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Voltage-dependent metabolic regulation of Kv2.1 channels in pancreatic β-cells

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ABSTRACT

Voltage-gated potassium channels (Kv channels) play a crucial role in formation of action potentials in response to glucose stimulation in pancreatic β-ells. We previously reported that the Kv channel is regulated by glucose metabolism, particularly by MgATP. We examined whether the regulation of Kv channels is voltage-dependent and mechanistically related with phosphorylation of the channels. In rat pancreatic β-cells, suppression of glucose metabolism with low glucose concentrations of 2.8 mM or less or by metabolic inhibitors decreased the Kv2.1-channel activity at positive membrane potentials, while increased it at potentials negative to −10 mV, suggesting that modulation of Kv channels by glucose metabolism is voltage-dependent. Similarly, in HEK293 cells expressing the recombinant Kv2.1 channels, 0 mM but not 10 mM MgATP modulated the channel activity in a manner similar to that in β-cells. Both steady-state activation and inactivation kinetics of the channel were shifted toward the negative potential in association with the voltage-dependent modulation of the channels by cytosolic dialysis of alkaline phosphatase in β-cells. The modulation of Kv-channel current–voltage relations were also observed during and after glucose-stimulated electrical excitation. These results suggest that the cellular metabolism including MgATP production and/or channel phosphorylation/dephosphorylation underlie the physiological modulation of Kv2.1 channels during glucose-induced insulin secretion.

1. Introduction

Potassium channels in pancreatic β-cells play a crucial role in glucose-stimulated insulin secretion. The resting potential of β-cells is approximately −70 mV at low glucose concentration (i.e., 2.8 mM). At this membrane potential, a class of potassium channel, ATP-sensitive K^+ channel (K_{ATP} channel), is open, thereby determining the resting potential [1–4]. Increase in external glucose concentration to 5.6 mM or higher leads to elevation of cytoplasmic ATP or ATP/ADP ratio within a few minutes, depolarizes β-cell membrane as a consequence of closure of the K_{ATP} channel and induces bursting spike-like short action potentials at membrane potentials positive from −50 to −40 mV. These action potentials are produced by orchestrated openings of voltage-dependent Ca^{2+} channels (VDCCs) and voltage-gated K^+ channels (Kv channels). Action potentials, overlapping on a slow membrane depolarization, appear with a short duration and peak amplitude reaching 0 mV or more positive range [5]. This initial response to glucose stimulation is known as the triggering pathway (K_{ATP}-channel dependent pathway) that exchanges metabolic signals to electrical responses upon increase in glucose concentration. In addition, under the condition with diazoxide, a K_{ATP} channel opener, unable K_{ATP} channels to close in response to glucose metabolism and with increased external K^+ concentration that depolarizes membrane, β-cells are also responsive to glucose metabolism, being recognized as the amplifying pathway or K_{ATP}-channel independent pathway [3,6].

Kv channels are composed of delayed-rectifier K^+ channels that are slowly activated upon depolarization [7–9], and of a distinct class of Kv channels with fast and transient activation during depolarization, being defined as A-current [10,11]. Delayed rectifying K^+ channels play a pivotal role in regulation of insulin secretion in response to glucose in both triggering and amplifying pathways, and their pharmacological inhibition and genetic ablation are likely to increase insulin secretion [12,13]. We have recently reported a novel regulation of the Kv channels in pancreatic β-cells by glucose metabolism, primarily via MgATP [14]. In the present report we further examined whether the metabolism-dependent regulation of the Kv-channel current takes place in the physiological membrane potential range between −50 and 0 mV during glucose stimulation.

2. Materials and methods

Male Wistar rats were housed according to our institutional guidelines and for animal care. Approval of animal experiments

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by institutional committee of ethics was obtained. Islets of Langerhans were isolated by collagenase digestion from the rats aged 8–10 weeks, as previously reported [14,15]. Collected islets were dispersed into single cells and maintained in short-term culture for up to 3 days in Eagle's minimal essential medium containing 5.6 mM glucose supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin in 95% air with 5% CO_2 at 37 °C. The cells were superfused with control HEPES-Krebs-Ringer bicarbonate buffer (HKRB) solution containing 2.8 mM glucose.

