

Voltage-gated potassium channels are involved in oxymatrine-regulated islet function in rat islet β cells and INS-1 cells

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ABSTRACT

Objective(s): Oxymatrine can regulate glucose metabolism. But the underlying mechanisms remain unclear. We investigated the relationship of oxymatrine and voltage-gated potassium (Kv) channel in rat islet β cells and INS-1 cells.

Materials and Methods: Insulin secretion and Kv channel currents were tested by radioimmunoassay and patch-clamp technique, respectively. The INS-1 cell viability was detected using cell counting kit-8 experiments. Flowcytometry analysis and western blot were employed for cell apoptosis and protein levels, respectively. INS-1 cell proliferation was assessed by the 5-Ethynyl-2'-deoxyuridine method.

Results: Oxymatrine potentiated insulin secretion at high glucose ($P<0.01$ vs 11.1 G, $P<0.01$ vs 16.7 G) and inhibited KV currents at 40 mV (45.73 ± 15.34 pA/pF for oxymatrine, 73.80 ± 19.23 pA/pF for control, $P<0.05$). After the INS-1 cells were treated with oxymatrine for 12 and 24 hr, KV2.1 channel protein was up-regulated ($P<0.01$ vs Control). At the same time, compared with the high glucose and high fat group, cell viability and proliferation ability were increased ($P<0.01$). The cell apoptotic rate was reduced, reaching $17.30\%\pm 1.00\%$ at 12 hr and $10.35\%\pm 1.52\%$ at 24 hr ($P<0.01$). These protective effects of oxymatrine were reversed by using Stromatoxin-1, a kv channel inhibitor.

Conclusion: The results indicate that oxymatrine can stimulate insulin secretion and decrease kv channel currents in islet β cells. Besides, oxymatrine also increases cell viability, proliferation, and reduces cell apoptosis in INS-1 cells. The effects of oxymatrine are related to kv channels. This finding provides new insight into the mechanisms of oxymatrine-regulated islet function.

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Introduction

Diabetes mellitus is a chronic progressive disease with high incidence. It is characterized by insufficient insulin secretion and/or cell dysfunction. It can lead to disability and death. According to a report by the International Diabetes Federation, the number of people with diabetes is on the rise in 2019 worldwide. The average growth rate is 51% (1). In diabetes, type 2 diabetes mellitus (T2DM) accounts for 90% (2). The etiology and pathogenesis are not fully understood. The basic pathophysiology is insulin resistance and pancreatic β cell hypofunction. How to control blood glucose, promote β cell proliferation, and inhibit β cell apoptosis is an important basis for treatments of T2DM.

Depending on the biophysical properties, potassium (K^+) channels are classified into different families and subfamilies (3). Delayed rectifiers, such as Kv1.2, Kv2.1, and Kv3.1, have higher activation thresholds and exhibit very slow inactivation (4, 5). In rat islet β cells, glucose increases the intracellular ATP levels. The resultant closure of ATP-sensitive potassium (K_{ATP}) channels causes cell membrane depolarization and activates voltage-gated calcium (Ca_v) channels. Subsequently, Ca^{2+} enters the cells and insulin secretion is promoted (6-8). Meanwhile, membrane depolarization also activates the

voltage-gated potassium (Kv) channels. The outflow of K^+ leads to cell membrane repolarization, which prevents Ca^{2+} entry through voltage-gated calcium channels and inhibits insulin secretion (7, 9, 10).

In rodents, many studies have indicated that the repolarization of pancreatic β cell action potentials is largely mediated by Kv2.1 (11). For example, a study demonstrated that adenovirus-mediated expression of a C-terminal truncated Kv2.1 subunit, specifically eliminating Kv2 family currents, inhibits delayed rectifier currents by 60–70% in rat islets cells. Glucose-stimulated insulin secretion from rat islets is increased by 60% (12). In Kv2.1^{-/-} C57BL/6 mice, glucose-stimulated action potentials recorded by the perforated patch-clamp show a significantly prolonged duration compared to control islets at high glucose concentration (14.4 mmol/l) (11).

Kv2.1 is also involved in the apoptosis processes of neuron and pancreatic β cells (13-15). Kv channels participate in staurosporine or thapsigargin-induced β cell apoptosis. Overexpression of Kv2.1 in INS 832/13 cells potentiate apoptosis in response to mitochondrial and endoplasmic reticulum stress (13). SP6616 (a Kv2.1 inhibitor) suppresses the streptozocin-induced INS 832/13 cell apoptosis in MTT assay. In addition,

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Western blot experiments show that SP6616 inhibits the streptozocin-induced increases in both the protein level of cleaved caspase 3 and the activity of caspase 3/7 (15).

Oxymatrine, the main component of a traditional Chinese herb, *Sophora flavescens* Ait, has anti-inflammatory, antiviral, liver-protecting, anti-tumor, immunomodulatory, and antioxidant effects (16-19). At present, oxymatrine has been used clinically in China, mainly for the treatment of liver diseases (20, 21). In recent years, some studies have found that oxymatrine can regulate glucose metabolism. Oxymatrine significantly increases the number of pancreatic islets, promotes insulin secretion, and improves insulin sensitivity in diabetic rats (22). After streptozotocin-induced diabetic rats were intragastrically administered oxymatrine for 7 weeks, the blood glucose levels were decreased and insulin levels were increased in a dose-dependent manner (23). Furthermore, oxymatrine has been shown to have anti-apoptotic effects. By reducing the caspase-3 level and increasing Bcl-2/Bax level, oxymatrine can suppress neuronal apoptosis, improve learning function and alleviate cognitive impairment in diabetic rats (24, 25). Oxymatrine also down-regulates the apoptosis of hepatocytes in rats with acute liver injury by inhibiting the TLR4/PI3K/Akt/GSK-3 β pathway (26). It has been indicated that oxymatrine can prevent cardiomyocyte apoptosis and improve the cardiotoxicity induced by doxorubicin and aldosterone in rats (27, 28).

Some studies have proved that oxymatrine can regulate the K⁺ channel. Oxymatrine decreases the peak amplitudes of delayed rectifier K⁺ currents in clonal pheochromocytoma (PC12) cells (29). In HEK293 cells that stably expressed the wild-type human ether-a-go-go-related gene (hERG), oxymatrine inhibits hERG tail current in a concentration-dependent manner (30). But, the role of K_v channels in oxymatrine-regulated islet cell function remains unclear. Therefore, we investigated the relationship of K_v channels and oxymatrine in rat islet β cells and rat insulinoma (INS-1) cells. The results demonstrate that oxymatrine increases insulin secretion, inhibits INS-1 cell apoptosis, promotes INS-1 cell viability and proliferation, which relates to the dual effects of oxymatrine on K_v channels.

