Effects of Vaspin on Pancreatic β Cell Secretion via PI3K/Akt and NF-κB Signaling Pathways

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Abstract

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Materials

- Cell culture medium by Contributed by users
- fetal bovine serum by Contributed by users
- ecombinant human vaspin by Contributed by users
- Antibodies against IRS-2, p-IRS-2 (Ser731), Akt, p-Akt (Thr308), mTOR, p-mTOR (Ser2448), p70S6K, p-p70S6K (Thr389), NF-κB P65 by Contributed by users
- Rat insulin ELISA kit by Contributed by users
- Cell Counting Kit-8 by Contributed by users

Protocol

Rat insulinoma cells (INS-1) were cultured in RPMI1640 medium supplemented with 12% fetal bovine serum (FBS), penicillin-streptomycin (500 U/ml), 10mM HEPES, 0.11 g/L sodium pyruvate, and 50 M β-mercaptoethanol in humidified 5% CO2 at 37℃.

Step 1.

For determining the appropriate concentration of vaspin, INS-1 cells were cultured and divided into five groups: (1) control group: treated with culture medium only; (2) PA group: treated with 0.5 mmol/L palmitic acid (PA); (3) PA + vaspin (80 ng/ml): treated with 0.5 mmol/L palmitic acid and 80 ng/ml vaspin; (4) PA + vaspin (160 ng/ml): treated with 0.5 mmol/L palmitic acid and 160 ng/ml vaspin; (5) PA + vaspin (320 ng/ml): treated with 0.5 mmol/L palmitic acid and 320 ng/ml vaspin.

Step 2.

In order to detect whether vaspin can improve insulin secretion function of INS- 1 cell through PI3K/Akt signaling pathway, cells were assigned into three groups: (1) PA group: treated with 0.5 mmol/L palmitic acid; (2) PA + vaspin group: treated with 0.5 mmol/L palmitic acid and 320 ng/ml vaspin; (3) PA + vaspin + ly294002 group: treated with 0.5 mmol/L palmitic acid and 320 ng/ml vaspin and 25 μmol/L ly294002 (PI3K inhibitor).

Step 3.

Based on the biological effects of mTOR/S6K1 signaling pathway, we further explored whether vaspin

can improve cell proliferation of INS-1 cells through mTOR/S6K1 signaling pathway. Cells were assigned into five groups: (1) NC group: treated with serum-free medium; (2) PA group: treated with 0.5 mmol/L palmitic acid; (3) PA + vaspin group: treated with 0.5 mmol/L palmitic acid and 320 ng/ml vaspin; (4) PA + vaspin + Rapamycin group: treated with 0.5 mmol/L palmitic acid and 320 ng/ml vaspin and 25 nmol/L rapamycin; (5) PA + Rapamycin group: treated with 0.5 mmol/L palmitic acid and 25 nmol/L rapamycin (mTOR inhibitor, served as positive control).

Step 4.

In order to determine whether vaspin can improve insulin secretion function of INS-1 cell via NF-κB signaling pathway, cells were assigned into three groups: (1) PA group: treated with 0.5 mmol/L palmitic acid; (2) PA + vaspin group: treated with 0.5 mmol/L palmitic acid and 320 ng/ml vaspin; (3) PA + TPCK group: treated with 0.5 mmol/L palmitic acid and 20 μmol/L TPCK (NF-κB inhibitor, serves as positive control).

Step 5.

Cells were lysed on ice for 10 min in RIPA buffer and then each emulsion was centrifuged (14,000 rpm, 4℃ for 15 min) and supernatant was collected. The protein concentrations were quantified by BCA protein assay kit. The protein of IRS-2, p-IRS-2 (Ser731), Akt, p-Akt (Thr308), mTOR, p-mTOR (Ser2448), p70S6K, p-p70S6K (Thr389), NF-κB P65 were determined by western blot analysis.

Step 6.

Total RNA was extracted from INS-1 cells using the Total RNA Extractor (Trizol) kit. The levels of IRS-2, Akt, NF-κB P65 mRNA were determined by Real-time PCR.

Step 7.

INS-1 cells of same amount (2.5×105 cell/well) were seeded in 24-well culture dishes and treated with glucose (16.7 mmol/L) for 1 h. The concentration of insulin from the supernatant was determined using ELISA kit.

Step 8.

The proliferation activity of INS-1 cells was measured using the Cell Counting Kit-8 (CCK-8).In each experiment, a blank and a negative control group were included. After the intervention, a volume of 10 μL of CCK-8 solution was added to each well 4 hours before the completion of incubation. The viability of the cells was determined using a spectrophotometer plate reader at an absorbance of 450 nm.

Step 9.

As a first step, the rats were randomly assigned into normal diet group (NC group, n=10) and high-fat-diet group (HFD group, n=20) after one week of adaptive feeding. The normal diet contained 57% carbohydrate, 18% protein, and 25% fat; the high-fat diet contained 37% carbohydrate, 13% protein and 50% fat. After 16 weeks of dietary manipulation, rats were randomly divided into high fat diet group (HF group, n=10) and vaspin treatment group (HF + vaspin group, n=10). Rats in the vaspin group were treated with 320 ng/ml (3ml/kg) vaspin intraperitoneally once daily for 4 weeks. The NC group and HF group were given the same volume of normal saline solution (as vehicle control) for the same duration as vaspin. The body weight was recorded weekly. Fasting blood glucose (FBG) was measured biweekly from tail vein using a Freestyle Blood Glucose Meter (Johnson & Johnson, New Jersey, USA).

Step 10.

The oral glucose tolerance test (OGTT) was performed in overnight-fasted rats at the end of intervention. Each experimental animal received a single dose of 1.5 g of 50% glucose solution/kg body weight via gavage. Blood samples were obtained from the tail vein and glucose values were measured by the glucose meter before glucose loading (t=0) and 30, 60, and 120 min after glucose administration. The area under the curve for glucose (AUC glucose) was used to assess the pancreatic

β cell function.

Step 11.

At the end of intervention, overnight-fasted rats were intraperitoneally injected with Recombinant Human Insulin (1 IU/kg body weight; Humulin R, Eli Lilly and Company, Indianapolis, IN, USA). Blood samples were obtained from the tail vein. The glucose values were measured with the glucose meter before loading (t=0) and 30, 60, 120 min after insulin administration.

Step 12.

Rats were fasted overnight and anesthetized with sodium pentobarbital (50 mg/kg body weight) via intraperitoneal injection. A catheter was placed in the right jugular vein for 50% glucose infusion. Blood samples were taken via tail vein for determination of insulin concentrations. The blood glucose was measured at time 0, immediately after the initial injection of glucose, and continuously monitored at 1, 5, 10, 15 min and every five minutes thereafter until it maintained at (14 ± 0.5) mmol/L for 5 consecutive time points by adjusting the infusion rate of the 50% glucose solution. In addition, approximately 100 l of blood was collected at 0, 1, 5, 10, 15 min and any time point in the steady state. Plasma was obtained by centrifuging blood sample (4500 rpm, 15 min, 4oC) and stored at -20℃. The reaction of islet β cells to glucose and the islet secretion capacity were assessed according to the GIR (glucose infusion rate). GIR(mg/kg·min) = Rate (μl/min) × glucose concentration (g/ml) ÷ 1000 ÷ B.W.(g). The insulin level was used to evaluate bidirectional regulating effects of islet β cells.

Step 13.