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Original Paper

Voltage-Gated Na^+ Channels are Modulated by Glucose and Involved in Regulating Cellular Insulin Content of INS-1 Cells

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Key Words
Glucose • Voltage-gated Na^+ channel • INS-1 cells • Tetrodotoxin • Insulin

Abstract
Background/Aims: Islet beta cells (β-cells) are unique cells that play a critical role in glucose homeostasis by secreting insulin in response to increased glucose levels. Voltage-gated ion channels in β-cells, such as K^+ and Ca^{2+} channels, contribute to insulin secretion. The response of voltage-gated Na^+ channels (VGSCs) in β-cells to the changes in glucose levels remains unknown. This work aims to determine the role of extracellular glucose on the regulation of VGSC. ***Methods:*** The effect of glucose on VGSC currents (I_{Na}) was investigated in insulin-secreting β-cell line (INS-1) cells of rats using whole-cell patch clamp techniques, and the effects of glucose on insulin content and cell viability were determined using Enzyme-Linked Immunosorbent Assay（ELISA）and Methylthiazolyldiphenyl-tetrazolium Bromide（MTT）assay methods respectively. ***Results:*** Our results show that extracellular glucose application can inhibit the peak of I_{Na} in a concentration-dependent manner. Glucose concentration of 18 mM reduced the amplitude of I_{Na}, suppressed the I_{Na} of steady-state activation, shifted the steady-state inactivation curves of I_{Na} to negative potentials, and prolonged the time course of I_{Na} recovery from inactivation. Glucose also enhanced the activity-dependent attenuation of I_{Na} and reduced the fraction of activated channels. Furthermore, 18 mM glucose or low concentration of tetrodotoxin (TTX, a VGSC-specific blocker) partially inhibited the activity of VGSC and also improved insulin synthesis. ***Conclusion:*** These results revealed that extracellular glucose application enhances the insulin synthesis in INS-1 cells and the mechanism through the partial inhibition on I_{Na} channel is involved. Our results innovatively suggest that VGSC plays a vital role in modulating glucose homeostasis.

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Introduction

Voltage-gated Na^+ channels (VGSCs) generate and conduct action potentials (APs) and regulate the electrical signaling in all types of excitable cells, including neurons, muscles, and endocrine cells [1-3]. Voltage-dependent Ca^{2+} channels typically begin to be activated near the peak of APs and its currents are largest during the falling phase of Aps [4]. The contribution of voltage-dependent potassium channels is typically greatest in the later stages of the repolarization phase of Aps [5-6]. Large rapid depolarization results in opening of the Na^+ channel and inward Na^+ current. Activation, deactivation, and inactivation in VGSCs link various physiological processes to electrical activity by controlling the action potentials in nerve, muscle and other excitable cells [3], such as adrenal chromaffin cells [7]. VGSCs mediate the influx of Na^+ into the cytosol of cells in response to local membrane depolarization, thereby generating the upstroke phase of an action potential [3].

Pancreatic islet beta cells (β-cell) are typical excitable endocrine cells that secrete insulin [8]. Functional failure in β-cells may cause diabetes [9]. Voltage-gated ion channels, such as K^+ and Ca^{2+} channels in β-cells, contribute to insulin secretion [10-16]; however, the role of Na^+ channels in β-cells remains unknown [11, 14, 17]. In this study, the effect of glucose on VGSC currents (I_{Na}) and its channel intrinsic kinetics was investigated in insulin-secreting β-cell line (INS-1) cells of rats by using whole-cell patch clamp techniques. INS-1 cells have a stable, robustly glucose responsive phenotype that allows scholars to perform biochemical and molecular research without the added complication of a phenotypic drift [18]. Furthermore, β-cells are hypersensitive to the changes in extracellular blood glucose. Elevated glucose levels significantly promote the synthesis of insulin in β-cells and thus improve the regulation of blood glucose [19, 20]. Protein synthesis is acutely stimulated during 1 h incubation at different glucose concentrations in MIN6 cells, that is, the concentration dependency of glucose-stimulated translation, in which the protein synthesis ratio reached the maximum value with 20 mM glucose [19]. Moreover, the high glucose-stimulation effect on protein synthesis in islets is highly specific to pro-insulin synthesis [20]. These findings suggest that the increase in extracellular blood glucose can promote the synthesis of intracellular insulin in β-cells. Thus, we combined patch clamp technique with insulin content assay and cell viability assay to clarify the role of VGSC in glycemic control in β-cells. The activity of VGSC and the insulin synthesis level regulated by glucose and tetrodotoxin (TTX, a VGSC-specific blocker) were compared. We aim to determine the role of extracellular glucose on the regulation of VGSC and VGSC in regulating insulin synthesis.

Materials and Methods

Cell culture

Rat INS-1 cells were grown in 10-cm tissue culture dishes at 37 °C and 5% CO_2 in a humid atmosphere. The cells were passaged every 2 days by using 0.7 mL of 0.05% trypsin-EDTA. The culture medium was DMEM with 11.1 mM D-glucose supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 10 mM HEPES, 4.0 mM L-glutamine, 110 mg sodium pyruvate, and 50 μM β- mercaptoethanol.

Solutions and drugs

The components (mM) of the external solution for recording of I_{Na} are as follows: 120 NaCl, 20 TEA-Cl, 5.6 KCl, 1.2 $MgCl_2$.6 H_2O, 2.6 $CaCl_2$, 0.5 $CdCl_2$, 5 HEPES, and 3 glucose. The pH level was adjusted to 7.4 using NaOH. The patch pipette solution for whole-cell patch recording was (mM): 110 CsCl, 5 NaCl, 1 $MgCl_2$.6 H_2O, 20 TEA-Cl, 3 Mg-ATP, and 5 HEPES. The internal solutions were adjusted to pH level of 7.2 using CsOH and TTX with purity of more than 99%. All drugs purchased from Sigma-Aldrich.

