Role of extracellular signal-regulated kinase 1/2 signal transduction pathway in insulin secretion by β-TC6 cells

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Abstract. The present study aimed to investigate the role of the extracellular signal-regulated kinase (ERK)1/2 signal transduction pathway in glucose-stimulated insulin secretion in β-TC6 mouse pancreatic cells. Insulin production by β-TC6 cells was stimulated with various concentrations of glucose, which was dose-dependently inhibited by mitogen-activated protein kinase inhibitor PD98059, as indicated by western blot analysis. Furthermore, glucose stimulation enhanced the phosphorylation of ERK1/2, which was dose-dependently inhibited by PD98059, as indicated by western blot analysis. These results indicated that the activation of the ERK1/2 signal transduction pathway may have an important role in glucose-stimulated insulin secretion in β-TC6 cells.

Introduction

Diabetes is a common disease that poses a serious threat to human health and has an increasing incidence (1). As a metabolic disorder, diabetes results from an inadequate amount of functional β-cells (2). In type 1 diabetes (T1D), β-cells are destroyed by the immune system (3), while type 2 diabetes (T2D) is associated with insulin resistance and β-cell dysfunction (4). The reduction of the amount of β cells is a common feature of T1D as well as T2D (5). According to Kim and Lee (6), apoptosis of islet β-cells has a key role in the pathogenesis of diabetes. Glucose-stimulated insulin secretion is one of the important physiological characteristics of islet β-cells. Extracellular-regulated protein kinase 1/2 (ERK1/2) is an important member of the mitogen-activated protein kinase (MAPK) family. It is activated by multiple extracellular stimuli and regulates cell growth, proliferation, differentiation and death (7). A previous study showed that glucose stimulation can activate the ERK1/2 signal transduction pathway in islet β-cells, while the role of ERK1/2 activation in insulin secretion has remained elusive (8). The present study aimed to investigate the role of the ERK1/2 signal transduction pathway in glucose-stimulated insulin secretion in β-TC6 mouse pancreatic cells.

Subjects and methods

Cell culture. β-TC6 mouse pancreatic cells (Shanghai Cell Bank of Chinese Academy of Sciences, Beijing, China) were cultured in high-glucose Dulbecco’s modified Eagle’s medium (HyClone, Logan, UT, USA) with fetal bovine serum, streptomycin and penicillin (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China) at 37°C in a humidified atmosphere containing 5% CO2. The medium was replaced once every three days. The cells were passaged at a split ratio of 1:2 every 7-10 days. The survival rate was >90% according to trypan blue staining.

Glucose-stimulated insulin secretion. β-TC6 cells were digested with 0.25% trypsin and 0.01% ethylenediaminetetraacetic acid (HyClone). The single-cell suspension (4x10^4/ml) was seeded into 24-well plates. After 48 h of growth, the cells were washed by phosphate-buffered saline (PBS; Fuzhou Maixin Biotechnology Development Co., Ltd.) and then...
cultured in serum- and sugar-free KRHB medium (4) (NaCl, 129 mM; KCl, 4.8 mM; NaHCO₃, 5 mM; MgSO₄, 1.2 mM; CaCl₂, 2 M) at 37°C for 30 min. The cells were washed with KRHB medium and further cultured at 37°C for 60 min in KRHB medium containing glucose at a concentration of 0, 1.38, 5.5 or 11.1 mM, respectively (9). Finally, the cell supernatant was collected and the insulin concentration was measured using a radioimmunoassay.

Intervention with MAPK inhibitor PD98059. β-TC6 cells were seeded into six-well plates and incubated for 47.5 h. The MAPK inhibitor PD98059 (Cell Signaling Technology, Inc., Danvers, MA, USA) was added to yield a final concentration of 2, 10 or 50 µM, followed by culture for 30 min. After washing with PBS, the cells were incubated in KRHB medium containing 1.38 mM glucose for 60 min. The cells were then lysed in lysis buffer (Fuzhou Maixin Biotechnology Development Co., Ltd.) and the level of phosphorylated ERK1/2 was measured by western blot analysis. Furthermore, insulin secretion was measured in the supernatant of centrifuged lysate using a radioimmunoassay.

Detection of insulin concentration in the cell supernatant. The insulin concentration in culture supernatant was detected using a radioimmunoassay employing a GC-1200 γ-radioimmunoassay instrument (USTC Chuangxin Co., Ltd., Hefei, China) and an insulin radioimmunoassay kit (Science and Technology Center, Beijing PLA General Hospital, Beijing, China) according to the manufacturer's instructions. Each group comprised three tubes (the same supernatant aliquoted into three tubes) and the average value was used as result.

Detection of ERK1/2 phosphorylation levels. The level of ERK1/2 phosphorylation was detected by western blot analysis. After culture with different concentrations of glucose and optionally with PD98059, the β-TC6 cells were collected and lysed with radioimmunoprecipitation assay buffer mixed with protein phosphatase inhibitor (Fuzhou Maixin Biotechnology Development Co., Ltd.), followed by centrifugation at 1,049 x g at 4°C for 5 min. The cell supernatant was collected and the protein concentration was determined. Equal amounts of protein were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis for 1.5 h to separate the protein, followed by electrotransfer onto a nitrocellulose membrane (Sigma-Aldrich St. Louis, MO, USA) and blocked at room temperature for 2 h. After washing of the membrane with PBS, it was incubated with NBT-BCIP solution (Fuzhou Maixin Biotechnology Development Co., Ltd.) in the dark. Images were captured and analyzed using the LAS3000 imaging system (Fuji Film Co., Tokyo, Japan).

