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Inhibition of voltage-gated potassium channels mediates uncarboxylated osteocalcin-regulated insulin secretion in rat pancreatic β cells

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Chemical compounds studied in this article:
NaCl (PubChem CID: 5234)
KH_2PO_4 (PubChem CID: 516951)
KCl (PubChem CID: 4873)
$CaCl_2{\cdot}2H_2O$ (PubChem CID: 6093260)
HEPES (PubChem CID: 23831)
Tetraethylammonium chloride (PubChem CID: 5946)
Poly-d-lysine hydrobromide (PubChem CID: 16219815)
EGTA (PubChem CID: 6207)
CsCl (PubChem CID: 24293)
Fluo 4AM (PubChem CID: 4060965)

ABSTRACT

Insulin secretion from pancreatic β cells is important to maintain glucose homeostasis and is regulated by electrical activities. Uncarboxylated osteocalcin, a bone-derived protein, has been reported to regulate glucose metabolism by increasing insulin secretion, stimulating β cell proliferation and improving insulin sensitivity. But the underlying mechanisms of uncarboxylated osteocalcin-modulated insulin secretion remain unclear. In the present study, we investigated the relationship of uncarboxylated osteocalcin-regulated insulin secretion and voltage-gated potassium (K_V) channels, voltage-gated calcium channels in rat β cells. Insulin secretion was measured by radioimmunoassay. Channel currents and membrane action potentials were recorded using the conventional whole-cell patch-clamp technique. Calcium imaging system was used to analyze intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The data show that under 16.7 mmol/l glucose conditions uncarboxylated osteocalcin alone increased insulin secretion and $[Ca^{2+}]_i$, but with no such effects on insulin secretion and $[Ca^{2+}]_i$ in the presence of a K_V channel blocker, tetraethylammonium chloride. In the patch-clamp experiments, uncarboxylated osteocalcin lengthened action potential duration and significantly inhibited K_V currents, but had no influence on the characteristics of voltage-gated calcium channels. These results indicate that K_V channels are involved in uncarboxylated osteocalcin-regulated insulin secretion in rat pancreatic β cells. By inhibiting K_V channels, uncarboxylated osteocalcin prolongs action potential duration, increases intracellular Ca^{2+} concentration and finally promotes insulin secretion. This finding provides new insight into the mechanisms of osteocalcin-modulated insulin secretion.

1. Introduction

Insulin secretion from pancreatic β cells is important in maintaining glucose homeostasis. A series of electrical activities are involved in glucose-stimulated insulin secretion. Metabolism of glucose increases intracellular ATP levels and leads to closure of ATP-sensitive potassium channels. The resultant depolarization activates voltage-gated calcium channels, increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and triggers insulin secretion. Voltage-gated potassium (K_V) channels are also activated by cell membrane depolarization. The outflow of K^+ is involved in the repolarization phase of action potential, which limits Ca^{2+} entry through voltage-gated calcium channels and limits insulin secretion (Ashcroft and Rorsman, 1989; Christensen et al., 2015; Jacobson et al., 2007; Kim et al., 2012; MacDonald et al., 2002b; MacDonald and Wheeler, 2003; Yoshida et al., 2009).

Osteocalcin is an abundant non-collagenous protein in bone. It is composed of 47–51 amino acids and molecular weight is 5200–

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5900 Da. Osteocalcin has high homology in a variety of species, especially three glutamic acid residues and Ca^{2+} binding sites are highly conservative (Hauschka et al., 1982; Lee et al., 2000; Lian et al., 1989).

Osteocalcin is also present in the blood. Blood osteocalcin includes carboxylated, undercarboxylated and uncarboxylated form (Booth et al., 2013; Lombardi et al., 2014). Previous studies have reported that uncarboxylated osteocalcin not only increases the expression of insulin genes (*insulin* 1, *insulin* 2), stimulates β cell proliferation in isolated mouse islets and mouse insulinoma (MIN6) cell line, but also enhances the expression of adiponectin and peroxisome proliferator-activated receptor gamma coactivator-1α in white and brown adipocytes. Furthermore, uncarboxylated osteocalcin improves glucose tolerance, insulin sensitivity and insulin secretion *in vivo* (Ferron et al., 2008, 2012; Ferron and Lacombe, 2014; Pi et al., 2011; Zhou et al., 2013). These findings suggest that uncarboxylated osteocalcin is an important regulator of glucose homeostasis. However, the underlying mechanisms of uncarboxylated osteocalcin-modulated insulin secretion are still not fully understood.

In the present study, we investigated the relationship of uncarboxylated osteocalcin-regulated insulin secretion and ion channels in rats. Our results demonstrate that in rat pancreatic β cells, uncarboxylated osteocalcin promotes insulin secretion by potently inhibiting K_V channels, extending action potential duration and increasing $[Ca^{2+}]_i$.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley (SD) rats (weight, 230 ± 20 g) were used for all experiments and supplied by the Animal Experimental Center of Shanxi Medical University (Taiyuan, PR China). Rats were housed in a climate-controlled animal cages (25 ± 2 °C, 55–60% humidity, 12 h light: 12 h darkness cycle) and could freely get regular chow and water. All procedures involving animals described below were approved by Animal Care and Use Committee of Shanxi Medical University and were carried out in accordance with the ethical guidelines for animal research of Shanxi Medical University.