Materials and Methods

Animals and cell line

Male Sprague-Dawley (SD) rats (250 \pm 20 g) were supplied by the Animal Experimental Center of Shanxi Medical University (Taiyuan, China). All rats were housed in air-conditioned animal cages (approximately 25 \pm 2 $^{\circ}$ C, 55–60% humidity, 12 hr of light-dark cycles). They could get food and water freely. The animals were carefully treated in accordance with ethical guidelines for Animal Use of Shanxi Medical University (Taiyuan, China). The INS-1 cell line was purchased from Shanghai AiYan Biological Technology Co., LTD (Shanghai, China).

Islet isolation and cell culture

As described in the previous experimental procedure (18, 19), pancreatic islets were acquired from SD rats by Collagenase P (1 mg/ml, Roche, Indianapolis, USA) digestion and histopaque-1077 (Sigma-Aldrich, USA)

density gradient separation. After digestion for 5 min by Dispase II (Roche, Indianapolis, USA), the isolated islets were dispersed to single islet cells. Pancreatic islets and islet cells were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA) containing glucose (11.1 mmol/l, Sangon Biotech Co. LTD., Shanghai, China), fetal bovine serum (10%, Gibco, Grand Island, NY, USA), penicillin (100 U/ml, Sigma-Aldrich, St. Louis, MO), and streptomycin (100 μ g/ml, Sigma-Aldrich, St. Louis, MO) at 37 $^{\circ}$ C in 5% CO₂ humidified atmosphere. The INS-1 cells were maintained in RPMI-1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 μ g/ml), sodium pyruvate (0.11 g/l, Sangon Biotech Co. LTD., Shanghai, China), β -mercaptoethanol (50 μ mol/l, Gibco, Grand Island, NY, USA) and fetal bovine serum (10%) at 37 $^{\circ}$ C in 95% air with 5% CO₂.

Insulin secretion experiments

The islets were pre-incubated with Krebs-Ringer bicarbonate-HEPES (KRBH) buffer containing 2.8 mmol/l glucose for 0.5 hr and the supernatant was discarded. Subsequently, the islets were incubated with KRBH buffer including 2.8 mmol/l, 11.1 mmol/l, or 16.7 mmol/l glucose in the presence or absence of oxymatrine (Sigma-Aldrich, St. Louis, MO) for 0.5 hr. The supernatant was collected and tested for insulin concentration by an Iodine [¹²⁵I] Insulin Radioimmunoassay Kit (Beijing North Institute of Biological Technology, China). The islets in each tube were lysed with 70% acid-ethanol solution (Ethanol/water/HCl (vol/vol) 150:47:3) and measured for insulin content. A composition of Krebs-Ringer bicarbonate-HEPES (KRBH) buffer was as follows (mmol/l): 128.8 NaCl; 1.2 KH₂PO₄; 4.8 KCl; 2.5 CaCl₂ · 2 H₂O; 1.2 MgSO₄; 10 HEPES; 5 NaHCO₃ and 2% bovine serum albumin (Solarboi, Beijing, China) at pH 7.4.

Patch-clamp experiments

In order to record voltage-gated potassium (K_v) channel currents, the solution was prepared. The intracellular solution included (mmol/l): 10 NaCl; 140 KCl; 10 HEPES; 0.05 EGTA; 1 MgCl₂, pH 7.3 adjusted with KOH. The extracellular solution contained (mmol/l): 141.9 NaCl; 5.6 KCl; 1.2 MgCl₂; 11.1 glucose; 5 HEPES, pH 7.4 with NaOH. The electrodes were pulled by Narishige MODEL PP-830 micropipette puller (Narishige Co., Tokyo, Japan) and polished to resistances of 4 to 7 M Ω by MICRO FORGE MF-200 (World Precision Instruments Inc., USA). K_v channel currents were recorded in whole-cell voltage-clamp mode with EPC-10 amplifier and PULSE software (HEKA Elektronik, Lambrecht, Germany) from a holding potential of -70 mV to various test pulses (-70 to 80 mV) in 10 mV steps. Islet β cells were recognized by their membrane capacitance [>7 picofarads (pF)] (31).

CCK-8 assay

Cell counting kit-8 (CCK-8, Beyotime Biotechnology, Shanghai, China) was used to detect the viability of the INS-1 cells. The INS-1 cells were seeded in 96-well plates with a density of 8 \times 10³ cells / well. The INS-1 cells were divided into 4 groups: control group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium (Sigma-Aldrich, St. Louis, MO))

group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) + oxymatrine (10 μ mol/l) group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) + oxymatrine (10 μ mol/l) + stromatoxin-1 (a K_v channel inhibitor, 100 nmol/L, MuseChem, New Jersey, USA) group. After the cells were incubated at 37 °C with 5% CO₂ humidified atmosphere for 12 hr or 24 hr, 10 μ l CCK-8 was added to each well for 4 hr and the optical density (OD) at 450 nm was determined by Berthold LB941 multifunctional microporous reader (Berthold Technologies, Germany).

Flow cytometry analysis

For cell apoptosis detection, Annexin V Alexa Fluor™ 488/Propidium Iodide (PI) kit (Invitrogen, California, USA) was employed. The INS-1 cells were divided into 4 groups: control group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) + oxymatrine (10 μ mol/l) group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) + oxymatrine (10 μ mol/l) + stromatoxin-1 (100 nmol/l) group. After treatment with the corresponding drugs for 12 hr or 24 hr, the cells were collected, washed with PBS, and centrifuged (1000 r/min, 5 min). The supernatant was discarded. Subsequently, the cells were resuspended in binding buffer (1 \times Annexin) to a density of 1×10^6 cells/ml and stained with Alexa Fluor 488 annexin V/PI according to the manufacturer's instructions. After incubation at room temperature for 15 min, the cells were detected by a FACS Calibur flow cytometer (BD Biosciences, USA).

Western blot experiments

The protein levels were tested by Western blot. Anti-Bax antibody (ab32503), Anti-Bcl-2 antibody (ab196495), Anti-K_v2.1 antibody (ab192761) were purchased from Abcam (Cambridge, UK); Anti-caspase-3 antibody (9662) was from Cell Signaling Technology (Boston, USA); β -actin antibody and corresponding secondary antibodies were obtained from Abcam (Cambridge, UK). The intensity of protein bands was determined using Image-Pro Plus 6.0.

