

TRP Genes

Candidates for Nonselective Cation Channels and Store-Operated Channels in Insulin-Secreting Cells

Feng Qian,¹ Ping Huang,¹ Li Ma,² Andrey Kuznetsov,² Natalia Tamarina,² and Louis H. Philipson²

Nonselective cation channels may play a role in insulin secretion by regulating pancreatic β -cell plasma membrane potential, Ca^{2+} homeostasis, and thereby glucose signaling. Transient receptor potential channel (TRPC)-related genes encode nonselective cation channels, some of which are similar to those described for β -cells. Some TRPC-like channels are activated via G-protein-coupled mechanisms, some have been reported to be calcium-store-operated channels (SOC), and others are activated by novel signaling molecules or are sensitive to pressure and osmotic strength. Here we report the cloning and expression of mSTRPC4 from a mouse insulinoma cDNA library. mSTRPC4 encoded a protein of 97 kd, expressed in both endocrine cells and the brain. Stable cell lines expressing mSTRPC4 showed abundant mSTRPC4 protein, but no reproducible currents could be detected. mSTRPC4 therefore probably functions as a heteromultimer. We also report that LTRPC2, a G-protein and adenosine 5'-diphosphoribose (ADPR)-activated nonselective cation channel, is also expressed in human islets. TRPC-like channels may provide a pathway for depolarization or Ca^{2+} entry in β -cells and may be interesting targets for manipulating β -cell function. *Diabetes* 51 (Suppl. 1):S183–S189, 2002

The concentration of intracellular free calcium ($[\text{Ca}^{2+}]_i$) is an evolutionarily conserved signal for the regulation of many aspects of cellular function. In both excitable and nonexcitable mammalian cells, $[\text{Ca}^{2+}]_i$ plays a crucial role in regulating complex Ca^{2+} signals and differentiated functions, such as excitability, secretion of hormones and release of transmitters, synaptic plasticity, gene expression, and apoptosis (1,2). In the "capacitative model" for nonexcitable cells (3), Ca^{2+} influx from the extracellular space replenishes Ca^{2+} stores that act like capacitors for calcium, a mecha-

nism termed capacitative calcium entry (CCE) (4). Store-operated channels (SOCs) have been described that facilitate CCE, functioning as calcium-selective or nonselective cation channels. These are widely if not ubiquitously expressed among different tissues and cell types (5). SOCs could play an important role in refilling intracellular calcium stores after stimulation, especially in maintaining $[\text{Ca}^{2+}]_i$ oscillations (6).

Nonselective cation currents were first described in insulin-secreting cells (the CRI-GI cell line) by Sturgess et al. (7,8). This channel was inhibited by adenine derivatives including AMP and ATP and was activated only with very high intracellular calcium (100 $\mu\text{mol/l}$). The open probability was voltage-dependent, and some rectification was observed. It was blocked by quinine and 4-aminopyridine, but was insensitive to tetraethylammonium, tetrodotoxin, or amiloride. It was uncertain what role such a channel might play in β -cell physiology (7). More recently, several groups have reinvestigated the expression and role of nonselective cation currents in primary cultures of rodent β -cells and cell lines (9–13). These currents bear some similarity to those reported for various members of the recently described TRPC gene families. We have previously summarized the evidence for a calcium-release activated nonselective cation current, termed I_{CRAN} , that could couple the state of intracellular Ca^{2+} stores to the plasma membrane potential (14). We found a similar current in βTC3 insulinoma cells and provided a mechanism for oscillatory calcium responses in the presence of glucose (12). This scheme suggested that regulation of I_{CRAN} via signaling from intracellular calcium pools could provide a mechanism to generate oscillations in plasma membrane potential, independent of K_{ATP} channel activity. Other similar currents were also linked to activation by thapsigargin-depletion of intracellular Ca^{2+} pools (15), activation by muscarinic stimulation (16), and activation by cAMP stimulation (10,17). A nonselective cation current could therefore underlie the depolarizing effects of both cholinergic stimulation and thapsigargin treatment, as both serve to deplete the $[\text{Ca}^{2+}]_i$ stores. SKF 96365 and La^{3+} , blockers of Ca^{2+} -store-operated channels, and removal of external Na^+ suppressed glucose-stimulated $[\text{Ca}^{2+}]_i$ oscillations (12). A maitotoxin-activated nonselective cation current in mouse islet cells and βTC3 cells was also attenuated by removal of extracellular Na^+ and by SKF 96365 (12,18).