Electrophysiological recordings

Electrophysiological recordings were performed by using the conventional whole-cell patch clamp recording configurations under voltage-clamp conditions. The recording electrodes were fabricated

from borosilicate glass pipettes (Sutter Instruments, USA) using a Flaming-Brown puller (P-97, Sutter Instruments, USA). The resistance was at a range of 3–7 MΩ, when the recording electrode was filled with the pipette solution. The macroscopic currents through VGSC were amplified by MultiClamp 700B (Axon Instruments, USA), low-pass filtered at 5 kHz, and digitized by Digidata 1440A (Axon Instruments, USA) at the sampling frequency of 20 kHz. Whole-cell series resistance was 10–20 MΩ for recordings, of which 70%–90% was compensated. When we set the holding potential at −80 or −120 mV, hyperpolarization triggered a dramatic increase of the Na^+ fast inward currents [17, 21]. All experiments were conducted at room temperature (22 °C–25 °C).

Insulin content assay

INS-1 cells were seeded in 12-well plates at a density of 1×10^5 cells per well and cultured for 24 h to measure the insulin synthesis in these cells. The cells were then pre-incubated for 1 h at 37 °C in fresh Krebs-Ringer bicarbonate (KRB) buffer containing 129 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 5 mM $NaHCO_3$, 10 mM HEPES, and 0.1% BSA at a pH level of 7.2 (glucose-free) and were subsequently stimulated for 1 h in 1 mL of KRB buffer (containing 2.8 mM glucose or TTX) at 37 °C, 5% CO_2. The total protein was collected, and the insulin content was measured by RayBio® Enzyme-Linked Immunosorbent Assay (ELISA) Kits (USA, ELR-Insulin). Insulin content was normalized to total protein from each well.

MTT cell viability assay

Methylthiazolyldiphenyl-tetrazolium Bromide (MTT Beyotime, Jiangsu, China) assay was applied to study the TTX-induced, dose-dependent cytotoxic effect. Cell viability was determined using the reduction assay. After treatment, the cells were incubated with MTT solution (5 mg/mL in PBS, pH 7.2) at 37 °C for 4 h. All culture media were removed, and the resulting formazan was dissolved in 150 mL DMSO. Cell viability was evaluated by colorimetric changes using MULTISKAN MK3 (Thermo Electron Corporation) at a test wavelength of 492 nm.

Data analysis

pClamp 10.4 (Axon Instruments, USA) and Origin Pro 8.0 (Origin Lab, USA) were used for data acquisition and analysis. For activation, and inactivation plots, the currents were normalized to the maximum values and plotted as a function of the potential. For the recovery from inactivation plots, the currents were normalized to the maximum values and plotted against the duration of the test pulse. For activity-dependent plots, the currents were normalized to the first amplitude of I_{Na} and plotted against the number of command steps. For the fraction of activated channels plots, the currents of test pulse (I_2) were normalized to the maximum values of the prepulse (I_1) and plotted against the duration of the prepulse. In order to obtain the steady-state activation kinetics of I_{Na}, the amplitude of current values (I) were transformed into conductance (G) by using the equation:

$$G=I/(V_m-V_{rev}) \quad (1)$$

where V_{rev} is the Na^+ equilibrium potential (V_{rev} = −5.03 ± 0.78 mV, n = 9, under normal control condition) and V_m is the membrane potential where I_{Na} was recorded. Normalized current conductance (G/G_{max}) and the data describing the fractional decrease in the normalized amplitude of currents during steady-state inactivation (I/I_{max}) were fitted with a Boltzmann equation:

$$I/I_{max} \text{ or } G/G_{max}= [1+\exp [(V_{1/2}-V_m)/V_c]]^{-1} \quad (2)$$

where I_{max} is the maximal current amplitude, G_{max} is the maximal conductance, V_m is the command or conditioning voltage (the values of V_m and V_{rev} are identical when I/I_{max} or G/G_{max}=1), $V_{1/2}$ is the potential of half-maximal activation or inactivation, and V_c is proportional to the slope at $V_{1/2}$. The time to peak value was used to analyze the activation kinetics. Inactivation and deactivation time constants were obtained by mono-exponentially fitting the current traces. All data were presented as the means ± SEM and compared using Student's t-test ($P < 0.05$ indicates statistical significance).

Results

Hyperpolarization increased Na^+ currents in INS-1 cells

To activate I_{Na}, membrane potential was held at −120 or −80 mV for 50 ms, and then depolarized to −10 mV for 50 ms [21]. Fig. 1 shows that the depolarization from holding

potential (V_{hold}) −120 mV induced large Na^+ currents in INS-1 cells. However, the depolarization-induced Na^+ current declined by 60% when V_{hold} was −80 mV. These results are similar to those for pancreatic islet β-cells [21], in which the fast-activation currents dramatically increased when the holding potential was reduced from −80 mV to −120 mV. The I_{Na} detected in the present study in INS-1 cells could be completely inhibited by 1 μM TTX (Fig. 8A), thereby indicating that the channel was VGSC. These results demonstrate that the Na^+ channels in INS-1 cells and pancreatic islet β-cells have a similar role. Thus in our subsequent experiments, the membrane potential was held at −120 mV to activate I_{Na}.

Glucose inhibited I_{Na} in a concentration-dependent manner

The membrane potential was first held at −120 mV for 50 ms then depolarized to −10 mV for 50 ms to activate I_{Na}. In this experiment, every I_{Na} was recorded at 30 s intervals. When I_{Na} reached relatively stable maximum amplitude, glucose was externally applied to the cells. As shown in Fig. 2, the amplitude of I_{Na} decreased by 36.06% ± 4.97% (n = 9, $P < 0.05$) within 1 min of exposure to 18 mM glucose. Glucose produced a concentration-dependent blockade of I_{Na}. Considering the maximal effects of 18 mM glucose on the amplitude of I_{Na}, this concentration was used in the following experiments.

