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BRIEF ARTICLES

Comparative identification of Ca^{2+} channel expression in INS-1 and rat pancreatic β cells

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Abstract

AIM: To identify and compare the profile of Ca^{2+} channel subunit expression in INS-1 and rat pancreatic β cells.

METHODS: The rat insulin-secreting INS-1 cell line was cultured in RPMI-1640 with Wistar rats employed as islet donors. Ca^{2+} channel subunit expression in INS-1 and isolated rat β cells were examined by reverse transcription polymerase chain reaction (RT-PCR). Absolute real-time quantitative PCR was performed in a Bio-Rad iQ5 Gradient Real Time PCR system and the data analyzed using an iQ5 system to identify the expression level of the Ca^{2+} channel subunits.

RESULTS: In INS-1 cells, the L-type Ca^{2+} channel 1C subunit had the highest expression level and the TPRM2 subunit had the second highest expression. In rat β cells, the TPRC4β subunit expression was dominant and the expression of the L-type 1C subunit exceeded the 1D subunit expression about two-fold. This result agreed with other studies, confirming the important role of the L-type 1C subunit in insulin-secreting cells, and suggested that non-voltage-operated Ca^{2+} channels may have an important role in biphasic insulin secretion.

CONCLUSION: Twelve major Ca^{2+} channel subunit types were identified in INS-1 and rat β cells and significant differences were observed in the expression of certain subunits between these cells.

Key words: L-type calcium channels; Expression profile; Insulin-secreting cells; Rats; pancreatic β cell; Reverse transcription-polymerase chain reaction

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INTRODUCTION

Recent theories portray type 2 diabetes mellitus (T2DM) as a heterogeneous disorder. In addition to insulin resistance, clinical studies in humans and animals have documented a variety of defects in β cell function[1], and most researchers agree that both insulin secretion impairment and insulin resistance contribute to the fully established disease[2]. Insulin produced by pancreatic islet β cells efficiently regulates glucose homeostasis in humans and other mammals. These cells are electrically excitable, and couple changes in blood glucose concentration to insulin release *via* electrical signals.

Glucose-stimulated insulin secretion is biphasic, with about a 10 min first phase and a several hour second phase[3]. Intracellular Ca^{2+} signals play a pivotal role in β cell function and, as insulin secretion is the most important role of these cells, knowledge of the intricacies of the signals involved in excitation-secretion coupling is important in understanding both normal β cell function and related pathological states. It has been reported that both voltage-dependent and non-voltage-operated Ca^{2+} channels are involved in these processes. The voltage-dependent Ca^{2+} channels include L-type, T-type, N-type and R-type channels[4,5]; the non-voltage-operated Ca^{2+} channels include ryanodine-sensitive Ca^{2+} channels[6-8],

transient receptor potential channels (TRP)[9-11], and inositol 1,4,5-trisphosphate (IP_3)-sensitive channels[12], the latter mobilizing Ca^{2+} from the endoplasmic reticulum. Impaired first-phase insulin secretion is an early feature of T2DM, whereas second-phase insulin secretion deteriorates with progression of the disease. A genetic study has indicated that polymorphisms in R-type channels in humans are associated with T2DM and impaired insulin secretion[13]. Moreover, most Ca^{2+} channels consist of various subunits which participate in different physiologic functions in different species or sub-cloned cell lines[12]. For example, T-type Ca^{2+} channels have little or no expression in rodents, but can be detected in humans[14]. Thus, the above suggests that Ca^{2+} channel subunits may be suitable candidates as pathogenetic factors in diabetes.

Considering the essential functions of Ca^{2+} signals for insulin secretion and the various Ca^{2+} channel subunits in β cells, a systemic identification of Ca^{2+} channel subunit expression in β cells is necessary to further the understanding of their functions in regulating insulin secretion and to find potential new therapy targets for T2DM. The aim of this study was to detect the expression profile of six voltage-dependent Ca^{2+} channel subunits, including L-type (α1C, D, S and 1F subunits), R-type (α1E subunit), and N-type (α1B subunit), and nine non-voltage-operated Ca^{2+} channel subunits including Ryr1, Ryr2, TRPC1, TRPC4α, TRPC4β, TRPM2, IP_3R1, IP_3R2 and IP_3R3, in an INS-1 cell line, and to compare the identified subunits with those detectable in rat primary pancreatic β cells. The INS-1 rat cell line was employed as a model of pancreatic β cells because INS-1 cells show susceptibility to glucotoxicity, similar to β cells.