Statistical analysis. Values are expressed as the mean ± standard deviation. SPSS 11.5 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. One-way analysis of variance was performed for comparison among multiple groups. The independent-samples t-test was used for comparison between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Glucose dose-dependently induces insulin secretion by β-TC6 cells. The insulin levels in the supernatants of β-TC6 cells treated with 1.38, 5.5 and 11.1 mM glucose were 151.0 ± 14.34, 130.67 ± 11.35 and 129.05 ± 4.71 µU/ml, respectively, which were significantly elevated compared with those in the 0 mM glucose group (119.77 ± 3.89 µU/ml; P<0.01 or P<0.05). In addition, the insulin levels in the 1.38 mM glucose group were significantly higher than those in the 11.1 mM glucose group (P<0.05) (Fig. 1).

MAPK inhibitor PD98059 dose-dependently reduces ERK1/2 phosphorylation induced by insulin. After intervention with 2, 10 and 50 µM PD98059, the levels of ERK1/2 phosphorylation induced by 1.38 mM glucose stimulation were decreased in a dose-dependent manner (Fig. 3). Furthermore, in the 1.38 mM glucose + 50 µM PD98059 group, the phosphorylation of ERK1/2 was almost completely inhibited and below the level in the untreated control group. β-actin was used as the intrinsic parameter to evaluate the amount of protein.

PD98059 inhibits glucose-induced insulin secretion by β-TC6 cells. As shown in Fig. 4, PD98059 suppressed glucose-stimulated insulin secretion by β-TC6 cells in a dose-dependent manner. The insulin levels in the 1.38 mM glucose + 10 µM PD98059 group and the 1.38 mM glucose + 50 µM PD98059 group were 128.27 ± 6.07 and 104.10 ± 9.83 µU/ml, respectively, which were significantly lower than those in the 1.38 mM glucose + 0 µM PD98059 group (151.0 ± 14.34 U/ml; P<0.01). The insulin level in the 1.38 mM glucose + 50 µM PD98059 group was lower compared with the untreated group, however, the difference was not significant (P>0.05).

Discussion

β-TC6 cells are derived from the insulinoma cells of a transgenic mouse and express SV40 t-antigen under the control of the insulin promoter. β-TC6 cells produce pro-insulin I and II, and effectively secrete insulin and a small amount of glucagon (10). The threshold of β cells refers to the amount
of glucose required for stimulation of insulin production. The maximum threshold of β-TC6 cells regarding glucose stimulation is lower than that of normal β-cells, with 1.38 mM glucose stimulating insulin secretion at peak levels, which are 1.6 times those of sugar-free insulin levels (stimulation index, 1.6) (9). In the present study, stimulation with 1.38 mM glucose led to insulin secretion at peak levels, which were 1.26 times those of sugar-free insulin levels (stimulation index, 1.26). Insulin secretion was stimulated to a lesser extent by higher concentrations of glucose (5.5 and 11.1 mM), indicating that 1.38 mM was the most suitable glucose concentration to stimulate insulin secretion.

MAPKs are a class of serine/threonine protein kinases which exist in most cells (11). ERK1/2 are important signaling proteins of the MAPK family that can be activated by extracellular stimuli such as ultraviolet irradiation, high osmotic pressure, heat shock and cytokines (12). A previous study reported that glucose stimulation can activate the ERK1/2 signal transduction pathway (13). Longuet et al (14) found that glucose stimulation can activate the ERK1/2 signal transduction pathway in rats, with the degree of activation regulating the concentration of glucose. In addition, glucose stimulation of the INS-1 rat pancreatic β-cell line and the MIN6 mouse pancreatic β-cell line, has been shown to activate the ERK1/2 signal transduction pathway (8). In line with these results, the present study reported that glucose stimulation can activate ERK1/2 signal transduction pathway in β-TC6 mouse pancreatic cells.

The association between the activation of the ERK1/2 signal transduction pathway and insulin secretion in β-TC6 cells has not been previously reported, to the best of our knowledge. A study from 1997 indicated that the ERK1/2 signal transduction pathway is not required for glucose-stimulated insulin secretion (15). However, it has been demonstrated that is found that in MIN6 mouse pancreatic β-cells and primary rat islet β cells, blocking of the ERK1/2 signaling pathway reduced glucose-stimulated insulin secretion (14,16). Furthermore, Vlacich et al (17) reported that the protein kinase Pim3 can inhibit the activation of the ERK1/2 signal pathway through suppressor of cytokine-induced signaling 6 and regulates glucose-stimulated insulin secretion. The present study showed that MAPK inhibitor PD98059 dose-dependently inhibited the activation of the ERK1/2 signaling pathway and decreased insulin secretion stimulated by glucose. Thus, it is concluded that the activation of the ERK1/2 signal transduction pathway is may be associated with insulin secretion in β-TC6 cells. In addition, a previous study (18) indicates that inhibition of the ERK1/2 pathway is associated with the apoptosis of β cells. The apoptosis induced by glucose via this pathway may be an underlying mechanism of diabetes development.
Defects of pancreatic β-cell function and reduced insulin sensitivity are important pathophysiological features during the onset of diabetes (19). When β-cell defects appear, the pancreatic island mass and/or volume cannot steadily maintain the glucose metabolism, resulting in high blood glucose levels (20). The present study indicated the role of the ERK1/2 signal transduction pathway in glucose-stimulated insulin secretion, which may represent an important underlying mechanism of the development of diabetes. However, this mechanism remains to be further elucidated and confirmed by future studies.

Acknowledgements

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References