Effect of glucose on I_{Na} steady-state activation

Active currents were evoked by a series of +10 mV voltage steps to potentials between −70 mV and 40 mV for 50 ms from a holding potential of −120 mV to test the effect of glucose on I_{Na} steady-state activation. Currents were measured at their peak. Representative traces from both control and 18 mM glucose

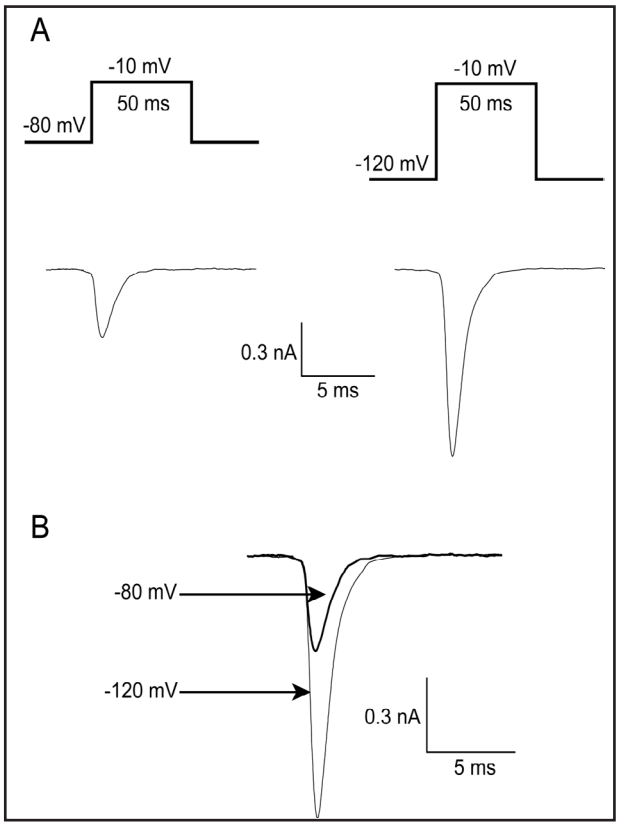

Fig. 1. Hyperpolarization increased Na^+ currents in INS-1 cells. (A) The fast inward Na^+ currents were induced by voltage pulses from either −80 mV or −120 mV for 50 ms and then depolarized to −10 mV for 50 ms in an INS-1 cell. (B) Depolarization from holding potential (V_{hold}) −120 mV induced large Na^+ currents in INS-1 cell. However, the depolarization-induced Na^+ current declined by 60% when V_{hold} was −80 mV.

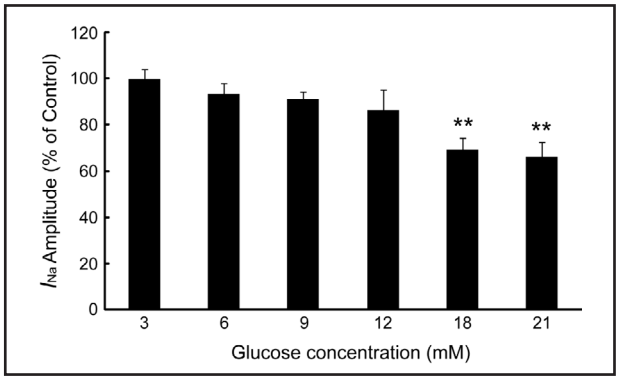

Fig. 2. Glucose inhibited I_{Na} in a concentration-dependent manner. The Na^+ current was 100% ± 3.85% (n = 6), 93.39% ± 4.81% (n = 5), 91.17% ± 3.09% (n = 6), 86.22% ± 8.85% (n = 6), 69.32% ± 5.29% (n = 6) and 66.14% ± 6.42% (n = 7) in 3, 6, 9, 12, 18 and 21 mM glucose, respectively; * P<0.05, ** P<0.01.

recordings are shown in Fig. 3A.

Current amplitudes (I) were represented as a percentage of the maximum current (I_{max}) recorded initially under control conditions to summarize the current-voltage plots (I/I_{max}, Where I is the amplitude of Na^+ current under the test pulse, I_{max} is the maximum amplitude of Na^+ current under the test pulse between −70 mV and +40 mV). As shown in Fig. 3B, the threshold for I_{Na} activation was approximately −40 mV, and the amplitude of I_{Na} was maximal at approximately −5.03 ± 0.78 mV (n = 9) and −10.34 ± 1.12 mV (n = 9, $P < 0.05$) in the control and 18 mM glucose conditions, respectively. Application of 18 mM glucose produced a negative shift in the activation curve (Fig. 3C). These results indicated that activation curve was shifted to more negative potentials by 18 mM glucose in INS-1 cells.