MATERIALS AND METHODS

Cell culture

INS-1 cells lines were glucose-responsive and a gift from Professor Tao Xu (Institute of Biophysics, Chinese Academy of Sciences, P. R. China) and were cultured in RPMI-1640 medium containing 11.2 mmol/L glucose, 100 mL/L fetal calf serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate[15], 100 000 U/L penicillin, 100 mg/L streptomycin, and 50 mmol/L β-mercapto-ethanol, in a fully humidified atmosphere containing 50 mL CO_2 per liter air at 37℃.

Preparation of rat pancreatic β cells and identification by reverse transcription-polymerase chain reaction (RT-PCR)

Pathogen-free, inbred, male Wistar rats weighing 300-350 g were used as islet donors. The animals were maintained on standard rat chow and acidified water *ad libitum*. For islet retrieval, individuals were sacrificed by cervical dislocation and the pancreas was quickly removed. Pancreatic islets were isolated using a standard collagenase digestion[16,17]. Briefly, islets were separated from exocrine tissue by centrifugation over a discontinuous dextran gradient after digestion with 0.5 g/L collagenase V (Sigma C9263) for 30 min, and further purification by handpicking under a microscope. Islets were collected and washed twice in phosphate-buffered saline and dispersed as single cells by mechanical shaking in 4 mL of Hank's solution (Ca^{2+} and Mg^{2+} free) before filtering the cell mixture over a 35 μm pore size filter (BD Falcon) and diluting to 200 mL. This preparation was divided into 20 mL per tube, and cDNA was synthesized from the total isolated RNA, as described below.

To distinguish glucagon-producing-cells from insulin-producing-cells, specific primer pairs were designed from the insulin and glucagon genes in GenBank. The sequences were: insulin-F, AAACAGCACCTTTGTGG TTCTCA; insulin-R, GTGCCACTTGTGGGTCCTCC; glucagon-F, TCGTGGCTG GATTGTTTG; and glucagon-R, TGGCGTTTGTCTTCGTTTAT. The PCR procedure was: the insulin gene at 95℃ for 30 s, 59℃ for 30 s, 72℃ for 30 s, and 35 cycles; and the glucagon gene at 95℃ for 30 s, 53℃ for 30 s, 72℃ for 30 s, and 35 cycles.

RT-PCR and absolute real-time quantitative PCR analysis

Total RNA was extracted from INS-1 cells and rat pancreatic β cells using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. One microliter aliquot of the RNA was incubated with Oligo (dT) 18 at 70℃ for 5 min, and then put on ice. RNase inhibitor (1 μL), 100 μmol/L dNTPs, 0.01 mol/L dithiothreitol, and 200 U of M-MLV reverse transcriptase (Promega, USA) were added to the mixture (20 μL final volume), incubated at 42℃ for 50 min, and then incubated at 85℃ for 5 min. Table 1 summarizes the primer pairs used for the amplification of Ca^{2+} channel subunits, PCR was performed in a standard 50 μL reaction volume, and the resulting products were visualized by Golden view after 3% agarose gel electrophoresis in TAE buffer (40 mmol/L Tris-acetate, 2 mmol/L EDTA, pH 8.5).