Transient receptor potential channel (TRPC)-related genes are excellent candidates for nonselective cation

From the ¹Department of Neurobiology, Physiology, and Pharmacology, the University of Chicago, Chicago, Illinois; and the ²Department of Medicine and Committee in Cell Physiology, the University of Chicago, Chicago, Illinois.

Address correspondence and reprint requests to l-philipson@uchicago.edu. Accepted in revised form 15 June 2001.

ADPR, adenosine 5'-diphosphoribose; cADPR, cyclic adenosine 5'-diphosphoribose; CCE, capacitative calcium entry; DMEM, Dulbecco's modified Eagle's medium; EYFP, enhanced yellow fluorescence protein; FBS, fetal bovine serum; I_{CRAN} , calcium-release activated nonselective cation current; IP_3 , inositol 1,4,5-trisphosphate; RACE, receptor-activated calcium entry; RT-PCR, reverse transcriptase-polymerase chain reaction; SOC, store-operated channel; SSC, standard saline citrate; TRPC, transient receptor potential channel.

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channels that may play a role in regulating insulin secretion. Several variants of mSTRPC1 message are expressed in MIN6 cells, and at least one was found in mouse islets (19). In Northern blot analysis of β TC3 cells, mSTRPC4 was most highly expressed (12). We sought to identify additional members of the TRPC family in insulin-secreting cells. We identified expression of STRPC1, -2, -4, and -6 in MIN-6 cells, STRPC1-6 in β TC3 cells, and STRPC1, -2, -3, -4, and -6 in mouse islets by reverse transcriptase-polymerase chain reaction (RT-PCR). The expression level of each gene varied considerably. We then cloned mSTRPC4 from a β TC3 cDNA library and compared its functional expression with hTRPC3. We also report the expression of LTRPC2, a newly described adenosine diphosphoribose (ADPR)-activated channel (20), in human islets.

RESEARCH DESIGN AND METHODS

Materials. A mouse β TC3 cell cDNA library was generously provided by R. Stein (Vanderbilt University). Mouse multiple tissue Northern blots and pEYFP-N1 were purchased from Clontech. Human pCDNA-Trp3-myc was a kind gift from Dr. Craig Montell (Johns Hopkins University).

cDNA cloning and sequence determination. The β TC3 cell cDNA library was screened with a cDNA fragment of mouse STRPC4 homolog, obtained by RT-PCR amplification from β TC3 insulinoma cell poly(A)⁺ RNA. PCR was performed with the oligonucleotide primer pair: 5'-CTGCAGATATCTCTGGG AAGG-3' and 5'-GCTTTGTTTCGAGCAAATTTC-3', based on the published partial sequences of TRP homolog cDNA isolated from mouse brain (X90697) (21). The PCR conditions were initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s with a final extension at 72°C for 8 min. The 417-bp fragment was subcloned into pCRII vector (Invitrogen) and sequenced. Screening of the β TC3 cell cDNA library (λ Ziplox; BRL Life Technologies) was performed as described (22) at a final stringency of 0.1 \times standard saline citrate (SSC), 0.1% SDS at 60°C. Both strands of the cDNA inserts were sequenced on an automated DNA sequencer (Perkin-Elmer). The complete clone of the alternative spliced form was constructed by ligation of the *PstI-NotI* fragment of the short splicing form with the *Sall-PstI* fragment of the unspliced form. Both clones were inserted between the *Sall* and *NotI* sites of the pBluescript II SK(+) phagemid.

Note on nomenclature. As the nomenclature for the TRPC channels has recently been revised, the channel cDNA originally termed mTRP4 in the GenBank entry is now termed mSTRPC4, where "m" indicates mouse, "S" indicates the "short" TRPC family, "TRP" is the designation from the *Drosophila* transient receptor potential gene, "C" indicates channel, "4" indicates the family member (currently numbered 1-7), and "L" and "S" indicate the particular splice variant (23).