5-Ethynyl-2'-deoxyuridine (EdU) assay

INS-1 cell proliferation was assessed by the 5-Ethynyl-2'-deoxyuridine (EdU) Cell Proliferation Detection Kit (Ribo Life Science, Suzhou, China) in accordance with the manufacturer's instructions. In brief, 8×10^3 cells/well were seeded in 96-well plates. INS-1 cells were divided into 4 groups: control group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) group; high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) + oxymatrine (10 μ mol/l) group; and high glucose (30 mmol/l glucose) + high fat (400 μ mol/l palmitic acid sodium) + oxymatrine (10 μ mol/l) + stromatoxin-1 (100 nmol/l) group. After incubation for 24 hr, the cells were treated with EdU (50 μ mol/l) for 2 hr. Then, the cells were fixed, discolored, and stained with Hoechst 33342. Photographs of cells were taken using a laser confocal microscope (LSM800, Carl Zeiss, Germany).

Statistical analysis

All data were presented as mean \pm standard deviation

(SD). Statistical significance was determined using analysis of variance (ANOVA) followed by Tukey's test. $P < 0.05$ was considered significant.

Results

Oxymatrine promotes glucose-stimulated insulin secretion in rat pancreatic islets

We tested the effects of different doses of oxymatrine (1 μ mol/l, 10 μ mol/l, and 100 μ mol/l) on insulin secretion. As shown in Figure 1, oxymatrine (1 μ mol/l, 10 μ mol/l, and 100 μ mol/l) had no effect on insulin secretion at low glucose level (2.8 mmol/l) ($P > 0.05$ vs 2.8 G). In contrast, at high glucose levels (11.1 mmol/l, 16.7 mmol/l), oxymatrine (10 μ mol/l and 100 μ mol/l) promoted insulin secretion ($P < 0.01$ vs 11.1 G, $P < 0.01$ vs 16.7 G). Furthermore, 10 μ mol/l oxymatrine increased insulin secretion (21.69 ± 0.92) by 31% under 11.1 mmol/l glucose conditions (16.52 ± 2.04) ($P < 0.01$ vs 11.1 G) and significantly potentiated insulin secretion (35.62 ± 2.20) by approximately 44% at 16.7 G (24.81 ± 3.46) ($P < 0.01$ vs 16.7 G).

Oxymatrine inhibits K_v channels in rat islet β cells

To study if K_v channels are involved in oxymatrine-regulated glucose-stimulated insulin secretion, we detected K_v channel currents by the whole-cell voltage-clamp technique. All currents recorded were represented as current density [picoampere (pA) /picofarad (pF)]. Figure 2A shows that 10 μ mol/l and 100 μ mol/l oxymatrine inhibited K_v channel currents compared with Control. Oxymatrine (10 μ mol/l) produced approximately 38% inhibition of K_v currents at 40 mV (45.73 ± 15.34 pA/pF for 10 μ mol/l oxymatrine, 73.80 ± 19.23 pA/pF for control, $P < 0.05$ vs Control, Figure 2B).

Oxymatrine increases the expression level of $K_v2.1$ channel protein in INS-1 cells

Compared with the control group, oxymatrine (1 μ mol/l) increased the expression level of $K_v2.1$

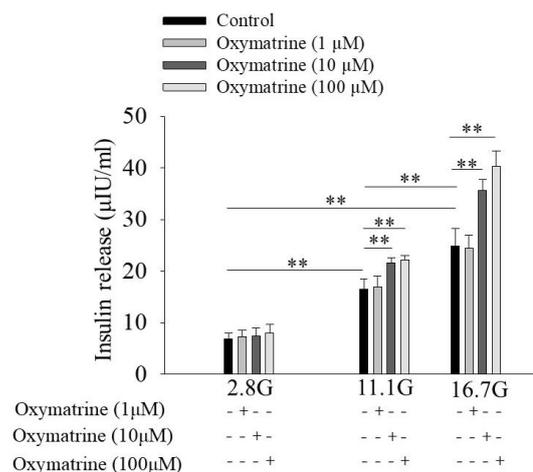


Figure 1. Oxymatrine promotes glucose-stimulated insulin secretion in rat pancreatic islets. Rat islets were incubated in Krebs-Ringer bicarbonate-HEPES buffer containing 2.8 mmol/l glucose (2.8 G), 11.1 mmol/l glucose (11.1 G), or 16.7 mmol/l glucose (16.7 G) in the absence or presence of oxymatrine as indicated. Each tube contains 6 islets, each group contains 7 tubes. ** $P < 0.01$

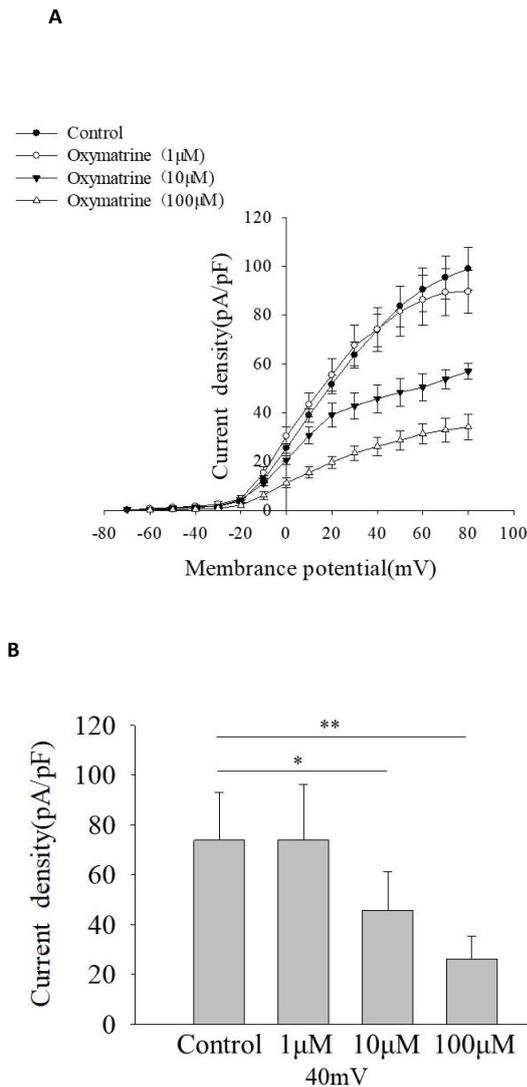


Figure 2. Oxymatrine inhibits kv channels in rat islet β cells. The cells were treated with or without oxymatrine (1 μ mol/L, 10 μ mol/l, and 100 μ mol/l). kv currents were recorded 10 min after different treatments from a holding potential of -70 mV to various test pulses (-70 to 80 mV) in 10 mV steps. (A) Current-voltage curves of Kv channel currents under different treatments. (B) Summary of the mean current density of Kv channel currents at 40 mV (each group represents different cells, n=6 β cells/group), * P <0.05, ** P <0.01

channel protein after INS-1 cells were incubated for 24 hr (Figures 3A, C, P <0.05 vs Control), but no effect at 12 hr (Figures 3A, B, P >0.05 vs Control). In contrast, oxymatrine (10 μ mol/l, 100 μ mol/l) significantly up-regulated $K_v2.1$ channel protein at 12 hr and 24 hr (Figures 3A-C, P <0.01 vs Control). But there was no distinct difference between the two groups (Figure 3A-C, P >0.05).

