane potential of a δ -cell recorded in the presence (*right*) and absence (*left*) of 10 mM glucose. (b) Simulation of the δ -cell membrane potential in response to changes of $g_{K,ATP}$. The inactivation parameters of the voltage-gated currents were adjusted to δ -cell values as listed in Table 1. During the simulation $g_{K,ATP}$ was reduced from 0.5 nS to 0 nS as indicated below the membrane potential trace.

Glucose could also have small effects on the inactivation properties of the voltage-gated currents and regulate the firing of action potentials not only via the resting conductance. Future experiments will reveal whether the gating of the Na^+ and T-type Ca^{2+} -currents is affected by changes in the glucose concentration.

6 Discussion

6.1 Fitting the Model

We have recently characterised the ion channels present in glucagons-secreting pancreatic α -cells and proposed a mechanism by which glucose suppresses glucagon release via closure of the KATP channels in the α -cell [2]. In the present paper we made use of the experimental data to establish a mathematical model for the electrical activity in α -cells. In order to prove our hypothesis concerning the regulation of the electrical activity it was necessary to stay as close as possible to the experimental data for the individual currents. In addition, only a model based on relevant data could be used to point out shortcomings in our knowledge about the system in question and in this way serve as a guide for future experiments. The inevitable divergence between simulated and experimentally recorded membrane potential traces resulting from this approach was not so much interpreted as an

indication of a poor model as an indication of the need to implement additional physiological mechanisms.

The modelling approach was to fit the parameters of the individual currents to the experimental results as accurately as possible, and subsequently to use the simulated currents to reproduce the independently recorded membrane potential from α -cells. It is clear that the experimental characterisation of the currents contributing to the membrane potential of the α -cell cannot be complete. In order to obtain regenerative electrical activity in the model, some parameter values were chosen that resulted in individual currents slightly outside the experimental error bars. In all relevant figures, the simulated current traces versus time were scaled by a factor in order to match the corresponding experimental traces. This approach facilitated comparison of the time courses, and was justified by the fact that even if the number of channel proteins varies in different cells, the voltage and time dependence of each single channel remains constant. Therefore, the current traces of different cells should coincide when scaled by a factor depending on the number of channels present in each cell. Inspection of the I–V curves, which were not scaled but plotted as they were produced by the model, shows that the amplitude of the currents implemented in the model are almost entirely within the error bars of the experimental data.

6.2 Model Results

The most important result obtained so far with using the simulation model is the validation of a novel theory for the KATP-dependent regulation of electrical activity in pancreatic α -cells described in the previous sections and [1]. A similar process has recently been reported for skeletal muscle cells. In an animal model for critical illness myopathy it was observed that excitability of muscle cells is reduced due to inactivation of the voltage-gated Na^+ currents [17]. It was also demonstrated that this reduction is due to a shift towards more negative voltages of the voltage-dependent inactivation, and that hyperpolarisation of the cells resulted in stronger excitability. Moreover, excitability was lost when the cells were depolarised to voltages where approximately 70% of the Na^+ channels were inactivated.

To our knowledge, this mechanism, using depolarisation in response to physiological stimuli to turn off electrical activity, represents a new mechanism for the regulation of action potential firing and has not been reported in other cells. Our model explains several apparently contradictory experimental findings. Glucose and tolbutamide have both been reported to inhibit [18–20], to increase [21], and to have no effect at all [22] on glucagon secretion, illustrating that the response of the α -cell depends strongly on the experimental conditions. In addition, perfused rat pancreas was reported to respond with a transient bout of glucagon release when tolbutamide was added, but only in the complete absence of glucose [23]. All of these findings can be explained as follows: under normal conditions, maximal stimulation of glucagon secretion is reached in the absence of glucose and the membrane potential of the α -cell is within the range where electrical activity is generated. Any experimental perturbation of the cell, e.g., compromising the cell metabolism with a subsequent reduction of the KATP-ratio, could lead to an exceptional high KATP conductance and hence hyperpolarise the cells corresponding to KATP conductances higher than 303 pS in our model. Under these conditions, addition of glucose or tolbutamide will

lead to a transient release of glucagon as predicted by the simulation of electrical activity (Figure 4).