Perforated whole-cell clamped currents were recorded using a pipette solution containing nystatin (150–200 μg/ml) dissolved in 0.1% DMSO, as previously reported [15,16]. Membrane currents were recorded using an amplifier (Axopatch, 200B, Foster, CA) in a computer using pCLAMP10.2 software. The resistances of patch pipettes ranged from 3 to 5 MΩ.

For perforated whole-cell clamp, pipette solution contained (in mM): K_2SO_4 40, KCl 50, $MgCl_2$ 5, EGTA 0.5 and HEPES 10 at pH 7.2 with KOH. For conventional whole-cell clamp experiments, pipette solution contained (in mM): KCl 50, K_2SO_4 35, $MgCl_2$ 5, EGTA 11, $CaCl_2$ 1, HEPES 11 and ATP-2Na (Rosh Diagnostic, Tokyo, Japan) 5 at pH 7.2 with KOH. The HKRB solution contained (in mM): NaCl 129, $NaHCO_3$ 5.0, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.0, $MgSO_4$ 1.2 and HEPES 10 at pH 7.4. Glucose was added to the solution at required concentrations. The experiments were performed at room temperature (25 °C). *p*-Trifluoromethoxyphenylhydrazone (FCCP) was from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Nystatin and adenosine 5′-(β,γ-imido)triphosphate (AMPPNP) were from Sigma-Aldrich (Tokyo, Japan).

Data represent the mean ± SEM. Statistical analyses were performed using the Student's *t*-test or one-way ANOVA as indicated with a software package of GrafPad Prism ver. 3.02. *P* values below 0.05 were considered statistically significant.

3. Results

3.1. Kv-channel current are regulated by glucose metabolism

Kv-channel currents were not only dependent on membrane potential, but also slowly increased with time by increasing glucose concentration from 2.8 to 16.6 mM, whereas they conversely decreased in the continuous exposure to 2.8 mM glucose in the external solution as long as the test potentials were continuously applied at +20 mV (Fig. 1A and B). After formation of nystatin-perforated whole-cell clamp mode in the presence of 2.8 mM glucose, the glucose concentration was either changed to 16.6 mM or maintained at 2.8 mM. To prevent contamination of K_{ATP} channel current in analyses of Kv-channel current, 100 μM tolbutamide was simultaneously administered in the superfusion solutions. Repetitive depolarized pulses to +20 mV were applied to elicit Kv currents every 10 s and the current amplitudes were normalized to that of time 0 min (Fig. 1B). The glucose-concentration dependent regulations of Kv-channel currents shown in Fig. 1A and B were also observed in conventional whole-cell mode (Fig. 1C, upper traces), confirming the previous results [14]. In these experiments 5 mM MgATP was contained in the pipette solution. Thus, MgATP at 5 mM was insufficient to prevent the glucose-dependent regulation of Kv-channel currents as long as the cells were continuously exposed to 2.8 mM glucose in the external solution (Fig. 1C, traces at +30 mV). Intriguingly, we found that the channel regulation by glucose metabolism at negative potentials was as opposed to that observed at positive potentials (Fig. 1C, traces at −20 mV). In the presence of 2.8 mM glucose for 5 min or more, the Kv-channel current increased with time at −20 mV, whereas it decreased at +30 mV. When we compared the current–voltage relations recorded at 0 and 10 min after formation of whole-cell clamp mode in the presence of 2.8 mM glucose, increases of the Kv current at both −30 and −20 mV and reductions of the current at voltages positive to 0 mV were found: the cross phenomenon of the current–voltage relations is the key feature in regulation of the Kv channels by glucose metabolism as depicted in Fig. 1D (the modulation of current–voltage relations). The modulation of the current–voltage relations was also observed when external glucose concentration was further decreased to 0 mM with 0 mM ATP in the pipette or when mitochondrial electron transport inhibitor, FCCP at 1 μM, was administered (Fig. 1E and F).

