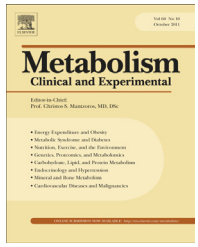
Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com



Calcium/calmodulin-dependent serine protein kinase is involved in exendin-4-induced insulin secretion in INS-1 cells

*Zheng-Qiu Zhu, Dong Wang, Dan Xiang, Yue-Xing Yuan, Yao Wang**

Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, Southeast University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 4 June 2013
Accepted 15 September 2013

Keywords:
CASK
GLP-1
PKA
Epac2

ABSTRACT

Objective. Exendin-4 (Ex-4) is an anti-diabetic drug that is a potent agonist of the glucagon-like peptide-1 (GLP-1) receptor. It has already been approved for the treatment of type 2 diabetes mellitus, but its underlying mechanisms of action are not fully understood. Calcium/calmodulin-dependent serine protein kinase (CASK), which plays a vital role in the transport and release of neurotransmitters in neurons, is expressed in pancreatic islet cells and β-cells. This study aimed to investigate whether CASK is involved in the insulin secretagogue action induced by Ex-4 in INS-1 cells.

Material/Methods. A glucose-stimulated insulin secretion (GSIS) assay was performed with or without siRNA treatment against CASK. The expression level and location of CASK were evaluated by real-time PCR, western blotting and immunofluorescence. With the use of a protein kinase A (PKA) inhibitor or an exchange protein directly activated by cAMP-2 (Epac2) agonist, immunoblotting was performed to establish the signaling pathway through which Ex-4 alters CASK expression.

Results. Knock-down of CASK significantly attenuated the Ex-4-enhanced insulin release, and we showed that Ex-4 could increase transcription of CASK mRNA and expression of CASK protein but did not change the cellular location of CASK. A PKA inhibitor reduced the ability of Ex-4 to stimulate CASK expression, but an Epac2 agonist had no effect suggesting that regulation was mediated by the cAMP/PKA pathway.

Conclusion. Our study suggests that the stimulation of β-cell insulin secretion by Ex-4 is mediated, at least in part, by CASK via a novel signaling mechanism.

1. Introduction

The underlying pathophysiologies of metabolic syndrome and diabetes are chronic changes in the availability of, or sensitivity to, insulin. The incretin hormone glucagon-like peptide-1 (GLP-1) stimulates glucose-dependent insulin secretion [1,2]. The antidiabetic drug, exenatide, otherwise known as exendin-4 (Ex-4), originally isolated from the venom of the Gila monster lizard [3], is a potent stimulator of the GLP-1 receptor [4] and, in contrast to GLP-1, is not a substrate for cleavage by dipeptidyl peptidase IV, and so, has a longer half-life in vivo [5]. Yet the mechanisms through which GLP-1 and Ex-4 exert their effects are incompletely understood.

The calcium/calmodulin-dependent serine protein kinase (CASK) is a membrane- associated guanylate kinase; it is a

Abbreviations: cAMP, cyclic adenosine monophosphate; CASK, calcium/calmodulin-dependent serine protein kinase; Epac2, exchange protein directly activated by cAMP-2; ESCA-AM, 8-pCPT-2′-O-Me-cAMP-AM; Ex-4, exendin-4; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; H-89, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide; PKA, protein kinase A.

* *Corresponding author.* Zhongda Hospital, Institute of Diabetes, Southeast University, Nanjing 210009, PR China. Tel.: +86 25 8328 5147.
E-mail address: wang_yao100@163.com (Y. Wang).

0026-0495/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.metabol.2013.09.009

scaffolding protein that recruits or organizes other proteins at the plasma membrane to co-ordinate signal transduction pathways within the cytoplasm and nucleus. CASK plays a crucial role in neuron development and function in both rodents and humans [6,7], and its interaction with the components of the exocytosis complex, which suggests a role in neurotransmitter release [8–10].

Pancreatic islet β-cells and neurons appear to have evolved from a common ancestor — a neuronal precursor. There is a high degree of overlap between the proteins known to be involved in neural development and those known to pancreatic islets [11,12]. Also, recent studies have demonstrated similarities between synaptic vesicle proteins crucial for neurotransmitter secretion, and components of the insulin secretory apparatus [13–15]. CASK and neuroligin, one of the synaptic cell-surface molecules, are involved in initiating the assembly of the presynaptic secretory machinery [16,17]. It is known that both neuroligin and CASK are expressed in pancreatic β-cells and neuroligin participates in insulin secretion [16,18], but the role of CASK there is not yet known. It is possible that the assembly of the machinery necessary for the release of insulin vesicles is also mediated by CASK, in a similar manner to how it has been postulated to mediate the release of neurotransmitter granules.