The expression levels of the subunits were quantified by absolute real-time quantitative PCR with the Bio-Rad iQ5 Gradient Real Time PCR system and all reactions performed in a 25 μL reaction volume containing 12.5 μL of 2xSYBR Green Master mix (Bio-Rad, USA), 1 μL of each primer pair (10 μmol/L), and 1 μL of cDNA templates. The products of PCR were cloned into the pGEM-T easy vector using the pGEM-T easy cloning kit (Promega, USA). Five microliters LB-broth cultures containing single colonies were grown up overnight at 37℃ with shaking at 200 r/min and the resulting plasmids were purified. Plasmid DNA was solubilized in 50 μL TE buffer and the sequences of the cloned products confirmed by DNA sequencing. Purified plasmid clones were quantified by Eppendorf Biophotometer and the copy number calculated as follows: 6.02×10^{23} (copies/mol) × DNA amount (g)/[DNA length (bp) × 660 (g/mol per becquerel)].

Based on the copy number and concentration of the plasmid DNA, the precise number of molecules added to subsequent real-time PCR runs was calculated, thus

Table 1 Sequences of primer pairs of Ca^{2+} channel subunits and RT-PCR

Primer	Sequence	Temperature (℃)
L-α1C (5')	5'-GTCCATAGTGGGTCGTCT-3'	50
L-α1C (3')	5'-TGGTTTGTTCTTGCTTTC-3'	
L-α1D (5')	5'-CAGGGATGCTGTGGAAGT-3'	51
L-α1D (3')	5'-TGGGCTGAGAACCTAGACG-3'	
L-α-S (5')	5'-ATGCCAGAGGATGACAACAAC-3'	55
L-α-S (3')	5'-CACCCAgAAAgACAATGATgAA-3'	
L-α1F (5')	5'-GACGGCAACTTGGCTTCT-3'	53
L-α1F (3')	5'-GCTGGCATGACTGCTGGT-3'	
N-α1β(5')	5'-GCTCGCTCTTCGTCTTCA-3'	52
N-α1β (3')	5'-AGGTTCCGTGTCATCCAGT-3'	
R-α1E (5')	5'-ATGTCCCTGAAGATGTATGG-3'	50
R-α1E (3')	5'-AACGACCTCAAAGATGCTG-3'	
Ryr1 (5')	5'-GGGCGGAGAATGAGAAAGA-3'	55
Ryr1 (3')	5'-CAGGGTCGTCACGGTTGT-3'	
Ryr2 (5')	5'-TGAGTATGCCCATTGAGT-3'	48
Ryr2 (3')	5'-CTTTGCTTTAGGCGTGAG-3'	
TRPC1 (5')	5'-GTCAGACATTAAGAGGCTGTG-3'	50
TRPC1 (3')	5'-AAGTTGCCAAGTAAAGGGA-3'	
TRPC4α (5')	5'-AATGGTTCTGCCCTGGTG-3'	50
TRPC4α (3')	5'-GAAGATTTGGTTTGCGTTT-3'	
TRPC4β (5')	5'-GCAGCATTCCTGGTCTCA-3'	51
TRPC4β (3')	5'-GGGCGTGTTTCTCCTTTG-3'	
TRPM2 (5')	5'-GTCATCACCATCGGCATAGC-3'	56
TRPM2 (3')	5'-TGTCCAGGCAGGTCAGGTT-3'	
IP_3R1 (5')	5'-CAACCGTTACTATGGAAACATC-3'	54
IP_3R1 (3')	5'-TCAGCCAGGCTCATCTCAC-3'	
IP_3R2 (5')	5'-CGATGCCAGGATACGATGT-3'	54
IP_3R2 (3')	5'-CACCCTTGAAGTACCGATT-3'	
IP_3R3 (5')	5'-AGGAGCTGGTGGACGTGAT-3'	55
IP_3R3 (3')	5'-TGCTTGTTGTGCCTGGAAA-3'	

Table 2 Expression profile and clone numbers of Ca^{2+} channel subunits in INS-1 and rat primary β cells (mean ± SE)

