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Short Communication

Glucose-sensitivity of the afterhyperpolarization potential: Role of SK1 channel in insulin-secreting cells

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ABSTRACT

The role of the small-conductance, calcium-activated SK potassium channel in regulating pancreatic β cell function remains controversial with conflicting pharmacological results. In this study, we used current clamp recordings to further characterize the function of SK channels in INS-1 cell line. We compared afterhyperpolarization potential (AHP) responses of SK1-downregulated cells with those of control INS-1 cells. They were tested with and without the presence of glucose. We found that cells in which SK1 channel subunit expression had been downregulated exhibited AHPs in the presence of 20 mM glucose while control INS-1 cells had AHPs only in the absence of glucose. Our findings show that the glucose-dependence of the AHP in the rat INS-1 cell line depends only on SK1 channel subunit expression.

1. Introduction

Pancreatic β cells utilize complex interactions of ion channels to respond to glucose concentrations >7 mM with release of insulin (see reviews: [10,14,18]). Members of the potassium channel family play a significant role in regulating β cells' electrical properties, especially the ATP-sensitive K^+ (K_{ATP}) channels which set the β cell resting membrane potential (∼−70 mV). These channels are inhibited by increases in the concentration of ATP produced by glucose metabolism. Inhibition of the K_{ATP} channels depolarizes the β cell membrane [2,4,20]. The voltage-gated K^+ channels (Kv) are equally important in defining the electrical properties of β cells. They respond to membrane depolarization and are responsible for mediating action potential repolarization [21]. Pharmacological inhibition [6] or genetic deletion of Kv channels [8] leads to enhanced insulin secretion.

Calcium-activated potassium channels have also been identified in β cells, though their role is controversial and warrants further investigation. An earlier study of the large-conductance, calcium-activated potassium channel (BK) suggests that it does not play a role in regulating the electrical activity of the β cell [12]; however, more recent studies demonstrated that BK channels control the amplitude of β cell action potentials [7,9]. Similarly, the role of calcium-activated, small conductance potassium channels (SK) in regulating β cell electrical activities is controversial. Initial studies showed β cells to be insensitive to apamin [5,11,13], a specific blocker of SK channels, while other studies showed that blockade of these channel types has an effect in promoting insulin secretion under certain conditions [1,9,23,26].

Two studies have identified the expression of the apamin-sensitive SK channel subtypes (SK1, SK2, and SK3) in primary β cells and β cell lines [1,23]. SK channels, as also do *Shaker*-like voltage-gated potassium channels, have a tetrameric structure that results from the assembly of four subunits [15]. SK channels can form homotetrameric channels (with identical subunits) or heterotetrameric channels (with different SK channel subunits). In a previous study [1], we found that down-regulating SK1 channel subunit expression results in a β cell line that lacks the AHP and these modified cells have depolarized resting membrane potential and longer durations of action potentials induced artificially by 5 ms 100 pA current injections. These down-regulated β cell lines also exhibited constitutive insulin secretion even in the absence of glucose.

In this study, we further investigate the role of SK channels in the INS-1 cells. This work again uses the SK1-downregulated cells as an *in vitro* model for studying the functional role of the SK1 channel subunit in β cells. Here, we find that expression of the SK1 channel subunit is crucial to linking β cell AHP responses to glucose levels.

2. Methods

2.1. Cell culture

Rat insulinoma INS-1 cells were cultured on culture flask in RPMI-1640 medium (Hyclone, Logan, UT) and incubated at 37 °C

Abbreviation: AHP, afterhyperpolarization.

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and at 5% $C0_2$ conditions. Cells were supplemented with 10% (v/v) FBS, 10 mM HEPES, 2 mM l-alanyl-l-glutamine, 50 μM 2-mercaptoethanol, and 1 mM sodium pyruvate. For siRNA transfection, plated cells (seeded at 1–5 × 10^5 density) were transfected with HP GenomeWide *KCNN1* (SK1) siRNA or with non-silencing control siRNA (Qiagen, Valencia, CA) using HiPerfect Transfection Reagent (Qiagen, Valencia, CA).

2.2. Electrophysiology

Whole-cell patch recordings were conducted in current clamp mode using the EPC-9 patch clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Electrodes were pulled to a resistance of 2–3 MΩ. For recording, the electrode solution contained (in mM): potassium-glutamate 110, KCl 10, NaCl 10, $MgCl_2$ 1, Mg-ATP 3, HEPES 5, EGTA 10, and $CaCl_2$ 9 to achieve 1 μM free [Ca^{2+}] (MaxChelator: http://www.stanford.edu/~cpatton/maxc.html); pH was then adjusted to 7.2. Culture medium was replaced with extracellular recording solution consisting of (in mM): NaCl 140, KCl 4, $NaHCO_3$ 2, NaH_2PO_4 1, $MgSO_4$ 1, HEPES 5, $CaCl_2$ 2.5, with pH adjusted to 7.4. High intracellular Ca^{2+} was used to activate the SK channels. Apamin (100 nM) was added to the external solution to confirm SK channel-mediated AHP. Patch clamp data were obtained using Pulse software (HEKA Elektronik) and later analyzed using PulseFit (HEKA ElektroniK) and IgorPro (WaveMetrics, Lake Oswego, OR, USA). Glucose is either omitted or added (at 20 mM concentration) to the external recording solution. Fast perfusion changes of external solution were achieved by using a SmartSquirt Micro-perfusion system (AutoMate Scientific, Berkeley, CA), having computer-controlled valves and a pressurized system for delivering solutions. All experiments were performed at 32–34 °C.

2.3. Statistical analysis

Differences among group means were statistically compared using Student t- test. *P*-values <0.05 were considered as statistically significant. Statistical analyses were performed using SAS statistical software (SAS Institute Inc., Cary, N.C.).

