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Inwardly rectifying Kir2.1 currents in human β-cells control electrical activity: Characterisation and mathematical modelling

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ABSTRACT

Pancreatic β-cells fire action potentials as do cardiac cells and neurons, and electrical activity plays a central role in glucose-stimulated insulin secretion, which is disturbed in diabetes. The inwardly rectifying Kir2.1 potassium channels (KCNJ2 gene) control cardiac electrical activity by stabilising the interspike interval. Loss-of-function abnormalities in cardiac Kir2.1 currents can lead to the long QT syndrome and alterations of cardiac excitability, and patients with some forms of long QT syndrome suffer from over-secretion of insulin, hyperinsulinemia and symptomatic hypoglycemia. The KCNJ2 gene is also expressed in human pancreatic islets, and we show that functional Kir2.1 currents are present in human β-cells. We characterised the human Kir2.1 β-cell current, and included it in a recent mathematical model of electrical activity in human β-cells. Based on our simulations we propose that Kir2.1 currents control the interspike interval, and predict that blocking Kir2.1 channels increases the action potential frequency, which should augment the rate of insulin secretion. Vice versa, the model suggests that hyperactive Kir2.1 channels may lead to reduced insulin secretion. Our findings provide a putative link between increased insulin secretion and the long QT syndrome, and give novel insight into normal and disturbed β-cell function.

1. Introduction

Electrical activity plays a central role in glucose-stimulated insulin secretion from human pancreatic β-cells. Glucose metabolism increases the ATP/ADP ratio, which closes ATP-sensitive potassium channels (K(ATP)-channels). As a consequence other currents can depolarize the cell, leading to action potential (AP) firing and Ca^{2+}-influx through voltage-gated calcium channels. The resulting increase in intracellular calcium leads to insulin release by Ca^{2+}-dependent exocytosis [1–4].

Inwardly rectifying Kir2.1 potassium channels are well-known to control cardiac electrical activity, e.g., by stabilizing the interspike interval [5]. The KCNJ2 gene coding for Kir2.1 channels is widely expressed [6], also in human pancreatic islets and β-cells (Beta-Cell Gene Atlas, www.t1dbase.org/page/AtlasHome, [7]). Hence, Kir2.1 currents might play a role in shaping action potential firing in human β-cells. Interestingly, long QT syndrome can be due to loss of functionality in cardiac Kir2.1 channels [5], and recently, it was reported that some patients with the KCNQ1 long QT syndrome suffer from increased insulin secretion, hyperinsulinemia and symptomatic hypoglycemia [8]. This finding suggests a possible link between loss-of-function abnormalities of Kir2.1 channels, which can cause KCNJ2 long QT syndrome, and increased insulin secretion. Here we investigate the contribution of Kir2.1 currents to electrical activity in human β-cells using a combination of patch-clamp experiments and mathematical modelling.

Mathematical modelling has played important roles in studying the dynamics of electrical activity in rodent β-cells [9,10]. We recently developed a mathematical model of electrical activity in human β-cells to obtain insight in heterogeneous and sometimes non-intuitive electrophysiological responses in human β-cells [11,12]. Of note, our model tended to fire APs too rapidly compared to experiments, indicating that the model was still incomplete, i.e.,

Abbreviations: K(ATP), ATP-sensitive potassium (channels, current); AP, action potential; *V*, membrane potential; I–V, current/voltage (relationship); AMPK, 5′ adenosine monophosphate-activated protein kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate.

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important human β-cell currents were still missing from the model. Since the Kir2.1 current tends to stabilize the interspike potential in cardiomyocytes and neurons, it might improve the performance of the model concerning AP frequency. We thus extend our previously published model of electrical activity in human β-cells [12] by including Kir2.1 channels with conductance and current/voltage relationship fitted to the experimental characterisation of human β-cell inwardly rectifying Kir2.1 currents presented here.