Oxymatrine promotes cell viability in INS-1 cells under high glucose and high-fat conditions

To evaluate the effect of oxymatrine on INS-1 cell viability, CCK-8 experiments were used. The results indicated that the INS-1 cell viability was significantly reduced in the high glucose and high fat group (P <0.01 vs Control, Figure 4). After the cells were treated with

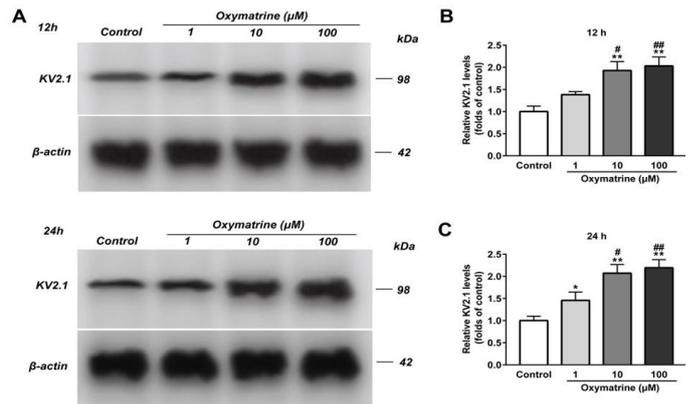


Figure 3. Oxymatrine increases the expression level of kv2.1 channel protein in INS-1 cells. The INS-1 cells were treated with or without oxymatrine (1 μ mol/l, 10 μ mol/l, and 100 μ mol/l). The expression level of kv2.1 channel protein was detected by Western blot assay, (A) Representative Western blot bands of kv2.1 channel protein were shown. (B) Analysis of the amount of kv2.1 channel protein (normalized to control). The experiments were repeated three times. Data represent an average of three experiments, 2×10^5 cells/well. * P <0.05 vs Control, ** P <0.01 vs Control, # P <0.05 vs Oxymatrine (1 μ mol/l), ## P <0.01 vs Oxymatrine (1 μ mol/l)

oxymatrine for 12 and 24 hr, the survival rate was increased (P <0.05 vs HG + PA, Figure 4A; P <0.01 vs HG + PA, Figure 4B). The protective effect of oxymatrine was inhibited by applying a K_v channel inhibitor, stromatoxin-1 (P <0.05 vs HG + PA + oxymatrine, Figure 4).

Oxymatrine increases the proliferation capacity of INS-1 cells in high glucose and high fat

To elucidate if oxymatrine affects the proliferation ability of INS-1 cells in high glucose and high fat, a 5-Ethynyl-2'-deoxyuridine (EdU) assay was performed. Compared with the control group (24.92% \pm 3.35%), the cell proliferation ability was significantly decreased in the

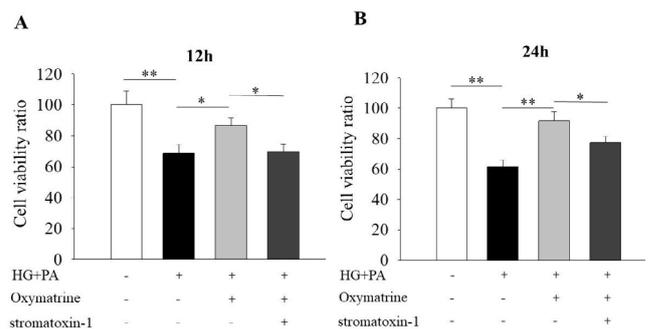


Figure 4. Oxymatrine promotes cell viability in INS-1 cells under high glucose and high-fat conditions. The INS-1 cells were treated with or without oxymatrine (10 μ mol/l) for 12 hr and 24 hr under different conditions. Data are normalized to control. INS-1 cells were preincubated with stromatoxin-1 for 30 min before the addition of oxymatrine, high glucose, and palmitic acid sodium. Then, the INS-1 cells were treated together with all corresponding drugs for 12 or 24 hr. The experiments were repeated three times. Data represent an average of three experiments, 8×10^3 cells/well. Control: not treated; HG: high glucose (30 mmol/l glucose); PA: palmitic acid sodium (400 μ mol/l); stromatoxin-1 (a kv channel inhibitor, 100 nmol/l). * P <0.05, ** P <0.01

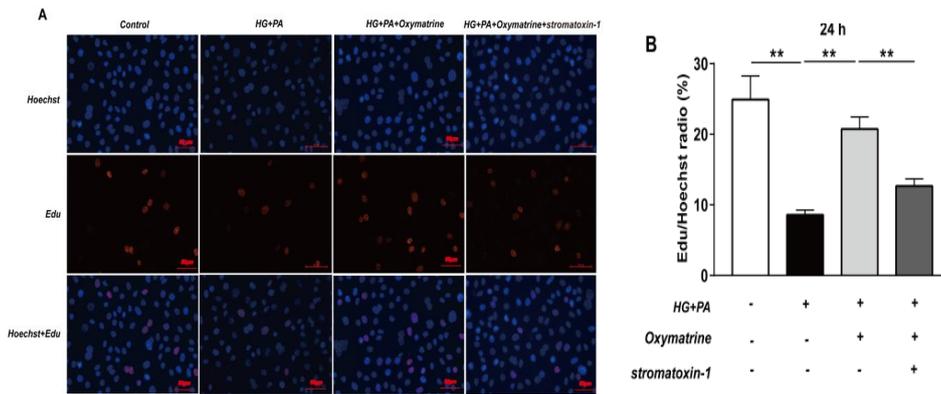


Figure 5. Oxymatrine increases the proliferation capacity of INS-1 cells in high glucose and high-fat. The proliferating nuclei were stained red with EdU and the nuclei of all cells were stained blue with Hoechst 33342. (A) Representative pictures from confocal microscopy were shown. (B) Analysis of the numbers of EdU positive and Hoechst 33342 positive cells. INS-1 cells were preincubated with stromatoxin-1 for 30 min before the addition of oxymatrine, high glucose, and palmitic acid sodium. Then, the INS-1 cells were treated together with all corresponding drugs for 24 hr. The experiments were repeated three times. Data represent an average of three experiments, 8×10^3 cells/well. Control: not treated; HG: high glucose (30 mmol/l glucose); PA: palmitic acid sodium (400 μ mol/l); stromatoxin-1 (100 nmol/l); 5-Ethynyl-2'-deoxyuridine: Edu, $**P < 0.01$

high glucose and high fat group ($8.58\% \pm 0.67\%$) ($P < 0.01$ vs Control, Figure 5). After the cells were incubated with oxymatrine for 24 hr, the proliferation ability was increased ($20.73 \pm 1.73\%$) ($P < 0.01$ vs HG + PA, Figure 5), which could be reversed by using Stromatoxin-1 ($P < 0.01$

vs HG + PA + oxymatrine, Figure 5).