3.2. Kv2.1 channels expressed in HEK293 cells are dependent on cell metabolism

In the previous report, we demonstrated that the Kv-channel current regulated by glucose metabolism is Kv2.1 channels, because the residual current after exposure to guangxitoxin-1E. a specific blocker of Kv2.1 channels, was uninfluenced by glucose-metabolic inhibition [14]. In HEK293 cells, Kv currents were little expressed at a basal state, whereas transfection of recombinant Kv2.1 channels to HEK293 cells (Supplementary file) showed voltage-dependent properties of the currents upon depolarizing the membrane potentials with 10 mV step from the holding potential of −70 mV (Fig. 2A–a and b). These voltage-dependent characteristics were identical to those of Kv-channel currents observed in β-cells. In HEK293 cells expressing Kv2.1 channels, we observed similar cellular metabolism-dependent increase and decrease of Kv2.1-channel currents at negative and positive potentials, respectively, in the absence of ATP in the pipette (Fig. 2B). These membrane potential-specific effects on the Kv2.1-channel currents by cellular metabolism were not observed in the presence of 10 mM MgATP in the pipette (Fig. 2C). These observations of MgATP-dependent channel regulations are consistent with those observed in Fig. 1 and in the previous report [14]. In the absence of MgATP at the cytoplasm, the Kv2.1-channel current increased 10 min after initiation of whole-cell mode as compared to that at 0 min and current–voltage relations demonstrated the cross phenomenon as observed in metabolic inhibition in β-cells (upper and lower traces of Fig. 2D and E). These results were statistically confirmed and were identical to the results in β-cells in Fig. 1 (Fig. 2F). When the relative current levels obtained by normalizing current amplitudes 10 min after formation of whole-cell mode to current amplitudes at time 0 were compared in the presence and absence of 10 mM MgATP in the pipette, the Kv2.1-channel currents were significantly increased at the potentials from −40 to −20 mV and decreased at +20 mV.

3.3. Dephosphorylation of the Kv-channel current is involved in the modulation of current–voltage relations: shift of activation and inactivation kinetics

MgATP was replaced with nonhydrolysable ATP analogue, AMPPNP, by dialyzing into the cell through the patch pipette. Following the dialysis, the Kv-channel current was increased at negative potentials in a manner similar to those under low glucose and 0 ATP conditions (Fig. 3A), and was reduced at positive potentials to a greater extent than low glucose and 0 ATP conditions (Fig. 1E). The result suggests that hydrolysis of MgATP is required to prevent the modulation of current–voltage relations. Direct phosphorylation of Kv2.1 channels influences channel kinetics [17–19]. Kv2.1 channels are highly phosphorylated and graded dephosphorylation reportedly shifts $G_{1/2}$ (membrane potential for half-maximal activation of channel current) in the G–V relations (chord conductance–voltage relations: activation curve) and the half-maximal effect for membrane potential ($V_{i1/2}$) of steady-state inactivation toward negative potentials as compared to that in con-