3. Results

We aimed to further characterize the function of SK channels in the insulinoma INS-1 cells by determining changes of the cell's AHP response in the presence and absence of glucose. As shown in Fig. 1A, control INS-1 cells in normal saline solution without glucose, when action potentials (AP) were invoked with a brief 5 ms 100 pA current injection, exhibited an AHP which is sensitive to apamin, a selective blocker of SK channels (Fig. 1A inset). In the cell shown in Fig. 1A, the AHP was completely eliminated by the subsequent addition of high glucose (20 mM). We compared the amplitudes of the AHPs in the absence and presence of glucose (0 mM and 20 mM) and found that the AHP is consistently absent or minimal in the presence of 20 mM glucose ($n = 6$ cells with pair recordings at both 0 mM and 20 mM glucose, $p = 0.0003$; Fig. 1B). Initially, we observed that blocking the SK channels with apamin (or knocking down expression of SK1 channel subunit) resulted in more depolarized resting membrane potentials [1]; similar ef-

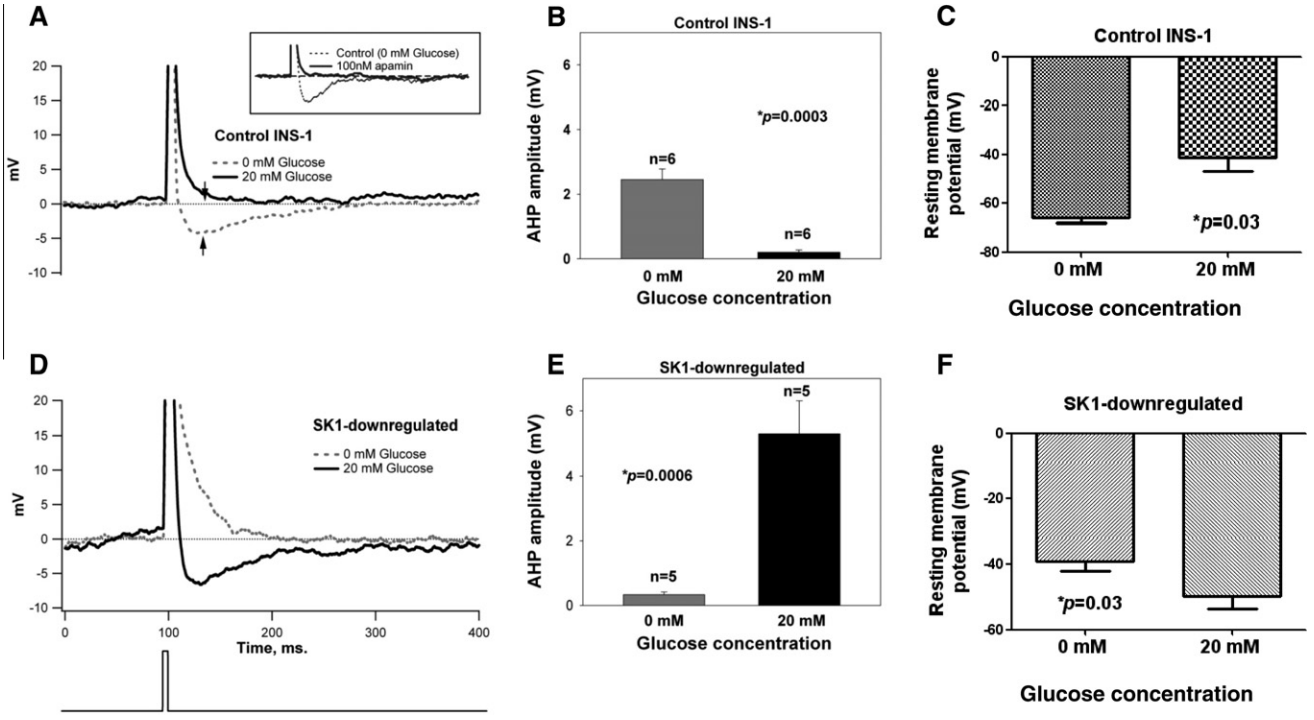

Fig. 1. Control INS-1 cells and SK1-downregulated cells exhibited different AHP responses and resting membrane potentials in the presence and absence of glucose. (A) Shown are representative current clamp recordings of the same (control) cell under zero-glucose (*broken gray line*) and 20 mM glucose (*black line*) conditions. Inset shows the AHP response of another control INS-1 cell treated under saline (0 mM glucose) and 100 nM apamin (in saline) solutions. Action potentials were induced by 5 ms 100 pA current injection. Recordings are shown in truncated form to highlight the AHP responses. (B) AHP amplitude summary of control cells recorded in glucose-omitted saline solution and subsequently in 20 mM glucose saline solution ($n = 6$). Amplitude was determined by the difference between the baseline and the peak hyperpolarized membrane potential as indicated by the arrows in A. (C) Shown are resting membrane potentials of INS-1 under zero-glucose and 20 mM glucose in Fig. 1B. (D) A representative SK1-downregulated cell lacked an AHP under zero-glucose conditions. Subsequent exposure of the same cell to high glucose (20 mM) induced the AHP. (E) SK1-downregulated cells ($n = 5$) exhibited increased AHP amplitude when exposed to 20 mM glucose ($n = 5$ cells with complete pairs (0 mM and 20 mM) of glucose recordings). (F) Resting membrane potentials of SK1-downregulated cells (from Fig. 1E) under different glucose conditions are different. Records in A and C were aligned at baseline for ease of comparison. Mean amplitude values (±SEM) are shown for all experimental conditions.