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Appendix

A Model Parameters

The basic framework of the mathematical model was introduced in the Methods section. Tables II–VI list the parameter values of the seven currents of the model. Two functions, a sigmoidal (sig) and a bell shaped (bell) function, constitute the building blocks of the voltage-gated currents. They are defined as follows:

$$\begin{aligned}\text{sig}(V, V^*, S^*) &= \frac{1}{1 + \exp\left(\frac{-(V-V^*)}{S^*}\right)} \\ \text{bell}(V, V^*, S^*, \tau^*, \tau_0) &= \frac{\tau^*}{\exp\left(\frac{-(V-V^*)}{S^*}\right) + \exp\left(\frac{(V-V^*)}{S^*}\right)} + \tau_0.\end{aligned}\quad (5)$$

Both the sigmoidal and the bell-shaped functions depend on the parameters V^* and S^* . V^* defines the inflection point of the antisymmetric sigmoid and the center of the symmetric bell-shaped function, while S^* is a slope parameter that expresses the steepness of the sigmoidal and bell-shaped functions. The maximal slope of the two functions is proportional to $\frac{1}{S^*}$. τ_0 is the asymptotic value of the bell-shaped function for infinite V , while the maximum of bell is $\frac{\tau_0 + \tau^*}{2}$.

m_i^∞ and h_i^∞ are the steady-state activation and inactivation of the voltage-gated channel i , respectively, and are functions of the membrane potential. However, the actual states of the voltage-gated channels are controlled by the time constants τ_i^m and τ_i^h :

$$\begin{aligned}\frac{dm_i}{dt} &= \frac{m_i^\infty(V) - m_i}{\tau_i^m(V)} \\ \frac{dh_i}{dt} &= \frac{h_i^\infty(V) - h_i}{\tau_i^h(V)}\end{aligned}\quad (6)$$

The ATP-dependent potassium channel and the leak channel in Table IV are considered to be passive charge carriers and therefore functions of the membrane potential. The value of $g_{K,ATP}$ given in the table corresponds to an active (i.e., low glucose concentration) state of the model.

Table II Default parameters for the inward ion currents of the α -cell model. The functions sig and bell are defined in Eq. (5).

$$\begin{aligned} I_{Ca,L} &= g_{Ca,L}^* m_{Ca,L}^2 h_{Ca,L} (V - V_{Ca}) \\ g_{Ca,L}^* &= 1 \text{ nS} \\ m_{Ca,L}^\infty(V) &= (sig)(V, -25 \text{ mV}, 10 \text{ mV}) \\ h_{Ca,L}^\infty(V) &= (sig)(V, -28 \text{ mV}, -5 \text{ mV}) \\ \tau_{Ca,L}^m(V) &= (bell)(V, -23 \text{ mV}, 20 \text{ mV}, 1 \text{ ms}, 0.05 \text{ ms}) \\ \tau_{Ca,L}^h(V) &= (bell)(V, 0 \text{ mV}, 20 \text{ mV}, 30 \text{ ms}, 21 \text{ ms}) \end{aligned}$$

$$\begin{aligned} I_{Ca,T} &= g_{Ca,T}^* m_{Ca,T}^3 h_{Ca,T} (V - V_{Ca}) \\ g_{Ca,T}^* &= 1 \text{ nS} \\ m_{Ca,T}^\infty(V) &= (sig)(V, -44 \text{ mV}, 4 \text{ mV}) \\ h_{Ca,T}^\infty(V) &= (sig)(V, -46 \text{ mV}, -5 \text{ mV}) \\ \tau_{Ca,T}^m(V) &= (bell)(V, -50 \text{ mV}, 12 \text{ mV}, 40 \text{ ms}, 0 \text{ ms}) \\ \tau_{Ca,T}^h(V) &= (bell)(V, -50 \text{ mV}, 15 \text{ mV}, 20 \text{ ms}, 5 \text{ ms}) \end{aligned}$$

$$\begin{aligned} I_{Na,V} &= g_{Na,V}^* m_{Na,V}^3 h_{Na,V} (V - V_{Na}) \\ g_{Na,V}^* &= 10 \text{ nS} \\ m_{Na,V}^\infty(V) &= (sig)(V, -25 \text{ mV}, 4 \text{ mV}) \\ h_{Na,V}^\infty(V) &= (sig)(V, -47 \text{ mV}, -8 \text{ mV}) \\ \tau_{Na,V}^m(V) &= (bell)(V, -50 \text{ mV}, 10 \text{ mV}, 6 \text{ ms}, 0.05 \text{ ms}) \\ \tau_{Na,V}^h(V) &= (bell)(V, -50 \text{ mV}, 8 \text{ mV}, 80 \text{ ms}, 0.5 \text{ ms}) \end{aligned}$$