Cell culture and transfection. Mouse β TC3 insulinoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose, 5% fetal bovine serum (FBS), 15% horse serum (GIBCO/BRL, Life Technologies). HEK293 cells were cultured in DMEM with high glucose, 10% FBS (GIBCO/BRL, Life Technologies), and transfected (2×10^6 cells/100-mm dish) with 5 μ g DNA by Superfect reagent (Qiagen).

Northern blot analysis. Mouse multiple tissue Northern blots (Clontech) were prehybridized and hybridized and washed to a final stringency of 0.1 \times SSC, 0.1% SDS at 60°C. A 2-kb human cDNA for β -actin was used as the probe to hybrid with the same blot as the control.

cDNA constructs. The 5' untranslated region of mTrp4 cDNA was excised and subcloned between the *XhoI* and *NotI* sites of a modified pBK-CMV vector (Stratagen). Then a three-primer PCR strategy was used to insert a human insulin C-peptide cDNA in-frame between the last amino acid and the stop codon (24). The *BamHI* and *NotI* fragment containing the entire insertion was cloned into pCMV5 expression vectors between the restriction sites *BglII* and *NotI*. The corresponding splicing isoforms were deposited in GenBank as mTrp4S (U50921) for the shorter form and mTrp4 L (U50922) for the longer form.

Production of antisera. The peptide N-CDSSIDYDLSPTDTAAHEDYV-C was synthesized and used to raise antisera in rabbits (Zeneca) in collaboration with R. Shehee, GlaxoWellcome Research Institute. The antisera from two animals, 523 and 524, were purified by affinity chromatography and tested in enzyme-linked immunosorbent assay (ELISA).

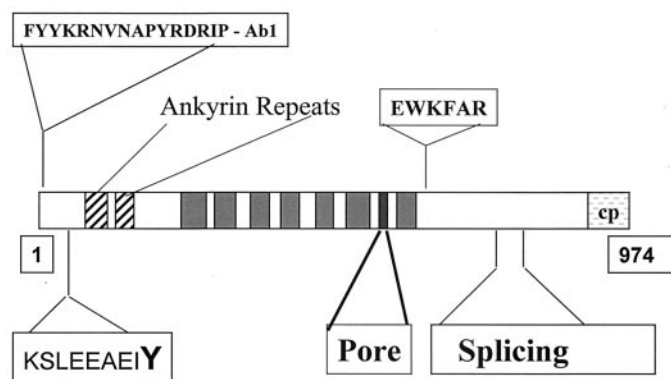


FIG. 1. Proposed transmembrane topology of TRPC-related channels. By analogy with voltage-dependent K⁺ channels, the functional TRPC channel would be a tetramer of this basic unit. Specific features of 974-amino acid STRPC4 protein are also shown, including the NH₂-terminal ankyrin repeat domains, transmembrane domains and pore location, the conserved Tyr-phosphorylation motif, the TRPC motif, and the area of variation due to RNA splicing. The NH₂-terminal peptide using for raising antisera is indicated, as is the COOH-terminal tag (cp) used in some experiments.

Recombinant expression in HEK293 cells. Cell lysates containing 40 μ g total protein, prepared 48 h after transfection, was fractionated on 7.5% (37.5:1) denaturing polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes (Amersham) and incubated with immunoglobulin G followed by detection with an enhanced chemiluminescence reagent (Amersham). For immunohistochemistry, cells were fixed in Dulbecco's phosphate-buffered saline (D-PBS)-buffered 4% paraformaldehyde solution, pH 7.4, for 10 min at 4°C. The primary and secondary antibodies were incubated with the fixed cells according to standard protocols (3% BSA, 0.1% Tween-20 in PBS, pH 7.4).