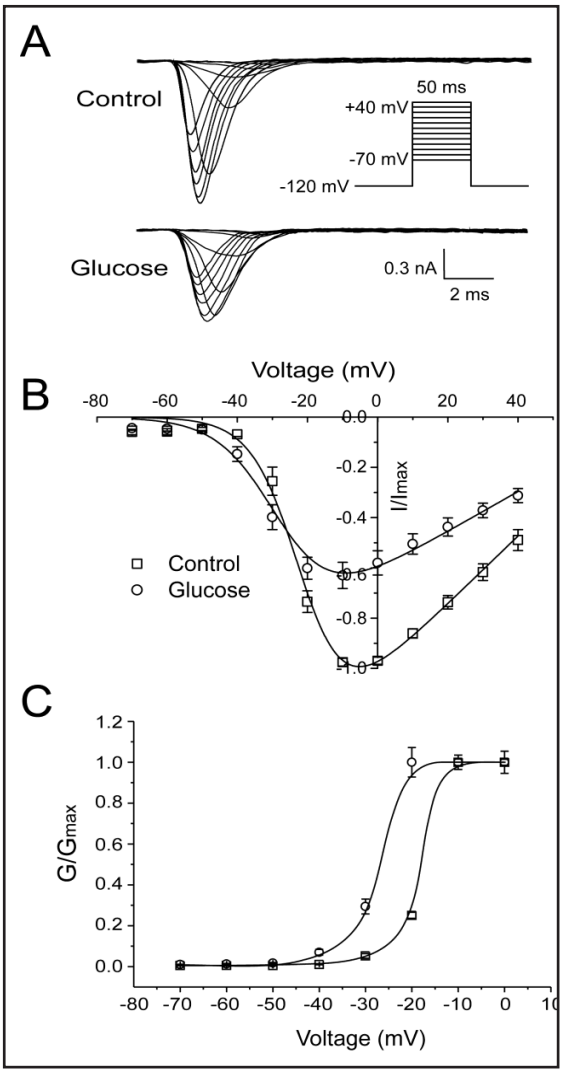

Fig. 3. Effects of glucose on steady-state activation of I_{Na}. (A) I_{Na} elicited in control (top traces) and in 18 mM glucose (bottom traces) conditions; glucose suppressed the amplitude of I_{Na} currents. (B) Current–Voltage relationship of I_{Na} in the control and application of 18 mM glucose. The amplitude of I_{Na} after 18 mM glucose treatment was lower than that of the control condition at most voltage points. (C) Comparison of steady-state activation of I_{Na} before and after 18 mM glucose treatment. The activation curves were fitted with a Boltzmann function (see text). The value of each point is mean ± SEM (n = 9).

Effect of glucose on I_{Na} steady-state inactivation

The effect of glucose on the voltage dependence of steady-state inactivation was examined with a double-pulse regime as presented in Fig. 4A. The membrane potential was conditioned to different potentials (ranging from −130 mV to 0 mV, with +10 mV increment) for 50 ms and then depolarized to a test potential of −10 mV for 50 ms. The typical traces of I_{Na} steady-state inactivation are shown in Fig. 4A. The inactivation curves before and after 18 mM glucose treatment were compared and were shown in Fig. 4B. Difference in $V_{1/2}$ was observed between the control (−66.7 ± 1.01 mV, n = 8) and glucose treated INS-1 cells (−78.0 ± 0.52 mV, n = 8, $P < 0.01$). Application of 18 mM glucose produced a 12 mV negative shift in the inactivation curve. These results indicate that inactivation curve was also shifted by 18 mM glucose in INS-1 cells.

Effects of glucose on I_{Na} recovery

The time course of recovery of the I_{Na} from inactivation was investigated using double-pulse protocols. As shown in Fig. 5A, a conditioning step (30 ms) from −120 to −10 mV was first applied to completely inactivate I_{Na}. After a pause at −120 mV from 1 to 20 ms (+1 ms increment，Δt=1ms), a test pulse (30 ms) to −10 mV was subsequently employed. After the progressively longer sojourns at −120 mV, the amplitude of I_{Na} gradually returned to the control value (Fig. 5A). Fig. 5B presents the percentages of amplitude of I_{Na} recovery from

inactivation for the control and 18 mM glucose conditions. The I_{Na} peak (normalized with respect to the initial amplitude) was plotted versus time under the control and 18 mM glucose conditions. In both cases, the time course of recovery from inactivation was well fitted by a single exponential function with recovery time constants of 1.52 ± 0.08 and 2.10 ± 0.07 ms in the control (n = 14) and 18 mM glucose (n = 14, P < 0.05) conditions, respectively. Glucose reduced the rate of I_{Na} recovery from inactivation. These results indicated that the recovery from inactivation of I_{Na} is slower in the 18 mM glucose than in the control.

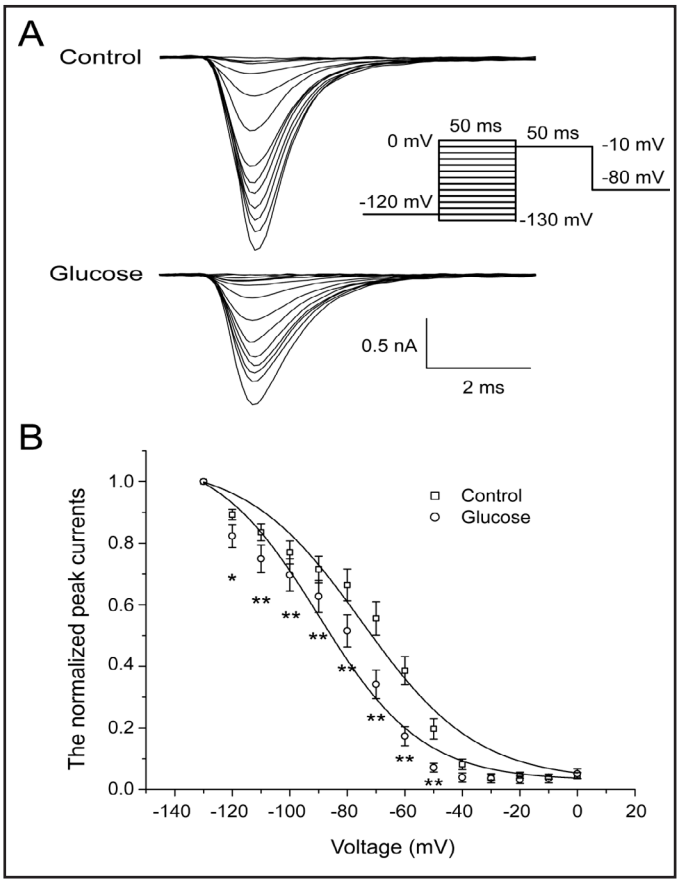

Fig. 4. Effects of glucose on steady-state inactivation of I_{Na}. (A) Current responses in control (top traces) and 18 mM glucose (bottom traces) conditions examined with a double-pulse regime. (B) The normalized amplitude of I_{Na} was plotted as a function of conditioning voltages. The inactivation curves were fitted with a Boltzmann function. The value of each point is mean ± SEM (n = 8). * P<0.05, ** P<0.01.