2. Materials and methods

2.1. Experiments

Human pancreatic islets were obtained with ethical approval and clinical consent from non-diabetic organ donors. All studies were approved by the Human Research Ethics Board at the University of Alberta. The islets were dispersed into single cells by incubation in Ca^{2+} free buffer and plated onto 35 mm plastic Petri dishes. The cells were incubated in RPMI 1640 culture medium containing 7.5 mM glucose for >24 h prior to the experiments. Patch-pipettes were pulled from borosilicate glass to a tip resistance of 6–9 MΩ when filled with intracellular solution. Potassium currents were measured with the patch-clamp technique in the whole-cell configuration, using an EPC-10 amplifier and Patchmaster software (HEKA, Lambrecht, Germany). The cells were constantly perifused with heated bath solution during the experiment to maintain a temperature of 31–33 °C. The extracellular solution consisted of (in mM) 140 NaCl, 3.6 KCl, 0.5 $MgSO_4$, 1.5 $CaCl_2$, 10 HEPES, 0.5 NaH_2PO_4, 5 NaHCO3 and 6 glucose (pH was adjusted to 7.4 with NaOH). The pipette solution consisted of (mM) 130 KCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 10 EGTA, 3.0 MgATP, 0.1 Na^+-GTP (pH 7.2 with KOH). β-cells were identified by immunostaining or by size when immunostaining was not possible (cell capacitance >6 pF, [3]).

2.2. Modelling

We build on the previously published Hodgkin-Huxley type model for human β-cells [11,12]. We include Kir2.1 channels in the model.

The membrane potential V (measured in mV) develops in time (measured in ms) according to

$$dV/dt = -(I_{SK} + I_{BK} + I_{Kv} + I_{Kir} + I_{Na} + I_{CaL} + I_{CaPQ} + I_{CaT} + I_{KATP} + I_{leak}). \quad (1)$$

All currents (measured in pA/pF), except the Kir2.1 current I_{Kir}, are modelled as in Refs. [12], where expressions and parameters can be found.

The Kir2.1 current I_{Kir} was assumed not to inactivate and to activate instantaneously in response to hyperpolarisation. Activation was modelled with a Boltzmann function, i.e.,

$$I_{Kir} = g_{Kir}(V - V_K)/(1 + \exp\{(V - V_{mKir})/n_{mKir}\}). \quad (2)$$

To model the data, we first fixed the K^+ reversal potential to $V_K = -85$ mV based on visual inspection of the data and the theoretically calculated value $V_K \approx -83$ mV. The other three parameters, g_{Kir}, V_{mKir}, and n_{mKir}, were then obtained by fitting the current expression in Eq. (2) to the I–V data in Fig. 1B. Since the perforated-patch recordings of electrical activity [3,13,14] are typically performed with intra- and extra-cellular K^+ concentrations different from the ones used for the Kir2.1 characterisation, the value of V_K was shifted to −75 mV in our model simulations [11,12]. The intra- and extra-cellular K^+ concentrations modify not only V_K, but also the location parameter V_{mKir}, which was estimated to $V_{mKir} = -100$ mV based on Fig. 1B. Because a 10 mV shift in V_K corresponds to a nearly identical shift in V_{mKir} [15], we used $V_{mKir} = -90$ mV for the simulations of electrical activity. The maximal conductance $g_{Kir} = 1$ nS ($g_{Kir} = 0.1$ nS/pF when normalized to cell capacitance ~10 pF [3]) and the slope parameter $n_{mKir} = 15$ mV were not changed from their estimated values.