Oxymatrine reduces high glucose and high fat-induced INS-1 cell apoptosis

As shown in Figure 6, the apoptotic rate of INS-1

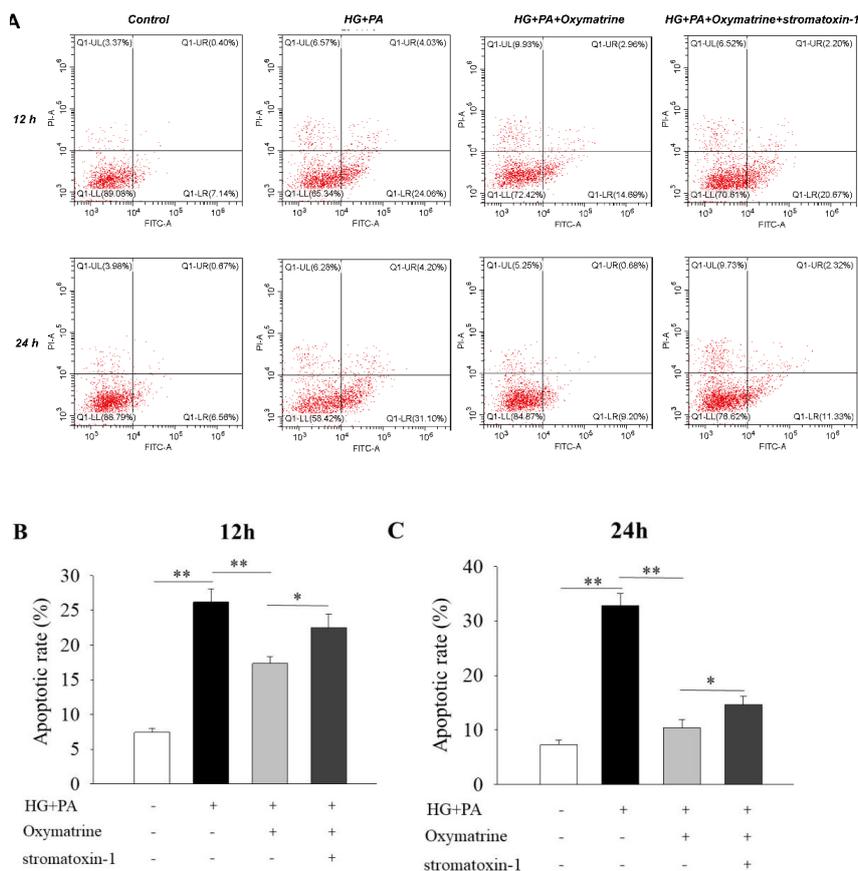


Figure 6. Oxymatrine reduces high glucose and high fat-induced INS-1 cell apoptosis. (A) Representative pictures from flowcytometry analysis were shown. (B) The apoptotic rate analysis of different groups. INS-1 cells were preincubated with stromatoxin-1 for 30 min before the addition of oxymatrine, high glucose, and palmitic acid sodium. Then, the INS-1 cells were treated together with all corresponding drugs for 12 or 24 hr. The experiments were repeated three times. Data represent an average of three experiments, 1×10^6 cells/ml. Control: not treated; HG: high glucose (30 mmol/l glucose); PA: palmitic acid sodium (400 μ mol/l); stromatoxin-1 (100 nmol/l); $*P < 0.05$, $**P < 0.01$