Ca^{2+} channel subunits	Length of PCR production (bp)	INS-1	β cells
L-α1C	147	$1.767 \times 10^7 \pm 1.763 \times 10^6$	$3.836 \times 10^6 \pm 1.087 \times 10^6$
L-α1D	197	$1.046 \times 10^6 \pm 2.074 \times 10^4$	$1.913 \times 10^6 \pm 1.329 \times 10^5$
L-α-S[1]	181	-	-
L-α1F[1]	144	-	-
N-α1β	188	$1.078 \times 10^4 \pm 6.477 \times 10^2$	$6.832 \times 10^3 \pm 3.220 \times 10^2$
R-α1E	102	$6.614 \times 10^3 \pm 1.477 \times 10^2$	$2.84 \times 10^3 \pm 1.943 \times 10^2$
Ryr1[1]	159	-	-
Ryr2	250	$1.367 \times 10^4 \pm 7.213 \times 10$	$6.520 \times 10^5 \pm 4.864 \times 10^4$
TRPC1	101	$5.631 \times 10^4 \pm 1.904 \times 10^3$	$3.260 \times 10^6 \pm 2.531 \times 10^5$
TRPC4α	196	$8.033 \times 10^4 \pm 5.405 \times 10^3$	$2.073 \times 10^6 \pm 2.207 \times 10^5$
TRPC4β	165	$2.226 \times 10^5 \pm 7.672 \times 10^4$	$6.666 \times 10^6 \pm 1.595 \times 10^6$
TRPM2	130	$1.028 \times 10^6 \pm 9.696 \times 10^4$	$1.538 \times 10^6 \pm 2.476 \times 10^5$
IP_3R1	159	$1.189 \times 10^5 \pm 1.650 \times 10^4$	$1.922 \times 10^6 \pm 7.584 \times 10^5$
IP_3R2	191	$3.339 \times 10^4 \pm 7.945 \times 10^3$	$8.293 \times 10^4 \pm 7.041 \times 10^3$
IP_3R3	130	$5.613 \times 10^5 \pm 1.009 \times 10^5$	$7.863 \times 10^5 \pm 3.894 \times 10^3$

[1]These three subunits were not identified in INS-1 and rat β cells.

providing a standard for specific cDNA quantification. All samples were prepared as 1:10, 1:100, and 1:1000 dilutions and each reaction at different dilutions performed in triplicate. The standard curve and data analysis were produced using Bio-Rad iQ5 software.

RESULTS

Preparation of rat pancreatic β cells and identification by RT-PCR

An average of 300-500 islets were produced from each rat, with about 1000 cells per islet and composed of 70% β cells. In the first 10 cDNA templates analyzed, six templates only expressed the insulin gene, three expressed the glucagon gene, and the last one expressed neither. The cDNA whose glucagon gene was positive was considered to be from contamination by alpha islet cells and could be ignored and, thus, the others were chosen as templates for analysis of β cell Ca^{2+} channel subunit expression.

Expression of Ca^{2+} channels subunits in INS-1 and rat pancreatic β cells

RT-PCR was performed to identify the expression of Ca^{2+} channel subunits in INS-1 and rat pancreatic β cells. Of the 15 subunit types, 12 types were amplified from INS-1 and rat pancreatic β cells and their identities confirmed (Figure 1A). Three types, not identified in either INS-1 or rat pancreatic β cells, were the L-type α1F, S and Ryr1 subunits. Under the same reaction conditions, these three were identified in cDNA from heart and skeletal muscle because these sources