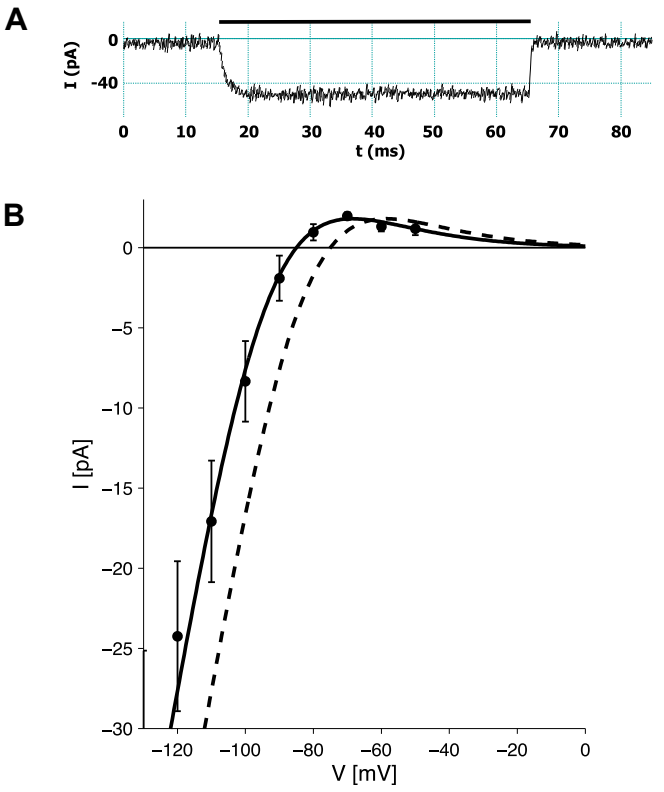

Fig. 1. A: Example trace of the tolbutamide-insensitive, barium-sensitive K^+ current in a human β-cell in response to a 50 ms depolarisation from −70 mV to −120 mV (black bar). B: Current-voltage relationship of the inwardly rectifying (Kir2.1) K^+ currents in human β-cells (dots and error bars indicate means and standard errors). The full curve shows a fit of Eq. (2) to the data points. This curve is right shifted to yield the dashed curve, which is used for the simulations of electrical activity, as explained in the Methods.

Simulations were done in XPPAUT [16] with the cvode solver. Computer code can be downloaded from http://www.dei.unipd.it/~pedersen.

3. Results

3.1. Experimental characterisation of Kir2.1 currents in human β-cells

The KCNJ2 gene coding for Kir2.1 channels is expressed in human islets and β-cells (Beta-Cell Gene Atlas, www.t1dbase.org/page/AtlasHome, [7]). Whole-cell voltage-clamp experiments were performed to investigate the Kir2.1 current/voltage (I–V) relationship in human β-cells. We found experimental evidence of a tolbutamide-insensitive, barium-sensitive K^+ current, which activated rapidly and showed little evidence of inactivation (Fig. 1A). The current developed at voltages close to the resting potential of human β-cells and at more hyperpolarized voltages, but depolarising pulses deactivated the current (Fig. 1B). These characteristics are typical of Kir2.x currents [17,18]. Further, Kir2.1 siRNA knockdown reduced the tolbutamide-insensitive, barium-sensitive K^+

current by ~70% (M. Braun, unpublished). In summary, our data show that human β-cells express functional inwardly rectifying Kir2.1 channels.

3.2. Mathematical modelling of the role of Kir2.1 currents in human β-cells

We included the Kir2.1 current in our previous model of human β-cells [12] based on the I–V curve found experimentally (Fig. 1B). We took into account that the intra- and extracellular K+ concentrations typically are different in the perforated patch experiments measuring electrical activity [3,13,14], compared to the whole-cell recordings of K^+ currents presented in Fig. 1. These changes of K^+ concentrations modify not only the Nernst K^+ potential V_K, but also the activation curve of the Kir2.1 current, which is right-shifted to the same extent as V_K, resulting in a characteristic 'cross-over' phenomenon (Fig. 1B; [15,18]).

Our previous version of the model tended to fire action potentials too rapidly (~5 Hz [12] vs. 1–3 Hz seen experimentally [13,14]). Our simulations showed that the Kir2.1 current stabilises the interspike interval, thus reducing the action potential firing frequency, compared to our previous version of the model (Fig. 2). With the Kir2.1 conductance estimated from data, the firing frequency is ~3 Hz, within the range of experimentally observed frequencies [13,14]. Blocking the Kir2.1 channels accelerates electrical activity, while increased Kir2.1 conductance reduces the AP firing frequency, and even abolishes spiking activity at high values (Fig. 2).