2.2. Islet isolation and cell culture

Pancreatic islets were obtained from male SD rats by 1 mg/ml collagenase P (Roche, Indianapolis, USA) digestion and histopaque-1077 (Sigma-Aldrich, USA) density gradient separation as described previously (Zhang et al., 2008). Islets were isolated and dispersed to single cells by Dispase II (Roche, Indianapolis, USA) digestion for 5 min. Pancreatic islets or islet cells were cultured in RPMI 1640 medium (HyClone, Beijing, China) containing 11.1 mmol/l glucose, 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin, at 37 °C in a 95% air and 5% CO_2 atmosphere (Zhang et al., 2009).

2.3. Glucose-stimulated insulin secretion in rat pancreatic islets

Insulin secretion was assayed using a Iodine [^{125}I] Insulin Radioimmunoassay Kit (Beijing north institute of biological technology, China) as described previously (Li et al., 2013). Pancreatic islets were cultured for 24 h before the experiments. For insulin assay, Krebs-Ringer bicarbonate-HEPES buffer was prepared with (mmol/l): 128.8 NaCl; 1.2 KH_2PO_4; 4.8 KCl; 2.5 $CaCl_2{\cdot}2H_2O$; 1.2 $MgSO_4$; 10 HEPES; 5 $NaHCO_3$ and 2% bovine serum albumin at pH 7.4. Pancreatic islets were pre-incubated in Krebs-Ringer bicarbonate-HEPES buffer including 2.8 mmol/l glucose for 30 min at 37 °C with 5% CO_2. The incubated supernatant was discarded. Successively, islets were incubated for 30 min at 37 °C with 5% CO_2 in Krebs-Ringer bicarbonate-HEPES buffer including 2.8 mmol/l, 11.1 mmol/l or 16.7 mmol/l glucose in the presence or absence of uncarboxylated osteocalcin (a peptide synthesized by Zoonbio Biotechnology Co., Ltd., Nanjing, China, purity was greater than 95%), tetraethylammonium chloride (TEA, Sigma-Aldrich, USA). Then, the incubated supernatant was collected and stored at −20 °C for insulin levels measurement. The islet pellets in per tube were lysed with 70% acid-ethanol solution (Ethanol/water/HCl (vol/vol)=150:47:3) and stored at −20 °C for test of insulin contents. All data were corrected by corresponding insulin contents.

2.4. Electrophysiology

The islet cells were cultured on Poly-D-lysine hydrobromide (Sigma-Aldrich, USA) coated coverslips for 24 h in medium including 11.1 mmol/l glucose, 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin before experiments. Currents and action potentials were recorded using the conventional whole-cell patch-clamp technique with an EPC-10 amplifier and PULSE software (HEKA Electronik, Lambrecht, Germany) at room temperature. The β cells were recognized by their size (> 7 pF) (Gopel et al., 1999). Patch electrodes were pulled from 1.5 mm-thin-walled outside diameter and 0.84 mm-thin-walled inside diameter borosilicate glass tubes by using a two-stage Narishige MODEL PP-830 micropipette puller (Narishige Co., Tokyo, Japan) and fire polished to resistances of 4–7 MΩ by MICRO FORGE MF-200 (World Precision Instruments Inc., USA). Then the electrodes were loaded with intracellular solution. For K_V currents recordings, the intracellular solution contained (mmol/l): 1 $MgCl_2{\cdot}6H_2O$; 140 KCl; 10 NaCl; 0.3 MgATP; 0.05 EGTA; 10 HEPES, adjusted to pH 7.3 with KOH. K_V extracellular solution consisted of (mmol/l): 138 NaCl; 5 HEPES; 1.2 $MgCl_2{\cdot}6H_2O$; 5.6 KCl; 2.6 $CaCl_2$; 11.1 glucose, pH 7.4 with NaOH. For the recordings of voltage-gated calcium currents, the intracellular solution contained (mmol/l): 1 $MgCl_2{\cdot}6H_2O$; 120 CsCl; 0.05 EGTA; 20 tetraethylammonium chloride; 10 HEPES; and 5 Mg ATP, pH 7.3 with CsOH. Extracellular solution was comprised of (mmol/l): 100 NaCl; 20 $BaCl_2$; 20 tetraethylammonium chloride; 4 CsCl; 5 HEPES; 1 $MgCl_2{\cdot}6H_2O$; and 3 glucose, pH 7.4 with NaOH. In the experiments, Ca^{2+} was replaced with Ba^{2+} in the extracellular solution because Ba^{2+} eliminates Ca^{2+}-dependent inactivation of the voltage-gated calcium channels. In current-clamp recordings, action potentials were elicited with 4 ms, 150 pA currents. Action potential duration was recorded as described previously (Li et al., 2013). The time was analyzed from initiation of the action potential to the membrane potential returned to within 10 mV of the resting membrane potential (Collier et al., 2004). The amplitude was calculated from initiation of the action potential to peak.

2.5. Measurements of $[Ca^{2+}]_i$

The islet cells were cultured on glass coverslips coated by Poly-D-lysine hydrobromide for 24 h before the experiments. Then the cells were incubated in Krebs-Ringer bicarbonate-HEPES buffer with 2 μmol/l Fluo 4AM (Dojindo Laboratories, Japan) for 30 min at 37 °C with 5% CO_2. The composition of the buffer was as follows (mmol/l): 1.2 KH_2PO_4; 128.8 NaCl; 1.2 $MgSO_4$; 4.8 KCl; 2.5 $CaCl_2{\cdot}2H_2O$; 5 $NaHCO_3$; 10 HEPES at pH 7.4. After this, cells were washed twice using the buffer without Fluo 4AM. Imaging was performed using OLYMPUS IX71 inverted microscope and analyzed with MetaFluor software 7.8 (Molecular Devices, USA). Fluorescent dye inside the cells was excitated at 494 nm and the emitted