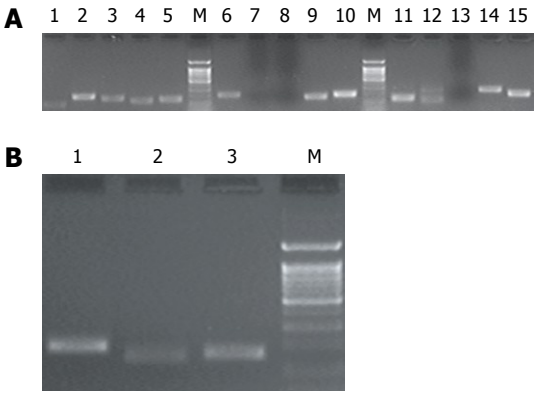

Figure 1 The Ca^{2+} channel subunits expression profile in INS-1 and rat β cells. A: 12 of 15 Ca^{2+} channel subunits in rat pancreatic β cells. 1-5: Separately represent subunits TRPC1, TRPC4α, TRPC4β, TRPM2, and L-α1C; 6-10: Separately represent subunits L-α1D, L-α-S, L-α1F, IP_3R1, and IP_3R2; 11-15: IP_3R3, R-α1E, Ryr1, Ryr2, and N-α1B; M: 100 bp ladder marker, (bottom band, 100 bp; the expression profile of INS-1 is the same as for rat β cells, not shown); B: L-α-S, L-α1F, and Ryr1 expressed in rat heart and skeletal muscle. 1-3: L-α-S (cDNA of skeletal muscle), L-α1F (heart), and Ryr1 (cDNA of skeletal muscle); M: 100 bp ladder marker; bottom band of M, 100 bp.

are composed of multiple tissues, including muscles, blood vessels, and nerve fibers (Figure 1B). It was found that 12 subunit types were expressed in INS-1 and rat pancreatic β cells (Table 2): L-type (α1C, α1D subunits), R-type (α1E subunit), and N-type (α1β subunit) preferentially in INS-1 cells; Ryr2, TRPC1, TRPC4α, TRPC4β, TRPM2, IP_3R1, IP_3R2, and IP_3R3 preferentially in β cells.

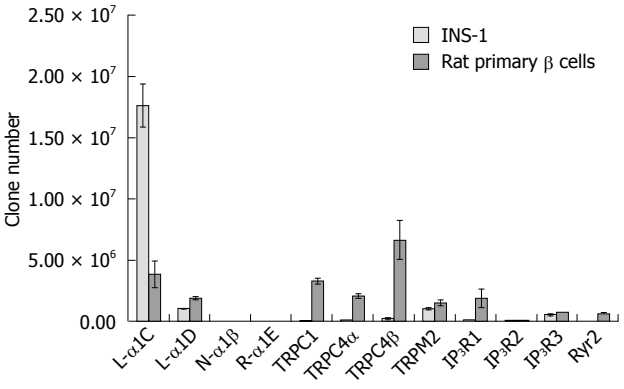

Figure 2 Expression level of 12 Ca^{2+} channel subunits in INS-1 and rat primary β cells.

mRNA level of Ca^{2+} channel subunits in INS-1 and rat pancreatic β cells

Melt curve analysis of all subunits revealed that there was a single peak at the expected melting temperature for PCR applications. The absolute real-time quantitative PCR analyses showed (Table 2, Figure 2) that, in INS-1, L-type α1C subunits were dominant and were expressed significantly more than other subunits. The R-α1E subunit was expressed at a very low level as in the β cells. In the latter cells, the expression levels of subunits were relatively similar, but the expression of Ryr2, TRPC1, TRPC4α, TRPC4β, TRPM2, and IP_3R1 were significantly higher than in INS-1.

DISCUSSION

As important regulatory factors in insulin secretion, Ca^{2+} channels have potential as targets for developing new T2DM therapies. Considering the variety of Ca^{2+} channel subunits present in different cell clones and species, the aim of this study was to perform a systematic identification of Ca^{2+} channel subunits in INS-1 and rat pancreatic β cells. Collectively, 12 of 15 subunit types were found to be expressed in INS-1 and primary rat islet β cells. The L-type α1C subunit expression exceeded that of α1D 10-fold in INS-1 cells, but only 2-fold in the β cells. These results were similar to recent research in INS-1 832/12 cells which confirmed that α1C subunit expression exceeded that of the α1D subunit by two-fold and that the α1C subunit had a critical role in insulin secretion[18]. The L-type α1C subunit performs a special function in the first phase of insulin secretion and glucose tolerance. In mice, α1C subunit deficiency decreased the whole-cell Ca^{2+} current by about 45% and abolished the first phase of insulin secretion, resulting in glucose intolerance[19]. In the present study, L-type α1C subunits were dominant among all subunit expressions, which was in accordance with its important biological function in both INS-1 and rat β cells. The L-type α1D subunit's roles in insulin secretion or proliferation were not confirmed until recently[18,20-22].