4. Discussion

Ion channels are widely expressed, but depending on the cell type they can have different roles in shaping electrical activity. Channelopathies, i.e., abnormalities in certain ion channels, might therefore affect function of several tissues. The long QT syndrome can be caused by loss-of-function in potassium channels such as the Kv7.1 channels (KCNQ1 gene) or – more rarely – the Kir2.1 channels (KCNJ2 gene) investigated here [5]. Interestingly, patients with the relatively frequent KCNQ1 long QT syndrome exhibit hyperinsulinemia caused by increased secretion of insulin [8].

Here, we characterised Kir2.1 currents in human β-cells. Besides the Kir2.1 coding gene KNCJ2, the genes KCNJ4 (Kir2.3) and, possibly, KCNJ12 (Kir2.2) are also expressed in human pancreatic islets and β-cells (Beta-Cell Gene Atlas, www.t1dbase.org/page/AtlasHome, [7]). Our data cannot rule out a contribution from Kir2.2 and Kir2.3 currents since they have I–V characteristics similar to the Kir2.1 current [19]. However, siRNA against Kir2.1 removed most of the inwardly rectifying K^+ current (M. Braun, unpublished), leading us to suggest that the observed current is due to Kir2.1 channels.

The inclusion of the Kir2.1 current in our model [12] resulted in a clear improvement of the model behaviour compared to the previous version; in particular, the new model exhibited slower spiking dynamics with frequency comparable to experiments [13,14]. Of note, the similarity between Kir2.x currents [19] means that the model results are valid also in case Kir2.2 and Kir2.3 channels contribute to the inwardly rectifying current. Our model simulations predicted that KCNJ2 long QT patients, who have reduced Kir2.1 currents, might show increased insulin secretion. Further, hyperactive Kir2.1 channels were predicted to decrease insulin release. We know of no data linking mutations in KCNJ2 to hyperinsulinemia, diabetes or impaired glucose tolerance, but our theoretical results suggest that such connections may exist. Genetic screening could test this hypothesis.

In summary, we have shown that human β-cells exhibit functional Kir2.1 currents. Simulations with our detailed and carefully constructed mathematical model of human β-cell electrophysiology predicted that Kir2.1 currents might play important roles in controlling insulin secretion in health and disease, and we speculate that KCNJ2 cardiac channelopathies may result in disturbed insulin secretion. Kir2.1 channels are regulated by components such as AMPK [20], which plays important roles in β-cells [21], and by PIP2 [22], itself a dynamic variable in β-cells [23]. Clearly, further studies should aim to clarify the role and control of Kir2.1 channels in β-cells.

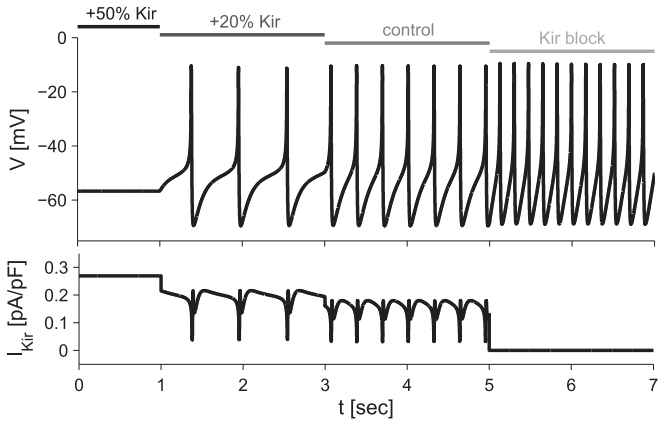

Fig. 2. Simulation of electrical activity in human β-cells with varying size of Kir2.1 currents as indicated above the bars. 'Control' corresponds to the data fit in Fig. 1B and corresponding parameters given in Materials and Methods. The case 'Kir block' (g_{Kir} = 0 nS/pF) corresponds to the previous version of our model [12].

Authors' contributions

MR developed the model, performed simulations, prepared figures, participated in the design of the study and contributed to drafting the manuscript; MB designed experiments, performed experiments, and analysed data; XW performed experiments; MGP designed the modelling study, participated to the development of the model, and wrote the manuscript.

Conflict of interest

None.

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