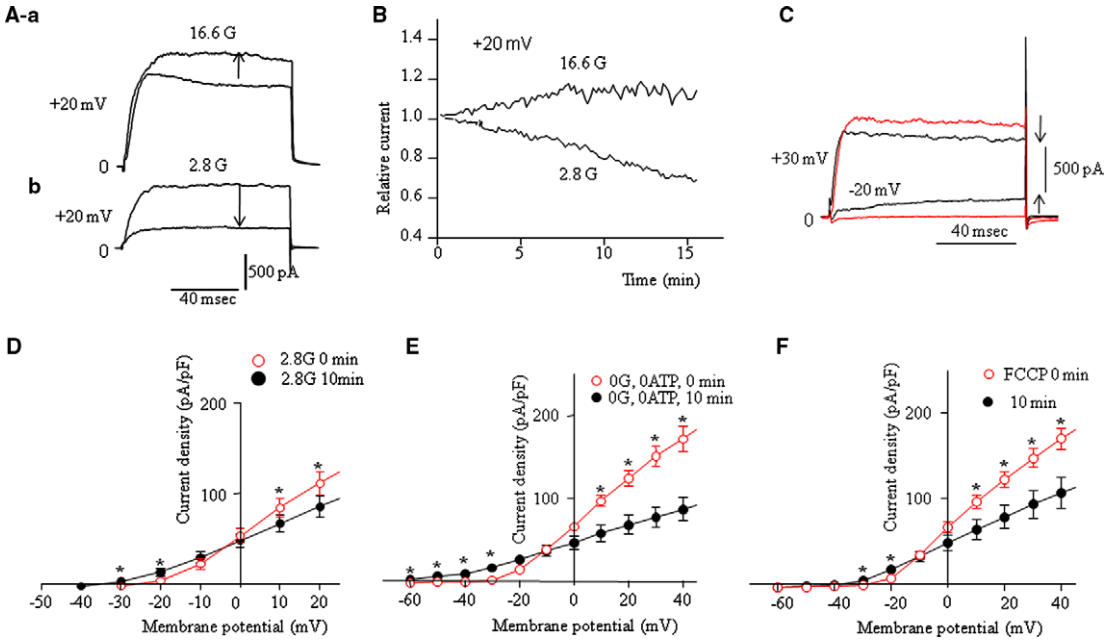

Fig. 1. Glucose-metabolism-dependent regulations of Kv-channel currents in rat pancreatic β-cells. (A) Effects of glucose concentration on the Kv-channel currents. Current traces in response to depolarization to +20 mV with 100 ms duration from a holding potential of −70 mV at intervals of 10 s were recorded after formation of perforated whole-cell clamp in the presence of 2.8 mM glucose in HKRB solution. Tolbutamide at 100 μM was added to the solution to inhibit K_{ATP} channels. At 3 min after exposure to this solution the time was reset to 0 min, the solution was changed to HKRB solution containing 16.6 mM glucose and recordings were initiated (upper traces: a). The current traces at time 0 and 12 min (arrow head) were depicted. In the lower traces (b), the cell was continuously superfused with HKRB solution containing 2.8 mM glucose from time 0. The current traces at time 0 and 12 min (arrow head) were depicted. (B) The Kv-channel current measured at the end (a current level averaged between 90 and 99 ms) of depolarized pulses was normalized to that at time 0 and plotted against time in HKRB solution with 2.8 mM (n = 5) and 16.6 mM glucose (n = 5). Only means of normalized data were plotted. (C) Kv-channel current traces recorded at +30 mV (upper traces) and −20 mV (lower traces) at time 0 (red) and 10 min (black) after initiation of exposure to 2.8 mM glucose were depicted. Arrows indicate changing direction. Data were from whole-cell clamp mode in experiments of C to F. (D) Current–voltage relations from the same voltage protocol as cells in C. Open symbols indicate 0 min and closed 10 min after superfusion of 2.8 mM glucose. Number of cells was 11. (E) Current–voltage relations with 0 mM MgATP in the pipette during exposure to 0 mM glucose at time 0 and 10 min (n = 4). (F) Current–voltage relations with 5 mM MgATP in the pipette by 10 min exposure to 1 μM FCCP in HKRB solution with 2.8 mM glucose (n = 7). Intrapipette MgATP concentration was 5 mM throughout the experiments in C–F. * $P < 0.02$ with paired tests between data at the same voltage indicated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

trol [19]. Application of alkaline phosphatase (AP) of 10 U/ml into the cytoplasm modulated the current–voltage relations and exhibited the cross phenomenon in current–voltage relations before and after the AP dialysis (Fig. 3B). These results were similar to those observed during metabolic inhibition (Fig. 1D–F). The shifts of the G–V relations (Supplementary file) to leftward direction as well as steady-state inactivation curve were also associated with AP effects (Fig. 3C and D). $G_{1/2}$ of G–V relations at time 0 and 5 min after AP dialysis were −3.1 ± 0.73 mV (n = 13) and −15.1 ± 1.6 mV (n = 13), respectively (Fig. 3C). $V_{i1/2}$ was shifted from −38.2 ± 1.6 mV (n = 10) at time 0 to −51.3 ± 1.2 mV at 5 min when AP was dialyzed (n = 10, in Fig. 3D). The experiments of AP dialysis in Fig. 3 were performed in the presence of 2.8 mM external glucose and 5 mM MgATP in the pipette. The current density was compared between those in the presence and absence of AP (Fig. 3E). The currents at the membrane potentials between −40 and −20 mV increased to a greater extent with AP dialysis than without it at 2.8 mM glucose. These results may suggest that the shift of current–voltage relations (the cross phenomenon) results from the dephosphorylation of the Kv2.1 channels rather than from metabolic inhibition itself.