The expression of the non-voltage-operated Ca^{2+} channel subunits Ryr2, TRPC1, TRPC4α, TRPC4β, and IP_3R1 in rat β cells exceeded those in INS-1 by 10-fold and TRPM2 expression was not significantly different between the β cells and INS-1. These results suggested that non-voltage-operated Ca^{2+} channels may have a greater role in regulating insulin secretion in comparison with voltage-dependent Ca^{2+} channels. The expression level of the TRPC4β subunit was dominant in the β cells and, as a member of the TRPC subfamily, TRPC4 shows four protein motifs (M1-M4) characteristic of the TRPC sub-family[23]. Specifically, TRPC4β lacks 84 amino acids in the C-terminus, which corresponds to putative binding sites for calmodulin and IP_3 receptors in TRPC4α. The ionic channels formed by TRPC4 appear to be Ca^{2+}-permeable, but there is a considerable discrepancy in the degree of Ca^{2+} selectivity. Studies with mice lacking TRPC4 suggest an important role for TRPC4 in supporting Ca^{2+} entry[24]. The defect in Ca^{2+} entry in TRPC4-/- mice appears to be associated with a reduction in arterial vasorelaxation, vascular permeability in the lung, and neurotransmitter release from thalamic dendrites[25]. Though the expression levels of subunits observed here were not absolutely coupled with their functions, the present results suggested that TRPC4β may be performing some functions in insulin biphasic secretion and that further clarification is needed.

The insulin-secreting INS-1 cell line was established by dispersion of a radiation-induced insulinoma from NEDH rats in 1992[15]. INS-1 cells can respond to glucose and are generally considered to be a β cell model, but an important drawback to this cell line is its polyclonal nature reflected by the presence of glucose-responsive and glucose-unresponsive subpopulations[26]. As was demonstrated here, there was a significant difference in the levels of expression of Ca^{2+} channel subunits between INS-1 and β cells, which probably reflected differences in the intracellular metabolism and/or secretory pathways. Taken together, these INS-1 cells may not have represented an exclusively insulin-producing β cell line.

The present study systematically identified the expression profile of Ca^{2+} channel subunits in INS-1 cells and rat pancreatic β cells and quantitatively characterized them by direct comparison. These results will be helpful in advancing the understanding of Ca^{2+} channel subunits and their roles related to insulin secretion.

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COMMENTS

Background

The proportion of people with type 2 diabetes has increased throughout the world. It is now recognized that abnormal insulin secretion precedes the onset of type two diabetes. Ca^{2+} channels have important roles in the progress of insulin secretion by β cells.

Research frontiers

Ca^{2+} channels are composed of several subunits and mainly control Ca^{2+} influx through different mechanisms because of different patterns of subunit composition. In islet β cells, the expression profile of these subunits has not

been systematically investigated. In this study, the authors systematically identified the expression of Ca^{2+} channels in primary β cells and INS-1, an insulin-secreting rat cell line.

Innovations and breakthroughs

This is the first study to report the expression profile of Ca^{2+} channel subunits in primary rat pancreatic β cells and INS-1. Furthermore, the real time PCR data suggested that the INS-1 cell line was not an ideal β cell bioelectrical model.

Applications

By understanding which types of Ca^{2+} channels are expressed in rat β and INS-1 cells, this study may have advanced the understanding of the possible functions of various Ca^{2+} channels in insulin secretion and provided clues to the physiopathology of diabetes.

Terminology

Ca^{2+} channels provide pores for the passive diffusion of ions across biological membranes, in particular Ca^{2+}. β cells are the unique cells of insulin production, and the INS-1 is an insulin-secreting cell line established by dispersion of a radiation-induced insulinoma from NEDH rats in 1992.

Peer review

This is an interesting study, the authors identified the expression profile of Ca^{2+} channel subunits in the INS-1 cell line and rat pancreatic β cells, by reverse transcription polymerase chain reaction.

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