Effect of glucose on I_{Na} activity-dependent attenuation

Repetitive depolarized pulses were applied to study the effect of glucose on the activity-dependent attenuation of I_{Na}. Fig. 6A presents 12 trains of depolarizing steps (the duration of the pulses is 50 ms, the duration of separation between pulses is 300 ms) to −10 mV from the holding potential of −120 mV in the control and 18 mM glucose conditions, respectively. As shown in Fig. 6A, the amplitude of the evoked currents significantly decreased when the number of steps increased. The gradual reduction of the current amplitude during repetitive depolarized pulses may be ascribed to the slow process of recovery from inactivation. The changes in the evoked I_{Na} during a train were expressed as the ratio of the amplitudes of the nth current in the train to the amplitude of the first current. I_{Na} amplitude decreased to 77.06% ± 1.05% (n = 12) and 72.79% ± 0.72% (n = 12, P < 0.05) in the control and 18 mM glucose conditions by the 12th step, respectively. Normalized amplitudes of I_{Na} as a function of the number of command steps are plotted in Fig. 6B. The results suggested that glucose enhances the activity-dependent attenuation of I_{Na}.

Effect of glucose on the fraction of activated channels

Fig. 7A shows the effect of varying pulse durations on glucose-induced inhibition. The conditioning pulse with varying durations from 30 ms to 160 ms (+10 ms increment，Δt=10 ms) to −10 mV from a holding potential of −120 mV was first employed to modulate the inactivation level of the I_{Na}. After a sojourn at −120 mV for 30 ms, a 30 ms test pulse to −10 mV was subsequently applied then holding potential to −80 mV. The fraction of channels available to open was tested after the conditioning prepulse by the test pulse. With this protocol, ionic

current markedly decreased during the test pulse when the conditioning prepulse was applied. This trend is consistent with the increase in the fraction of inactivated channels. A longer duration of prepulse led to a larger fraction of inactivated channels. The I_{Na} of the test pulse decreased with the increasing duration of the conditioning pulse, and the glucose-induced inhibition gradually enhanced when the prepulse duration increased. For example, the I_{Na} amplitudes were both 100% (n = 10) in the control and 18 mM glucose conditions at 30 ms duration of conditioning pulse. By contrast, the I_{Na} amplitudes were 74.04% ± 1.07% and 70.48% ± 1.88% (n = 10, P < 0.05) in the control and 18 mM glucose conditions, respectively, at 160 ms duration of conditioning pulse (Fig. 7B).

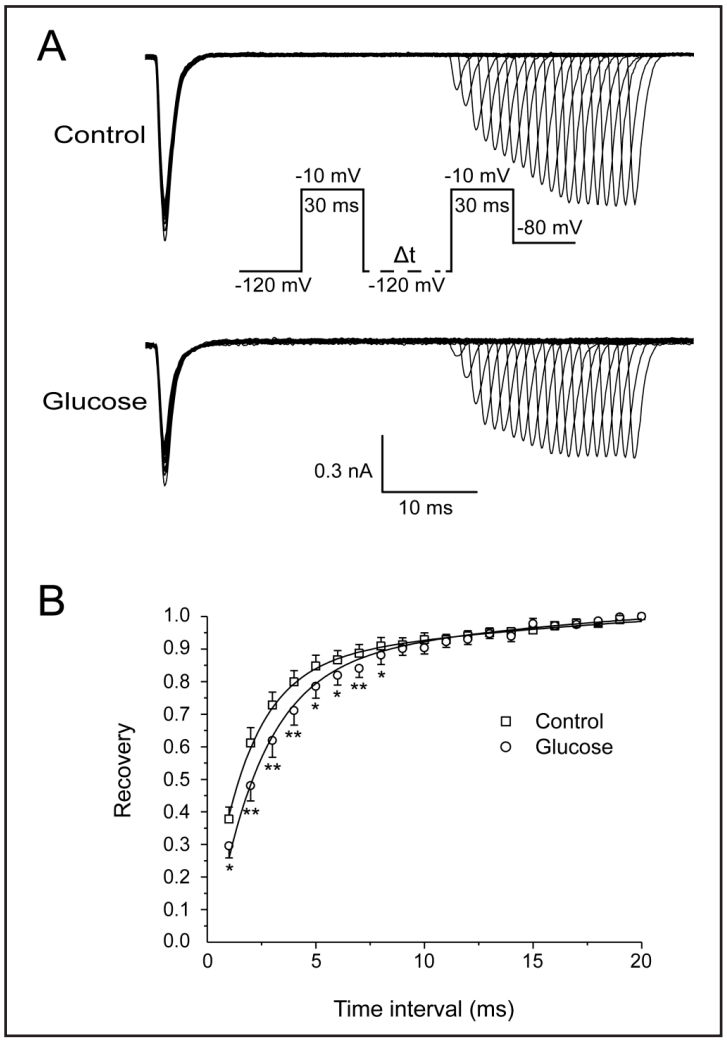

Fig. 5. Effects of glucose on the I_{Na} recovery from inactivation. (A) Representative current traces of I_{Na} recovery from inactivation in control (top traces) and 18 mM glucose (bottom traces) conditions. Inactivation recovery was examined using double-pulse protocols. (B) Time courses of the I_{Na} recovery from inactivation as a function of prepulse duration at −120 mV. Data were fitted with a single exponent. The value of each point is mean ± SEM (n = 14); * P<0.05, ** P<0.01.