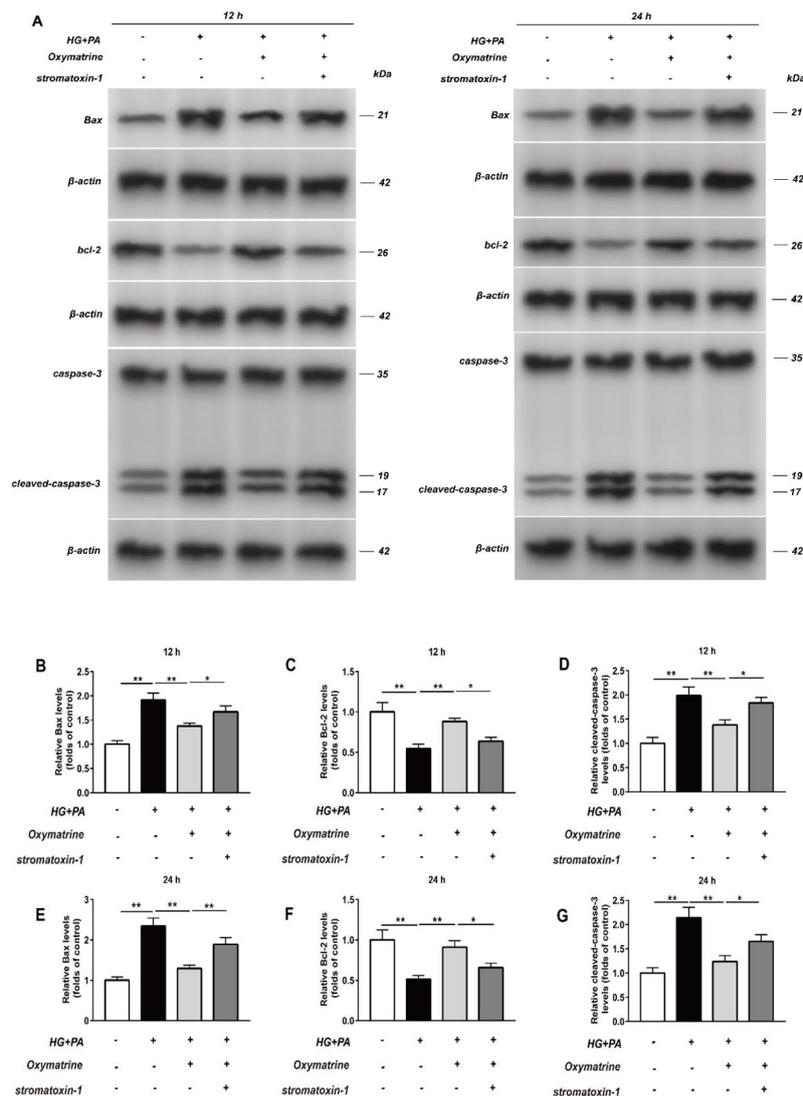


Figure 7. Oxymatrine affects the protein expressions of Bax, Bcl-2, and Caspase-3 in INS-1 cells under high glucose and high-fat conditions. The INS-1 cells were treated with or without oxymatrine (10 $\mu\text{mol/l}$) for 12 hr and 24 hr under different conditions. Data are normalized to Control. INS-1 cells were preincubated with stromatoxin-1 for 30 min before the addition of oxymatrine, high glucose, and palmitic acid sodium. Then, the INS-1 cells were treated together with all corresponding drugs for 12 or 24 hr. The experiments were repeated three times. Data represent an average of three experiments, 2×10^5 cells/well. Control: not treated; HG: high glucose (30 mmol/l glucose); PA: palmitic acid sodium (400 $\mu\text{mol/l}$); stromatoxin-1 (100 nmol/l); (A) Representative Western blot bands of Bax, Bcl-2 and Caspase-3 were shown. (B, E) Analysis of the amount of Bax protein. (C, F) Analysis of the amount of Bcl-2 protein. (D, G) Analysis of the amount of Caspase-3 protein. * $P < 0.05$, ** $P < 0.01$

cells in high glucose and high-fat groups was obviously increased compared with the control group ($P < 0.01$ vs Control, Figure 6). After the cells were treated with oxymatrine for 12 hr and 24 hr, the apoptotic rate was significantly reduced, reaching $17.30\% \pm 1.00\%$ at 12 hr and $10.35\% \pm 1.52\%$ at 24 hr ($P < 0.01$ vs HG + PA, Figures 6 B, C). By employing Stromatoxin-1, the effect of oxymatrine on INS-1 cell apoptosis was suppressed ($P < 0.05$ vs HG + PA + oxymatrine, Figures 6 B, C).

Oxymatrine affects the protein expressions of Bax, Bcl-2, and Caspase-3 in INS-1 cells under high glucose and high-fat conditions

As indicated, the protein expressions of Bax, Bcl-2, and Caspase-3 from different groups were detected by Western blot assay. In the high glucose and high fat group, the protein expressions of Bax and Caspase-3

were significantly increased ($P < 0.01$ vs Control, Figures 7A, B, D, E, and G), but the Bcl-2 level was decreased ($P < 0.01$ vs Control, Figures 7A, C, and F). As expected, after the cells were incubated with oxymatrine for 12 and 24 hr, Bax and Caspase-3 protein levels were reduced ($P < 0.01$ vs HG + PA, Figures 7A, B, D, E, and G). At the same time, the Bcl-2 level was increased ($P < 0.01$ vs HG + PA, Figures 7A, C, F). The effects could be reversed by adding Stromatoxin-1 ($P < 0.01$ vs HG + PA + oxymatrine, Figure 7E; $P < 0.05$ vs HG + PA + oxymatrine, Figures 7B, C, D, F, and G)

Discussion

In the current study, we investigated the role of oxymatrine in rat islet cells. The results indicated that oxymatrine stimulated insulin secretion in isolated rat islets, which was similar to the previous report (22,

23). Furthermore, we found that oxymatrine-regulated insulin secretion depended on glucose concentrations. Because oxymatrine increased insulin secretion under high glucose conditions (11.1 mmol/l and 16.7 mmol/l) but had no effect at low glucose (2.8 mmol/l). We also studied the role of oxymatrine in INS-1 cells. Our data showed that oxymatrine promoted cell viability and proliferation, and suppressed cell apoptosis in INS-1 cells incubated with high glucose and high fat. Oxymatrine decreased the protein levels of Bax and Caspase-3, and increased Bcl-2 level, which suggests that these apoptosis-associated proteins are involved in oxymatrine-inhibited INS-1 cell apoptosis.

K_v channels are related to insulin secretion, cell proliferation, and apoptosis. The insulinotropic effects of Kv channel inhibition depend on glucose concentrations (9,32), which is in line with our secretion results. Therefore, we tested if K_v channels affected the effects of oxymatrine on isolated rat islets β cells and INS-1 cells. The results showed that oxymatrine suppressed the $K_v2.1$ channel in rat islet β cells, but increased the expression level of $K_v2.1$ protein in INS-1 cells. We have no immediate explanation for this discrepancy. It is likely explained by acute and chronic treatment of oxymatrine. Studies have found that the $K_v2.1$ channel may have acute and chronic manifestations in different conditions. There may be oxidation of $K_v2.1$ channels and reduction of currents immediately following oxidative damage (33, 34), followed by soluble NSF attachment receptor (SNARE)-dependent trafficking of $K_v2.1$ channels to the plasma membrane, resulting in the increase of K^+ current (35, 36). Similarly, oxymatrine also shows acute and chronic effects on K^+ channels. A study recorded delayed rectifier K^+ channel from clonal pheochromocytoma (PC12) cells with automatic patch-clamp. Each concentration of oxymatrine was added once to the cells and lasted at least 300 sec until currents reached equilibrium. The results show that oxymatrine decreases the peak amplitudes of delayed rectifier K^+ currents in PC12 cells (29). Hu *et al.* also studied the acute effect of oxymatrine on the HEK293 cells that stably expressed the wild-type hERG gene. Oxymatrine at 30 °C inhibits hERG tail current in a concentration-dependent manner (cells were superfused continuously for about 10 min) (30). But a study indicates that the acute application of oxymatrine has no effect on probucol-induced hERG deficiency (data not shown). This demonstrates that the acute effects of oxymatrine may depend on the regulation of the kinetics of hERG channel gating (37). Zhang *et al.* investigated the effects of oxymatrine on the rapidly activating, delayed rectifier potassium channel (IKr) encoded by hERG. They show that the expression of the hERG protein and hERG currents are increased when hERG-human embryonic kidney (HEK 293) cells (a stably transfected HEK 293 cell line expressing high-level functional hERG) were cultured for 24 hr in the presence of oxymatrine (1 μ mol/l) (38).

It seems that oxymatrine cannot be described as a Kv channel inhibitor or a Kv channel activator. Research proved that Ca^{2+} influx through voltage-gated Ca^{2+} channels contributes to β cell apoptosis in mouse primary β cells and in a pancreatic β cell line (39). Ca^{2+} has similar effects on pancreatic β cells from ob/

ob mice treated with high concentrations of glucose (40). Activation of L-type Ca^{2+} channels is related to DNA fragmentation characteristic of apoptosis and the blockers of these channels suppress endonuclease activation (41). When K^+ channels are activated, the cell membrane is hyperpolarized and Ca^{2+} is prevented from entering the cell through a voltage-dependent Ca^{2+} channel (3). At the same time, glucose-stimulated insulin secretion was compromised (9).