In summary, we hypothesize that the mechanism by which Ex-4 increases insulin secretion is by regulating the expression of CASK via the cyclic AMP/protein kinase A (cAMP/PKA) or the exchange protein directly activated by cAMP-2 (Epac2) signaling pathway in pancreatic β-cells. This study investigates whether CASK is involved in Ex-4-induced insulin secretagogue action.

2. Material and Methods

2.1. Reagents

Exendin-4 and H-89(N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide) were purchased from Sigma-Aldrich, USA. RPMI-1460 medium and fetal bovine serum (FBS) were purchased from Gibco, USA. The Lipofectamine 2000 reagent and Trizol reagent were obtained from Invitrogen Life Technologies, USA. The Avian Myeloblastosis Virus Reverse Transcription System (AMV RT) was obtained from Promega, USA. The SYBR Green Real-Time PCR Master Mix and ReverTra Ace-α reverse transcription kit were purchased from Toyobo, Japan. The rabbit monoclonal antibodies against CASK (rat) were purchased from Santa Cruz Biotechnology, USA. The mouse monoclonal anti-α-tubulin (rat) and horseradish peroxidase (HRP)-conjugated IgG secondary antibodies (anti-rat and anti-mouse) were obtained from Amersham Pharmacia Biotech, USA. ESCA-AM (8-pCPT-2′-O-Me-cAMP-AM) was obtained from Biolog Life Science, Germany.

2.2. Cell culture

INS-1, a rat insulinoma cell line [19], was obtained from American Type Culture Collection (ATCC), USA. The cells were grown in RPMI-1640 medium supplemented with 10% FBS, 10 mmol/L HEPES, 2 mmol/L L-glutamine, 50 μmol/L β-mercaptoethanol, 1 mmol/L sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin. INS-1 cells were cultured at 37 °C in a Thermo tissue-culture incubator that provided a humidified atmosphere containing 95% air and 5% CO_2. Before addition of Ex-4, INS-1 cells were gently washed in phosphate-buffered saline (PBS). Cells were exposed to various concentrations (10–1000 nmol/L) of Ex-4 for the indicated times.

2.3. Knock-down of CASK by RNA interference

CASK-specific small interfering RNA (siRNA) and control siRNA were designed and synthesized by RIBOBIO (RiboBio, China). The sequence of the CASK siRNA used was (5′–3′): GCUGAAAGGAUCACUGUUUtt. INS-1 cells were transiently transfected with siRNA using the Lipofectamine 2000 reagent according to the manufacturer's instructions. Twenty-four hours after transfection, the cells were cultured for further studies. Transfection efficiency was monitored using 100 nmol/L of Cy3-Negative Control (fluorescence labeled siRNA; RiboBio) for 24 h.

2.4. Glucose-Stimulated Insulin Secretion (GSIS) assay

Briefly, 7.0×10^4 INS-1 cells were seeded into 500 μl RPMI-1640 medium with a standard glucose concentration (11.1 mmol/L) in a 48-well plate and treated with corresponding siRNA for 24 h as described above. After incubation for 1 h in glucose-free Krebs-Ringer bicarbonate (KRB) buffer (115 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4 \cdot 7H_2O$, 1.2 mmol/L KH_2PO_4, 20 mmol/L $NaHCO_3$, 16 mmol/L HEPES, 2.56 mmol/L $CaCl_2$, and 0.2% BSA), the cells were treated for 1 h in KRB buffer with low (2.8 mmol/L) or high (11.1 mmol/L) glucose with or without drug solutions. The supernatants were obtained and INS-1 cells were then washed twice with PBS (pH 7.4) at 4 °C and extracted with acid/ethanol (0.15 M HCl in 75% ethanol in H_2O) for 16 h at 4 °C. Supernatants were collected and stored at −80 °C until insulin determination was carried out by a radio-immunoassay (RIA) as described previously [20]. The results were normalized against the total insulin content.

2.5. Real-time PCR

INS-1 cells were cultured and treated as described above. The total RNA was extracted using Trizol reagent. First-strand cDNA synthesis was performed using 1 μg of total RNA and the AMV RT system. The cDNA from the reverse transcription reactions was used for amplifications with the following protocol: 1 min at 95 °C, 40 cycles of 10 s at 95 °C, 1 min at 60 °C and 10 s at 95 °C. The primers were designed using Primer Express Software (Applied Biosystems, USA). Real-time quantitative PCR was performed using the SYBR Green PCR Master Mix and LightCycler 480II Real-Time PCR SYSTEM (Roche Diagnostics, USA). The specific primers were (5′–3′): CASK — forward primer: AAGGAGAAAACTAAAGGGTGC; reverse primer: GGAGGTAGGGTCTTCGGAG); β-actin, the endogenous control — forward primer: GAACACGGCATTGTCACCAACT; reverse primer: GCCTGGATGGCTACGTACATG. All data were analyzed using β-actin expression as an endogenous control.