Table III Default parameters for the voltage-gated outward ion currents of the α -cell model. The functions sig and bell are defined in Eq. (5).

$$\begin{aligned} I_{K,A} &= g_{K,A}^* m_{K,A} h_{K,A} (V - V_K) \\ g_{K,A}^* &= 20 \text{ nS} \\ m_{K,A}^\infty(V) &= (sig)(V, 0 \text{ mV}, 10 \text{ mV}) \\ h_{K,A}^\infty(V) &= (sig)(V, -68 \text{ mV}, -4 \text{ mV}) \\ \tau_{K,A}^m(V) &= 0.5 \text{ ms} \\ \tau_{K,A}^h(V) &= (bell)(V, 5 \text{ mV}, 20 \text{ mV}, 30 \text{ ms}, 21 \text{ ms}) \end{aligned}$$

$$\begin{aligned} I_{K,DR} &= g_{K,DR}^* m_{K,DR}^4 (V - V_K) \\ g_{K,DR}^* &= 4.9 \text{ nS} \\ m_{K,DR}^\infty(V) &= (sig)(V, -18.5 \text{ mV}, 23 \text{ mV}) \\ \tau_{K,DR}^m(V) &= (bell)(V, -10 \text{ mV}, 25 \text{ mV}, 1.5 \text{ ms}, 15 \text{ ms}) \end{aligned}$$

Table IV Default parameters for the ohmic ion currents of the α -cell model. The functions sig and bell are defined in Eq. (5).

$$\begin{aligned} I_{K,ATP} &= g_{K,ATP}^* (V - V_K) \\ g_{K,ATP}^* &= 0.2 \text{ nS} \\ I_{Leak} &= g_{Leak}^* (V - V_{Leak}) \\ g_{Leak}^* &= 0.2 \text{ nS} \end{aligned}$$

Table V Reversal potentials for ion currents carried by sodium (V_{Na}), calcium (V_{Ca}), potassium (V_K), and the compound leak current (V_{Leak}).

$$\begin{aligned} V_{Na} &= 110 \text{ mV} & V_{Ca} &= 60 \text{ mV} \\ V_K &= -80 \text{ mV} & V_{Leak} &= -20 \text{ mV} \end{aligned}$$

Table VI Capacitance of the α -cell plasma membrane.