Effects of TTX and glucose on I_{Na} and insulin content

To further determine the role of the VGSC of INS-1 cells, we detected and compared the effects of TTX and 18 mM glucose on I_{Na} and insulin content of the INS-1 cells. As shown in Fig. 8A, application with low TTX concentration (0.1 and 0.2 μM) partially inhibited I_{Na} (0.1 μM TTX: 79.29% ± 4.51% of control, P < 0.05; 0.2 μM TTX: 69.94% ± 4.22% of control (contain 2.8 mM glucose), P < 0.01). The inhibition ratio was similar to that induced by 18 mM glucose (68.56% ± 3.98% of control, P < 0.01). More interesting, 0.1 and 0.2 μM TTX can increase the insulin content (0.1 μM TTX: 199.62% ± 11.61% of control, P < 0.01; 0.2 μM TTX: 264.59% ± 28.58% of control, P < 0.01), which is similar to the effects of 18 mM glucose (141.38% ± 14.85% of control, P < 0.01) in INS-1 cells. These results are consistent with the findings of Lipson who reported that on the short-term scale (3 h), exposure to elevated glucose levels results in phosphorylation of IRE1α and enhancement of insulin synthesis rate [22]. Moreover, the high glucose-stimulation effect on protein synthesis in islets is highly specific to proinsulin synthesis [19, 20]. However, the application of high TTX concentration (1 μM) completely inhibited I_{Na} and reduced insulin content (73.83% ± 10.75% of control, P < 0.01). This inhibition effect on insulin content could be attributed to the impaired cell health caused

by the high TTX concentration. This assumption was confirmed by our subsequent study shown in Fig. 8B. The low TTX concentration (0.1 and 0.2 μM) did not damage the cell viability within 1 h (0.1 μM: 101.20% ± 0.90% of control, $P > 0.05$; 0.2 μM: 104.78 % ± 0.12% of control, $P > 0.05$), whereas the high TTX concentration (1 μM) significantly inhibited the cell survival (88.90% ± 1.46%, $P < 0.05$), indicating that high TTX concentration at 1 μM impaired cell health within 1 h of application. These results also suggest that low TTX concentrations increase the insulin content via partial inhibition of I_{Na} without promoting cell proliferation. These results generally reveal that application of high glucose concentration at 18 mM can improve insulin synthesis in INS-1 cells, and the mechanism underlying partial inhibition of the I_{Na} channel is strongly recommended.

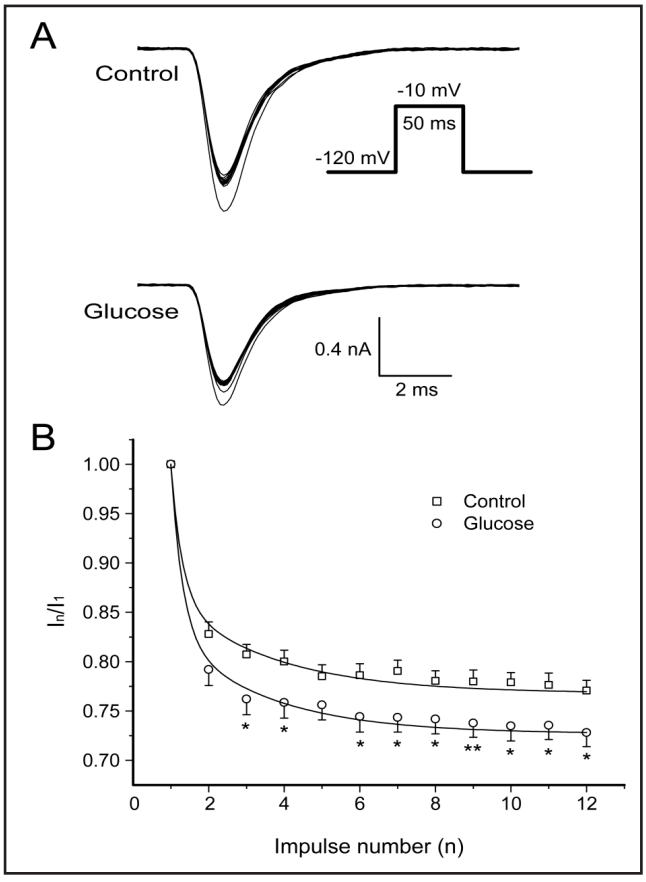

Fig. 6. Effects of glucose on the activity-dependent attenuation of I_{Na}. (A) Current traces evoked by trains of depolarizing steps in control (top traces) and 18 mM glucose (bottom traces) conditions. The amplitude of evoked currents was attenuated gradually with the increasing impulses. (B) Normalized amplitude of I_{Na} as a function of the number of command steps. Amplitudes were normalized to that of the first current in each train. 18 mM glucose significantly enhanced the activity-dependent attenuation. The value of each point is mean ± SEM (n = 16); * P<0.05, ** P<0.01.

Discussion

In the present study, the role of the VGSC in INS-1 cells response to the extracellular glucose levels were studied by using whole-cell patch clamp techniques and cell biology methods (insulin content assay and cell viability assay). We found that the amplitude of I_{Na} decreased with the increase in extracellular glucose level (Fig. 2). I_{Na} of the steady-state activation was suppressed with the negative shift in activation curves (Fig. 3). The inactivation curves of I_{Na} shifted to more negative potentials (Fig. 4), and the time course of I_{Na} recovery from inactivation was prolonged (Fig. 5). The activity-dependent attenuation of I_{Na} was enhanced (Fig. 6). The fraction of activated channels of I_{Na} decreased with application of 18 mM glucose (Fig. 7). Our results also show that the 18 mM glucose application induced the partial inhibition effect on I_{Na} but also increased the insulin content in INS-1 cells (Fig. 8), indicating that the VGSC of INS-1 cells were involved in the regulation of the glucose homeostasis. This assumption was further supported by the evidence that the insulin content was increased by treatment with low concentration of VGSC-specific blocker TTX (0.1 and 0.2 μM) coupled with the partial inhibition effect on I_{Na} (Fig. 8).