3.4. Glucose-metabolism regulation of Kv channels during electrical excitations in β-cells

We examined whether Kv-channel modulations by low and high glucose concentrations are observed during glucose-stimulated electrical excitations. During the superfusion of β-cells with 2.8 mM glucose, the membrane potential recorded in nystatin-perforated whole-cell mode was stabilized at approximately −70 mV (Fig. 4). The current-clamp mode was changed to the voltage-clamp mode with the holding potential of −70 mV. The current recordings by depolarized step pulses with 10 mV increment before (2.8 mM glucose) and during action potentials (8.3 mM glucose) were depicted. The Kv-channel currents induced by depolarized pulses between at −40 and −10 mV revealed smaller levels during depolarization at 8.3 mM glucose than at 2.8 mM glucose before the depolarization. Current–voltage relations showed more current at negative potentials at 2.8 mM glucose before depolarization than that at 8.3 mM during depolarization (open and closed circles in the figure). After the glucose concentration was restored to 2.8 mM from 8.3 mM glucose and repolarization was observed, current–voltage relations changed to a pattern similar to those at 2.8 mM glucose before depolarization (closed triangles).

4. Discussion

In the present report, we demonstrated the regulation of Kv-channel currents by a change in cellular metabolism that occurs in physiological ranges of glucose concentrations. Previously we have reported that the Kv-channel current increases at high glucose concentration (16.6 mM) and decreases (down-regulation) upon prolonged exposure to low glucose (2.8 mM) in both perforated and conventional whole-cell clamp modes [14]. In this report, mitochondrial metabolism was central for the ability of the cellar metabolism to maintain the Kv-current levels. An increase in internal MgATP concentration to 10 mM in whole-cell pipette