On the other hand, cell shrinkage is an early prerequisite for apoptosis, and intracellular K^+ loss is a major cause for cell shrinkage because K^+ is the primary cation inside the cell determining intracellular osmolarity. Increased K^+ efflux has been proven to be related to the early stage of apoptosis in many cell types (3, 42-44). As previously discussed by Chu *et al.*, increased expression of the Kv channel may be a protective mechanism in the β cell to inhibit insulin exhaustion and cell death but only for a period of time. The β cells treated with high glucose for more than 7 days showed irregular cell shape (45).

Activation of K^+ channels prevents Ca^{2+} from entering the cell, which inhibits cell proliferation (3). But in tumor cells, the effects of K^+ channels-promoted cell proliferation are observed (46-49). One explanation is that membrane hyperpolarization increases the driving force for Ca^{2+} entry into the cell and subsequently modulates cell proliferation (3). It is unclear whether the same mechanism operates in other cells. Therefore, the complicated relationships between oxymatrine and K^+ channels, and β cell growth remain to be further studied.

There is a limitation in our study. In the insulin secretion and electrophysiological experiments, we only observed the acute effects of oxymatrine. The chronic effects of oxymatrine on the electrical activity of the K_v channel and insulin secretion should be investigated.

Conclusion

Taken together, our results demonstrate that oxymatrine enhances insulin secretion, which is related to its influence on $K_v2.1$ channels. In addition, oxymatrine also promotes cell viability and proliferation, and inhibits cell apoptosis in INS-1 cells under high glucose and high-fat conditions, which has a relationship with the $K_v2.1$ channel. The double mechanism of oxymatrine on the $K_v2.1$ channel needs to be further studied.

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Conflicts of Interest

The authors declare no conflicts of interest associated with this manuscript.

References

1. International Diabetes Federation. IDF Diabetes Atlas-9th Edition. <https://diabetesatlas.org/en/>.
2. Ji L, Guo X, Guo L, Ren Q, Yu N, Zhang J. A multicenter evaluation of the performance and usability of a novel glucose

- monitoring system in Chinese adults with diabetes. *J Diabetes Sci Technol* 2017;11:290-295.
3. Wang Z. Roles of K⁺ channels in regulating tumour cell proliferation and apoptosis. *Pflugers Arch* 2004;448:274-286.
 4. González C, Baez-Nieto D, Valencia I, Oyarzún I, Rojas P, Naranjo D, *et al.* K(+) channels: function-structural overview. *Compr Physiol* 2012;2:2087-2149.
 5. Leung YM. Voltage-gated K⁺ channel modulators as neuroprotective agents. *Life Sci* 2010;86:775-780.
 6. Tarasov A, Dusonchet J, Ashcroft F. Metabolic regulation of the pancreatic beta-cell ATP-sensitive K⁺ channel: a pas de deux. *Diabetes* 2004;53 Suppl 3: S113-122.
 7. Prinz P, Goebel-Stengel M, Teuffel P, Rose M, Klapp BF, Stengel A. Peripheral and central localization of the nesfatin-1 receptor using autoradiography in rats. *Biochem Biophys Res Commun* 2016;470:521-527.
 8. Ashcroft FM, Rorsman P. K (ATP) channels and islet hormone secretion: new insights and controversies. *Nat Rev Endocrinol* 2013;9:660-669.
 9. MacDonald PE, Wheeler MB. Voltage-dependent K (+) channels in pancreatic beta cells: role, regulation and potential as therapeutic targets. *Diabetologia* 2003;46:1046-1062.
 10. Maejima Y, Horita S, Kobayashi D, Aoki M, O'hashi R, Imai R, *et al.* Nesfatin-1 inhibits voltage gated K⁺ channels in pancreatic beta cells. *Peptides* 2017;95:10-15.
 11. Jacobson DA, Kuznetsov A, Lopez JP, Kash S, Ammälä CE, Philipson LH. Kv2.1 ablation alters glucose-induced islet electrical activity, enhancing insulin secretion. *Cell Metab* 2007;6:229-235.
 12. MacDonald PE, Ha XF, Wang J, Smukler SR, Sun AM, Gaisano HY, *et al.* Members of the Kv1 and Kv2 voltage-dependent K (+) channel families regulate insulin secretion. *Mol Endocrinol* 2001;15:1423-1435.
 13. Kim SJ, Widenmaier SB, Choi WS, Nian C, Ao Z, Warnock G, *et al.* Pancreatic β -cell prosurvival effects of the incretin hormones involve post-translational modification of Kv2.1 delayed rectifier channels. *Cell Death Differ* 2012;19:333-344.
 14. Pal S, Hartnett KA, Nerbonne JM, Levitan ES, Aizenman E. Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. *J Neurosci* 2003;23:4798-4802.
 15. Zhou TT, Quan LL, Chen LP, Du T, Sun KX, Zhang JC, *et al.* SP6616 as a new Kv2.1 channel inhibitor efficiently promotes β -cell survival involving both PKC/Erk1/2 and CaM/PI3K/Akt signaling pathways. *Cell Death Dis* 2016;7:e2216.
 16. Liu Y, Xu Y, Ji W, Li X, Sun B, Gao Q, *et al.* Anti-tumor activities of matrine and oxymatrine: literature review. *Tumour Biol* 2014;35:5111-5119.
 17. Wang W, Pei X, Xu M, Sun S, Zhang C, Mu K, *et al.* The protective effect of sodium ferulate and oxymatrine combination on paraquat-induced lung injury. *Iran J Pharm Res* 2015;14:573-583.
 18. Zhang YY, Yi M, Huang YP. Oxymatrine ameliorates doxorubicin-induced cardiotoxicity in rats. *Cell Physiol Biochem* 2017;43:626-635.
 19. Xiao TT, Wang YY, Zhang Y, Bai CH, Shen XC. Similar to spironolactone, oxymatrine is protective in aldosterone-induced cardiomyocyte injury via inhibition of calpain and apoptosis-inducing factor signaling. *Plos One* 2014;9:e88856.
 20. Lu LG, Zeng MD, Mao YM, Li JQ, Wan MB, Li CZ, *et al.* Oxymatrine therapy for chronic hepatitis B: a randomized double-blind and placebo-controlled multi-center trial. *World J Gastroenterol* 2003;9:2480-2483.
 21. Wang YP, Zhao W, Xue R, Zhou ZX, Liu F, Han YX, *et al.* Oxymatrine inhibits hepatitis B infection with an advantage of overcoming drug-resistance. *Antiviral Res* 2011;89:227-231.
 22. Guo C, Zhang C, Li L, Wang Z, Xiao W, Yang Z. Hypoglycemic and hypolipidemic effects of oxymatrine in high-fat diet and streptozotocin-induced diabetic rats. *Phytomedicine* 2014;21:807-814.
 23. Huang Y, Li X, Zhang X, Tang J. Oxymatrine ameliorates memory impairment in diabetic rats by regulating oxidative stress and apoptosis: involvement of NOX2/NOX4. *Oxid Med Cell Longev* 2020;2020:3912173.
 24. Wang SB, Jia JP. Oxymatrine attenuates diabetes-associated cognitive deficits in rats. *Acta Pharmacol Sin* 2014;35:331-338.
 25. Zhao P, Zhou R, Li HN, Yao WX, Qiao HQ, Wang SJ, *et al.* Oxymatrine attenuated hypoxic-ischemic brain damage in neonatal rats via improving antioxidant enzyme activities and inhibiting cell death. *Neurochem Int* 2015;89:17-27.
 26. Zhang X, Jiang W, Zhou AL, Zhao M, Jiang DR. Inhibitory effect of oxymatrine on hepatocyte apoptosis via TLR4/PI3K/Akt/GSK-3 β signaling pathway. *World J Gastroenterol* 2017;23:3839-3849.
 27. Zhang YY, Yi M, Huang YP. Oxymatrine ameliorates doxorubicin-induced cardiotoxicity in rats. *Cell Physiol Biochem* 2017;43:626-635.
 28. Xiao TT, Wang YY, Zhang Y, Bai CH, Shen XC. Similar to spironolactone, oxymatrine is protective in aldosterone-induced cardiomyocyte injury via inhibition of calpain and apoptosis-inducing factor signaling. *Plos One* 2014;9:e88856.
 29. Wang Y, Yuan J, Yuan X, Wang W, Pei X, Zhao Q, *et al.* Observation of antinociceptive effects of oxymatrine and its effect on delayed rectifier K⁺ currents (Ik) in PC12 cells. *Neurochem Res* 2012;37:2143-2149.
 30. Hu MQ, Dong ZX, Zhao WX, Sun J, Zhao X, Gu DF, *et al.* The novel mechanism of oxymatrine affecting HERG currents at different temperatures. *Cell Physiol Biochem* 2010;26:513-522.
 31. Göpel S, Kanno T, Barg S, Galvanovskis J, Rorsman P. Voltage gated and resting membrane currents recorded from B-cells in intact mouse pancreatic islets. *J Physiol* 1999;521 Pt3:717-728.
 32. MacDonald PE, Sewing S, Wang J, Joseph JW, Smukler SR, Sakellaropoulos G, *et al.* Inhibition of Kv2.1 voltage-dependent K⁺ channels in pancreatic beta-cells enhances glucose-dependent insulin secretion. *J Biol Chem* 2002;277:44938-44945.
 33. Cotella D, Hernandez-Enriquez B, Wu X, Li R, Pan Z, Leveille J, *et al.* Toxic role of K⁺ channel oxidation in mammalian brain. *J Neurosci* 2012;32:4133-4144.
 34. Wu X, Hernandez-Enriquez B, Banas M, Xu R, Sesti F. Molecular mechanisms underlying the apoptotic effect of KCNB1 K⁺ channel oxidation. *J Biol Chem* 2013;288:4128-4134.
 35. Pal S, Takimoto K, Aizenman E, Levitan E. Apoptotic surface delivery of K⁺ channels. *Cell Death Differ* 2005;13:661-667.
 36. Shah NH, Aizenman E. Voltage-gated potassium channels at the crossroads of neuronal function, ischemic tolerance, and neurodegeneration. *Transl Stroke Res* 2014;5:38-58.
 37. Shi YQ, Fan P, Zhang GC, Zhang YH, Li MZ, Wang F, *et al.* Probuco-induced hERG channel reduction can be rescued by matrine and oxymatrine *in vitro*. *Curr Pharm Des* 2020;25:4606-4612.
 38. Zhang Y, Dong Z, Jin L, Zhang K, Zhao X, Fu J, *et al.* Arsenic trioxide-induced hERG K(+) channel deficiency can be rescued by matrine and oxymatrine through up-regulating transcription factor Sp1 expression. *Biochem Pharmacol* 2013;85:59-68.
 39. Juntti-Berggren L, Larsson O, Rorsman P, Ammälä C, Bokvist K, Wähländer K, *et al.* Increased activity of L-type Ca²⁺ channels exposed to serum from patients with type I diabetes. *Science* 1993;261:86-90.
 40. Efanova IB, Zaitsev SV, Zhivotovsky B, Köhler M, Efendić