The I_{Na} of the steady-state activation was suppressed with the negative shift in activation curve, suggesting faster activation to the maximum amplitude of I_{Na} by glucose. Glucose produced a 12 mV negative shift in the inactivation curve, indicating a lower membrane potential threshold for closing VGSC. Faster activation on VGSC with the lower membrane

potential threshold for closing VGSC will shorten the opening time of the sodium channel, which may contribute to the inhibition of I_{Na} amplitude. Slower recovery from inactivation implies that VGSC in 18 mM glucose requires a long time in the transition from the inactivation to the closed state and reduces the fraction of available VGSC during a spike train [23]. The gradual reduction of current amplitude during repetitive stimulation may be attributed to the slow recovery from inactivation [24, 25]. Thus, the enhanced activity-dependent attenuation of I_{Na} by glucose might result from the slow I_{Na} recovery from inactivation. Consistent with the amplitude reduction, the leftward shift of steady-state inactivation, and the slowed recovery, the activity-dependent attenuation of I_{Na} was enhanced, and the fraction of activated channels was also reduced by glucose. Application of 18 mM glucose partially inhibits I_{Na} through the alternation in the channel intrinsic kinetics. Indeed, it is previously reported that intracellular ATP (2–8 mM) regulates the peak amplitude and kinetics of I_{Na} in a dose-dependent manner [17]. These findings seem to contrast to our results because of extracelluar glucose induces increment of intracellular ATP, however, 12 mM ATP decreases the peak amplitude of I_{Na} [17]. 18 mM glucose may improve the content of ATP exceed 8 mM then inhibit I_{Na}. This hypothesis should be explored in future work.

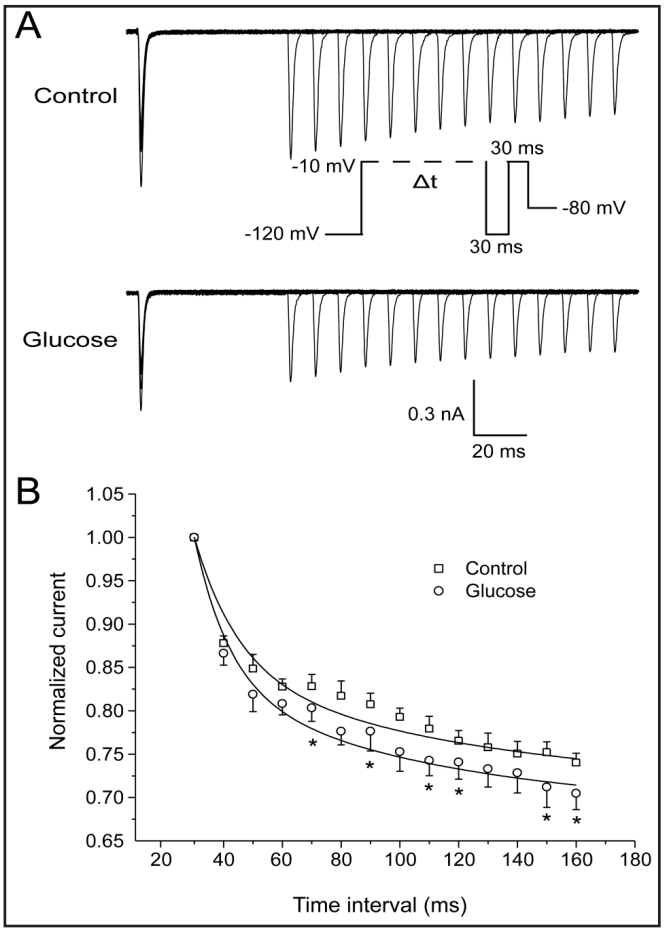

Fig. 7. Effects of glucose on the fraction of activated channels. (A) Representative traces obtained in control (top traces) and 18 mM glucose (bottom traces) conditions. The conditioning pulse was first employed to modulate the level of inactivated channels. (B) The normalized currents were plotted as a function of the duration of conditioning pulse. 18 mM glucose decreased the fraction of activated channels. The value of each point is mean ± SEM (n = 10); * P<0.05.

VGSC is responsible for both action potential generation and propagation and therefore plays a crucial role in β-cell activity [11]. VGSC may be a clinically relevant target; application of 1 μM TTX with 5 and 6 mM glucose in the islets reduced the amplitude and broadened the width of the action potential of β-cells, and simultaneously decreased insulin secretion [26]. As expected, TTX (0.1 μg/mL, 0.31 μM) reduced the activity of VGSC in β-cells; however, insulin secretion was impaired when the islets were applied with 6 and 20 mM glucose [27]. Velasco et al. also confirmed that TTX (0.1 μg/mL, 0.31 μM) decreases the amplitude of voltage spikes in pancreatic β-cells cells and the I_{Na} [28]. However, partial inhibition in VGSC will not suppress the action potential but will decrease its amplitude, prolong the duration, increase the excitation threshold, and delay the appearance of the next action potential [25]. In the present research, the relatively low concentrations of TTX (0.1 and 0.2 μM) induced a partial inhibitory effect of VGSC. This result is similar to the inhibition of I_{Na} by 18 mM glucose.