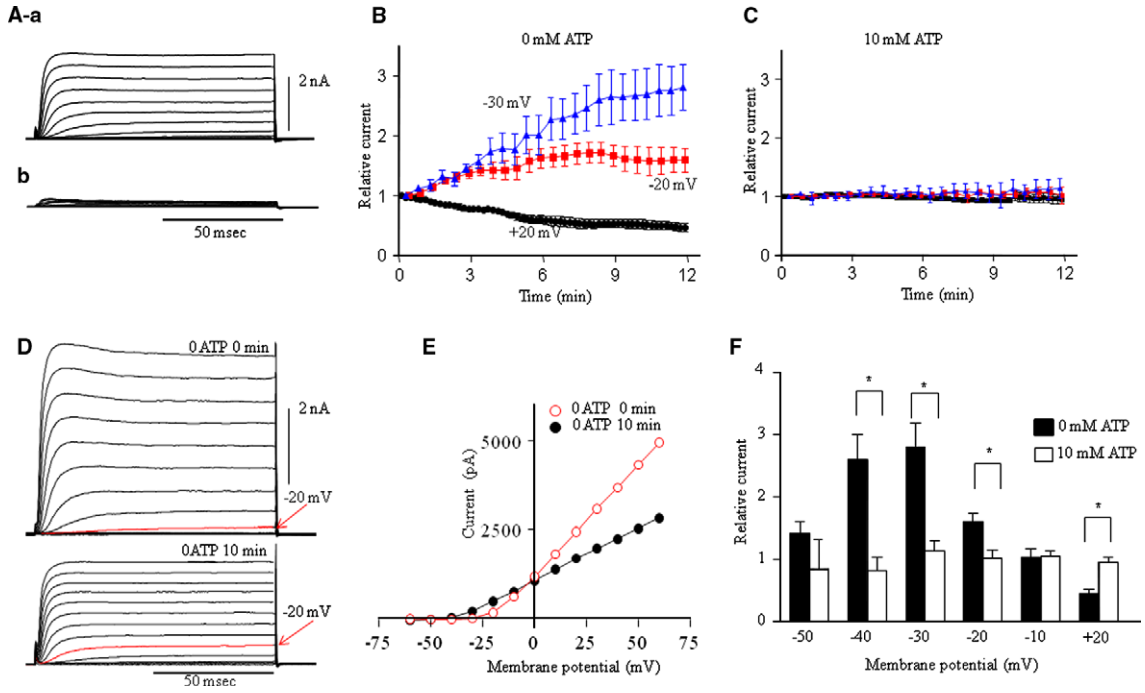

Fig. 2. Kv2.1-channels transfected on HEK293 cells are glucose-metabolism dependent. (A) Transfected Kv2.1-channel currents showed delayed rectifying properties similar to those in pancreatic β-cells (a). Untransfected HEK293 cells had little voltage-gated outward currents (b). (B) Relative Kv2.1-channel currents at +20 mV (black), −20 mV (red) and −30 mV (blue) plotted against time revealed increasing and decreasing time courses similar to the changes in Fig. 1B. The protocol performed in these experiments was the same as those in Fig. 1B except that MgATP in the pipette was 0 mM. $n = 5$ in each symbol. External glucose concentration was 2.8 mM throughout these experiments (B, C and D). (C) The relative Kv2.1-channel currents were uninfluenced when 10 mM ATP was included in the pipette solution ($n = 4$). (D and E) A series of current traces obtained from Kv2.1-channel transfected cells in whole-cell mode with 0 mM ATP at time 0 (upper traces) and 10 min after the initiation of the recording (lower). Note the current increase indicated by arrows (−20 mV) on traces at time 0 and 10 min dialysis with 0 mM ATP. (E) Current–voltage relations at 0 min (red circles) and 10 min (black circles) after initiation of dialysis of 0 mM ATP in the pipette solution. (F) The data of transfected Kv2.1 channel amplitudes at voltages between −50 and −10 mV and +20 mV were measured at 10 min after formation of whole-cell mode and normalized to those at time 0. The relative current levels showed current increases at negative potentials and a decrease at +20 mV in the absence of MgATP. Column bars indicated with black were data from 10 min dialysis of 0 mM MgATP in the pipette and white of 10 mM MgATP. $^{*}P < 0.02$ (unpaired test). Data were recorded in whole-cell clamp mode in these experiments. Number of cells for data collection was 6–9. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

solution stabilized the channel activity, while FCCP and 0 mM ATP with or without 11.2 mM glucose produced down-regulation of the Kv channel at +20 mV. Nonhydrolysable analogue of ATP, AMPPNP, did not mimic the ability of high glucose to prevent the Kv-channel down-regulation [14]. Thus, MgATP plays a pivotal role in maintaining the activity of the channel. In the present report, we have found that metabolic regulations of current amplitudes of Kv channels were voltage-dependent. The channel currents were decreased at positive potentials as we have demonstrated [14], whereas they conversely increased at negative potentials between −50 and −20 mV, the potential range physiologically during glucose-stimulated action potentials in pancreatic β-cells. At these negative membrane potentials Kv-channel currents decreased during exposure to supra-threshold glucose concentration (8.3 mM) in association with a shift of activation and inactivation curves toward the positive potential, thereby revealing less outward current during firings of action potentials. These changes resulted in the cross phenomenon of current–voltage relations at high vs. low glucose concentrations. The modulation of Kv-channel current–voltage relations by cellular metabolism is mediated primarily by MgATP, presumably in part due to involvement of phosphorylation at high glucose and dephosphorylation at low glucose concentration of the channel protein. Deletion of MgATP at the cytoplasm, lowering of glucose concentration, use of AMPPNP or alkaline phosphatase produced the similar modulation of current–voltage relations. These might be the consequence of the negative shifts of $G_{1/2}$ and $V_{i1/2}$. However, in the prolonged presence of 2.8 mM glucose with 5 mM MgATP in the pipette, the negative shift of $G_{1/2}$ but not $V_{i1/2}$ was observed [14], whereas addition of AP in the pipette with 5 mM MgATP during exposure to 2.8 mM glucose produced both $G_{1/2}$ and $V_{i1/2}$ shifts toward negative potentials. These differences may suggest that Kv channels were modulated by both phosphorylation/dephosphorylation processes and MgATP *per se*.

The present findings suggest that the Kv-channel modulation by glucose metabolism is playing a pivotal role in glucose-stimulated insulin secretion that includes the triggering and amplifying pathways. After closure of K_{ATP} channels following to elevation of glucose concentration at the external side, the modulation of Kv2.1 channels in terms of currents levels at negative potentials in association with rightward shift of activation curve may further depolarize the membrane potential and consequently promote more entry of Ca^{2+} ions. Conversely, changes of glucose concentration to subthreshold levels may increase Kv2.1-channel currents to exaggerate hyperpolarization in concert with initiation of openings of K_{ATP} channels.