- S, Orrenius S, *et al.* Glucose and tolbutamide induce apoptosis in pancreatic beta-cells. A process dependent on intracellular Ca^{2+} concentration. *J Biol Chem* 1998;273:33501-33507.
41. Chandra J, Zhivotovsky B, Zaitsev S, Juntti-Berggren L, Berggren PO, Orrenius S. Role of apoptosis in pancreatic beta-cell death in diabetes. *Diabetes* 2001;50 Suppl 1:S44-S47.
42. Hughes FM Jr, Cidlowski JA. Potassium is a critical regulator of apoptotic enzymes *in vitro* and *in vivo*. *Adv Enzyme Regul* 1999;39:157-171.
43. Bortner CD, Hughes FM Jr, Cidlowski JA. A primary role for K^+ and Na^+ efflux in the activation of apoptosis. *J Biol Chem* 1997;272:32436-32442.
44. Bortner CD, Cidlowski JA. Caspase independent/dependent regulation of K^+ (+), cell shrinkage, and mitochondrial membrane potential during lymphocyte apoptosis. *J Biol Chem* 1999;274:21953-21962.
45. Chu KY, Cheng Q, Chen C, Au LS, Seto SW, Tuo Y, *et al.* Angiotensin II exerts glucose-dependent effects on K_v currents in mouse pancreatic beta-cells via angiotensin II type 2 receptors. *Am J Physiol Cell Physiol* 2010;298:C313-323.
46. Abdul M, Santo A, Hoosein N. Activity of potassium channel-blockers in breast cancer. *Anticancer Res* 2003;23:3347-3351.
47. Rouzair-Dubois B, Gérard V, Dubois JM. Involvement of K^+ channels in the quercetin-induced inhibition of neuroblastoma cell growth. *Pflugers Arch* 1993;423:202-205.
48. Rouzair-Dubois B, Dubois JM. A quantitative analysis of the role of K^+ channels in mitogenesis of neuroblastoma cells. *Cell Signal* 1991;3:333-339.
49. Van Coppenolle F, Skryma R, Ouadid-Ahidouch hr, Slomianny C, Roudbaraki M, Delcourt P, *et al.* Prolactin stimulates cell proliferation through a long form of prolactin receptor and K^+ channel activation. *Biochem J* 2004;377:569-578.