TTX (0.1 and 0.2 μM) and 18 mM glucose can improve insulin synthesis (Fig. 8). These results reveal that the partial inhibitory effect of I_{Na} might be involved in improving insulin synthesis. It has also been reported that INS-1 cells release insulin even in the absence of glucose [29, 30], which might be attributed to the activity of Na^+, Ca^{2+} and K^+ channels. It is possible that blocking the Na^+ channels could be inhibiting the release of insulin through its effects on the membrane potential, thus allowing the cell to accumulate insulin. Barnett et al. reported that the application of high TTX concentration (1 μM) completely inhibits I_{Na} and insulin synthesis [26]. This finding is also supported by the results of the present research (Fig. 8). We hypothesized that high TTX concentration completely inhibits VGSC activity and impairs the health of INS-1 cells. This assumption was confirmed by the following study on cell viability (Fig. 8B). As shown in Fig. 8B, low TTX concentrations (0.1 and 0.2 μM) do not damage cell viability within 1 h. However, high TTX concentration (1 μM) significantly inhibits cell survival and thus impairs cell health within 1 h of application. These results also suggest that low TTX concentrations increase the insulin content via the partial inhibition on I_{Na} without promoting cell proliferation. Hirart and Matteson reported that TTX (0.2 μM) partially inhibits glucose-induced insulin secretion [31], TTX may enhance inhibition of VGSC by glucose, leading to decrease insulin secretion. Furthermore, some studies reported that high VGSC activity may be predisposed to β-cell death after inflammation [28, 32]. Thus, glucose-induced partial inhibition on VGSC may improve the survival rate of β-cells and enhance insulin synthesis. Glucose modulates the function of β–cell transcription factors and thus tightly regulates insulin synthesis [33, 34]. The results of the present study reveal that the application of high glucose concentration at 18 mM can increase the insulin content in INS-1 cells, and the mechanism through the partial inhibition on I_{Na} channel is involved. The mechanism of glucose-modulating insulin synthesis is complex. However, through our

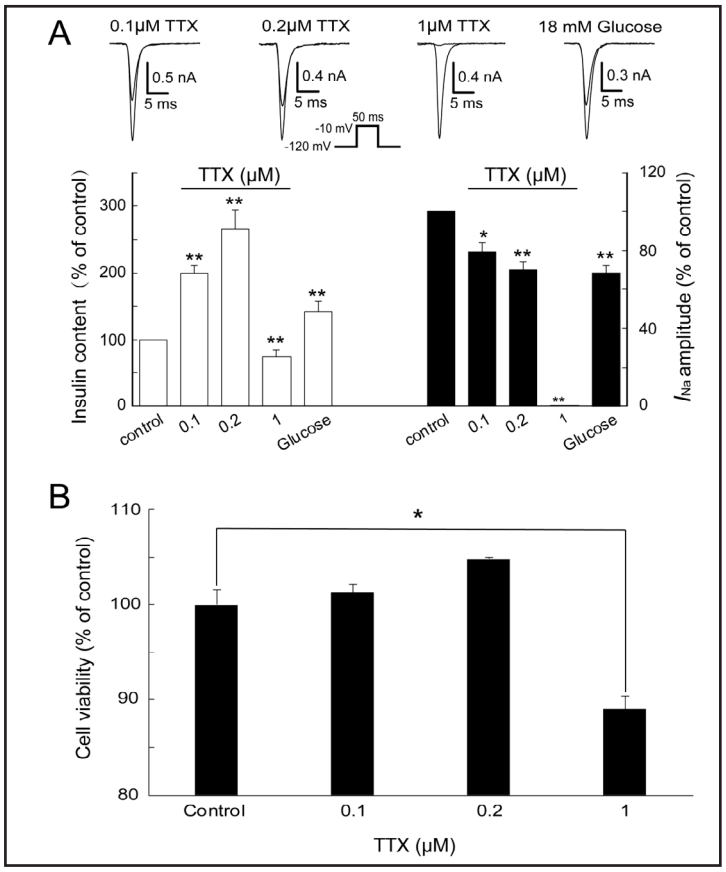

Fig. 8. Effects of TTX and glucose on the I_{Na} and insulin content. (A) The representative current traces of I_{Na} before and after the application of TTX and glucose are shown above the graph. Right black histogram shows that TTX (0.1 and 0.2 μM) and 18 mM glucose partially inhibited I_{Na}, and 1 μM TTX completely inhibited I_{Na} (n = 5 for each group). Left white histogram displays the changes of insulin content after the application of TTX and glucose. TTX (0.1 and 0.2 μM) and 18 mM glucose improved insulin content, and 1 μM TTX decreased the insulin content compared to control condition (n = 3 for each group). (B) The effect of TTX on INS-1 cell viability. Low TTX concentration (0.1 and 0.2 μM) does not affect cell survival, whereas high TTX concentration (1 μM) significantly decreases cell viability (n = 4 for each group); * P<0.05, ** P<0.01 vs. control. Control group contains 2.8 mM glucose.

results, we observed that the partially inhibitory effect of I_{Na} is involved in the process of insulin synthesis. Definite modulating mechanism of I_{Na} involved in insulin synthesis will be further investigated in our future work.

Conclusion

In conclusion, the present work is the first to present direct evidence on the role of VGSC in INS-1 cell response to changes in extracellular glucose levels. We observed that 18 mM glucose can partially inhibit VGSC activity and improve insulin synthesis. Considering that the application of relatively low TTX concentration can partially reduce VGSC currents and improve insulin synthesis, we concluded that glucose application can increase the insulin synthesis in INS-1 cells, and the partial inhibition on I_{Na} channel is involved. Our results innovatively suggest that VGSC plays a vital role in modulating glucose homeostasis.

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Disclosure Statement

All authors declare that they have no Disclosure Statements.

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