5. Conclusion

The present report is the first that demonstrated cellular-metabolism-dependent regulation of Kv2.1 channels in pancreatic β-cells. In contrast to the regulation of K_{ATP} channels that is characterized as changes in the open-state probability of the channel by ATP/ADP ratio, the modulation of the Kv2.1 channels by cellular metabolism is associated with the shift of voltage-dependent current–voltage relations. Alterations of activation and inactivation kinetic properties may underlie these modulations of Kv2.1 chan-

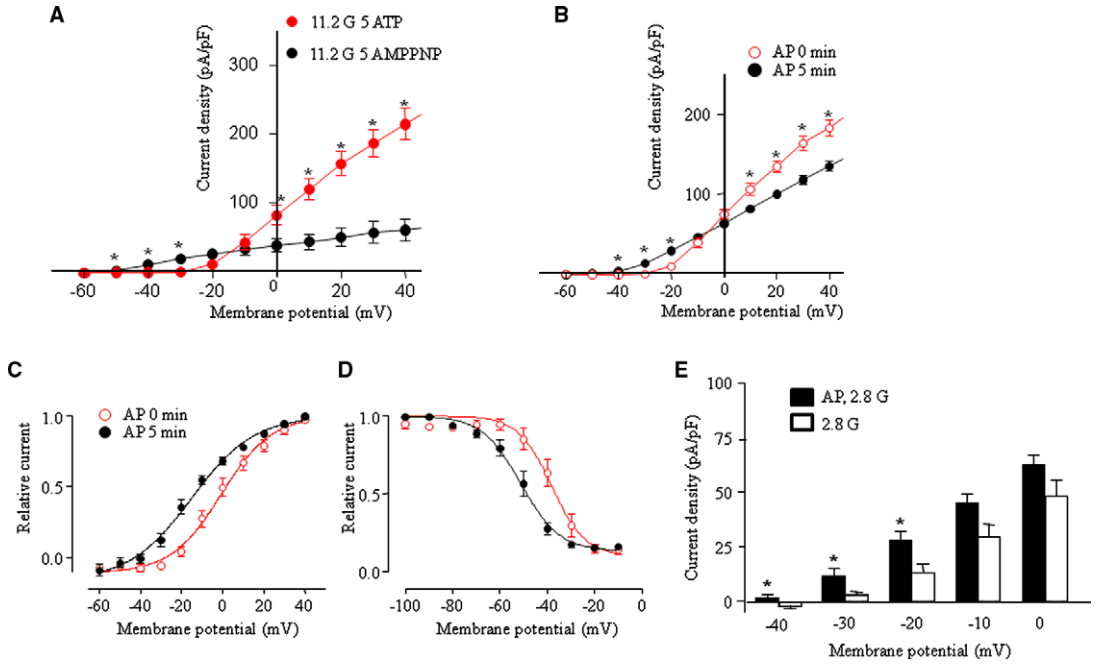

Fig. 3. Cross phenomenon of current–voltage relations is related to dephosphorylation of Kv channels. (A) Effects of 5 mM ANPPNP in the pipette on Kv-channel current–voltage relations. The cross phenomenon of both current–voltage relations in the absence (control: red circle, $n = 12$) and presence (black circles; $n = 5$) of 5 mM AMPPNP. Glucose concentration was 11.2 mM. (B) Dialysis of alkaline phosphatase (AP) mimicked the current–voltage relations observed in glucose-metabolism inhibited conditions (A). External glucose concentration was 2.8 mM and intrapipette MgATP was 5 mM. Number of tested cells was 7. (C and D) G–V relations for activation curve (C; $n = 6$–9) and inactivation curves (D; $n = 5$). Red symbols indicate data at 0 min after formation of whole-cell mode and black symbols 10 min after AP dialysis. (E) Current densities (pA/pF) in the presence (black bars; $n = 7$) and absence (white bars; $n = 11$) of AP in the pipette were compared at the membrane potentials as indicated on abscissa. The currents were recorded at 10 min after formation of whole-cell mode in the presence of 2.8 mM glucose in HKRB solution and 5 mM MgATP in the pipette. * indicates $P < 0.02$ vs. data from AMPPNP (A) and AP (B and E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