$$c_m = 5,000 \text{ fF}$$

References

- Göpel, S., Kanno, T., Barg, S., Weng, X.-G., Gromada, J., Rorsman, P.: Regulation of glucagon release in mouse α -cells by k_{ATP} channels and inactivation of TTX-sensitive Na^+ . *J. Physiol.* **528**(3), 509–520 (2000)
- Göpel, S.O., Kanno, T., Barg, S., Rorsman, P.: Patch-clamp characterisation of somatostatin-secreting δ -cells in intact mouse pancreatic islets. *J. Physiol.* **528**(3), 497–507 (2000)
- Gromada, J., Bokvist, K., Ding, W.G., Barg, S., Buschard, K., Renstrom, E., Rorsman, P.: Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca^{2+} current and the number of granules close to the L-type Ca^{2+} channels. *J. Gen. Physiol.* **110**, 217–228 (1997)
- Rorsman, P., Hellman, B.: Voltage-activated currents in guinea pig pancreatic α_2 cells. evidence for Ca^{2+} -dependent action potentials. *Br. J. Pharmacol.* **91**, 223–242 (1988)
- Berts, A., Ball, A., Gylfe, E., Hellman, B.: Suppression of Ca^{++} oscillations in glucagon-producing α_2 -cells by insulin/glucose and amino acids. *Biochim. Biophys. Acta* **1310**, 212–216 (1996)
- Nadal, A., Quesada, I., Soria, B.: Homologous and heterologous asynchronicity between identified α -, β - and δ -cells within intact islets of Langerhans in the mouse. *J. Physiol.* **517**, 85–93 (1999)
- Bokvist, K., Olsen, H.L., Hoy, M., Gottfredsen, C.F., Holmes, W.F., Buschard, K., Rorsman, P., Gromada, J.: Characterization of sulfonylurea and ATP-regulated K^+ channels in rat pancreatic A-cells. *Pflügers Arch.* **438**(4), 428–436 (1999)
- Rajan, A.S., Aguilar-Bryan, L., Nelson, D.A., Nichols, C.G., Wechsler, S.W., Lechago, J., Bryan, J.: Sulfonylurea receptors and ATP-sensitive K^+ channels in clonal pancreatic alpha cells. evidence for two high-affinity sulfonylurea receptors. *J. Biol. Chem.* **268**(20), 15221–15228 (1993)
- Detimary, P., Dejonghe, S., Ling, Z., Pipeleers, D., Schuit, F., Henquin, J.: The changes in adenine nucleotides measured in glucose-stimulated rodent islets occur in β cells but not in α cells and are also observed in human islets. *J. Biol. Chem.* **273**, 33905–33908 (1998)
- Östenson, C.G., Agren, A., Brolin, S.E., Petersson, B.: Adenine nucleotide concentrations in A2-cell rich and normal pancreatic islets from guinea pig. *Diabetes Metab.* **6**, 5–11 (1980)
- Bertram, R., Previte, J., Sherman, A., Kinard, T.A., Satin, L.S.: The phantom burster model for pancreatic beta-cells. *Biophys. J.* **79**(6), 2880–2892 (2000)
- Chay, T.R., Keizer, J.: Minimal model for membrane oscillations in the pancreatic beta-cell. *Biophys. J.* **42**(2), 181–190 (1983)
- Hille, B.: *Ion Channels of Excitable Membranes*. Sinauer Associates, Sunderland, Massachusetts (2001)
- Macianskiene, R., Viappiani, S.: Slowing of the inactivation of cardiac voltage-dependent sodium channels by the amiodarone derivative 2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl) benzofuran (kb130015). *J. Pharmacol. Exp. Ther.* **304**, 130–138 (2003)
- Ronner, P., Matschinsky, F.M., Hang, T.L., Epstein, A.J., Buettger, C.: Sulfonylurea-binding sites and ATP-sensitive K^+ channels in alpha-tc glucagonoma and beta-tc insulinoma cells.
- Conley, E.C., Brammar, W.J.: *The Ion Channel Facts Book: Voltage-gated Channels* (volume 4). Academic, San Diego, California (1999)
- Rich, M.M., Pinter, M.J.: Sodium channel inactivation in an animal model of acute quadriplegic myopathy. *Ann. Neurol.* **50**, 26–33 (2001)
- Massi-Benedetti, F., Flaorni, A., Luyckx, A., Lefebvre, P.: Inhibition of glucagon secretion in the human newborn by simultaneous administration of glucose and insulin. *Horm. Metab. Res.* **6**, 392–396 (1974)
- Samols, E., Tyler, J.M., Mialhe, P.: Suppression of pancreatic glucagon release by the hypoglycaemic sulphonylureas. *Lancet* **7587**, 174–176 (1969)
- Vance, J.E., Buchanan, K.: Interrelationship between glucagon and insulin release from isolated islets of Langerhans. *Diabetes* **17**, 311–312 (1968)
- Samols, E., Tyler, J.M., Marks, V.: Stimulation of glucagon secretion by oral glucose. *Lancet* **7425**, 1257–1259 (1965)
- Pek, S., Fajans, S.S., Floyd, J.C., Knopf, R.F., Conn, J.W.: Failure of sulfonylureas to suppress plasma glucagon in man. *Diabetes* **21**, 216–223 (1972)
- Loubatieres, A.L., Loubatieres-Mariani, M.M., Alric, R., Ribes, G.: Tolbutamide and glucagon secretion. *Diabetologia* **10**(4), 271–276 (1974) *Diabetes* **42**(12), 1760–1772 (1993)