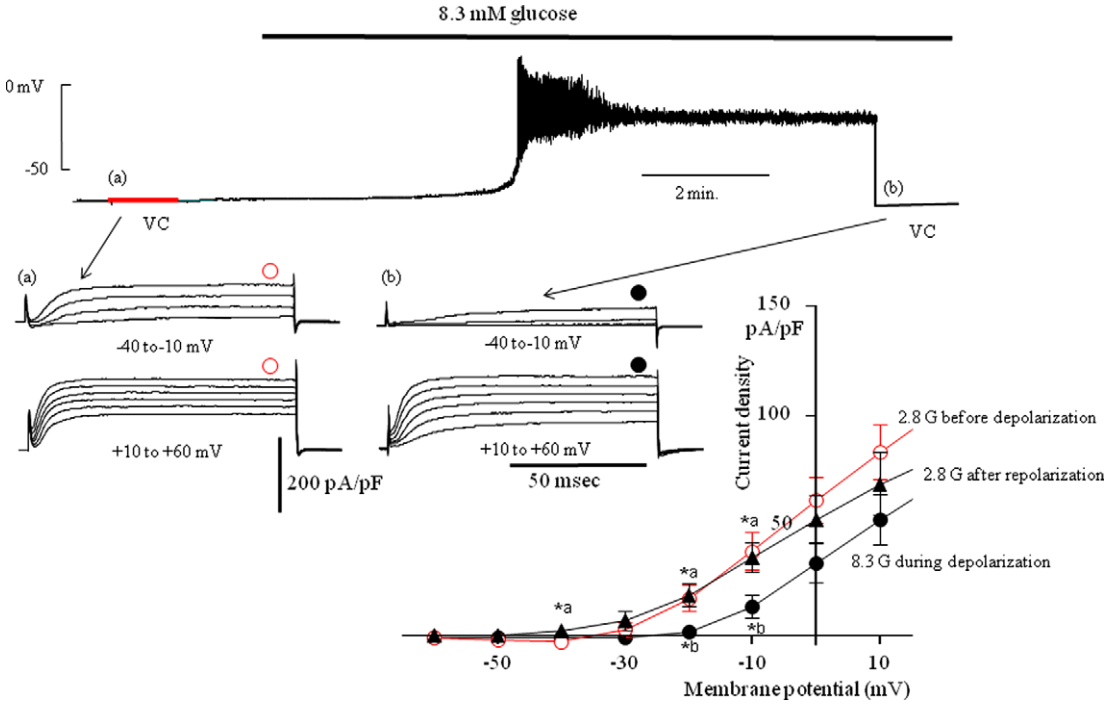

Fig. 4. Changes in current–voltage relations at low and high glucose concentrations in glucose-stimulated electrical excitations. Membrane potentials were recorded in the current-clamp mode at the glucose concentration of 2.8 mM. The membrane was approximately −70 mV under this condition and the recording mode was changed to the voltage clamp at (a). The Kv-channel currents were recorded by the voltage pulses with 10 mV increments from the holding potential of −70 mV (open circles) during exposure to HKRB solution in the presence of 0 mM Ca^{2+} and 100 μM tolbutamide to exclude contaminations of K_{ATP}-channel and Ca^{2+}-activated K^+ currents. After changing back to the current-clamp mode the external glucose concentration was increased to 8.3 mM at the bar indicated above the current trace. At this time the solution was reversed to HKRB solution with the external Ca^{2+} concentration of 2 and 0 mM tolbutamide. The membrane potential was slowly depolarized to reach the threshold potential, and revealed action potential firings as illustrated in the upper trace. In steady-state excitations as shown in the upper trace in the presence of 8.3 mM glucose, the membrane potential was voltage-clamped to −70 mV to record the Kv-channel current at (b) in the same HKRB solution as in (a). The current–voltage relations at 2.8 mM glucose before depolarization, at 8.3 mM glucose during depolarization and 2.8 mM glucose after repolarization obtained from the separate cells ($n = 15$) were plotted. *a: $P < 0.05$ vs. closed circles at the same voltage and *b vs. closed triangles.

nels by glucose metabolism that is presumably related to phosphorylated state or MgATP *per se*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.04.088.

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