Measurement of [Ca²⁺]_i and [Sr²⁺]_i. Cells were plated on glass coverslips coated with poly-L-lysine (Sigma) and/or with Pronectin F plus (Protein Polymer Technologies) at least 1 day before transfection. Transiently transfected cells were identified by yellow-shifted green fluorescence of enhanced yellow fluorescence protein (EYFP) (Clontech) cotransfected with the desired cDNA at a ratio of 1:9. This allowed direct identification of transfected cells that did not overlap with the fura-2 emission. Control cells were transfected

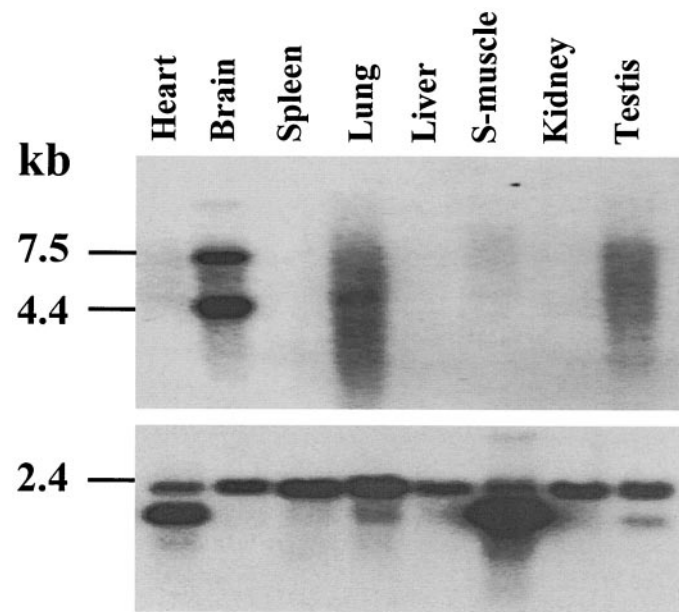


FIG. 2. Tissue distribution of mSTRPC4. Autoradiograph of Northern blot hybridization analysis with the mSTRPC4 cDNA probe of poly(A)⁺ RNA isolated from various tissues. From lane 1 (left) to lane 8 (right), tissues are as follows: heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. A, hybridized with mSTRPC4 cDNA probe; B, hybridized with human β -actin cDNA.

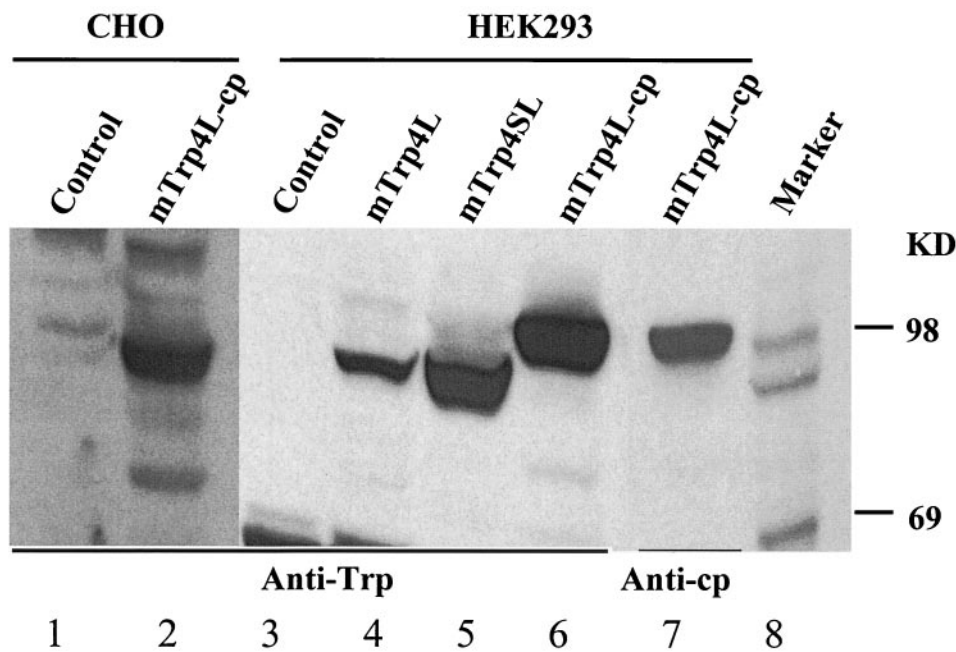


FIG. 3. mSTRPC4 1 protein expressed in CHO and HEK293 cells. Cell lysates were prepared from stable cell lines expressing mSTRPC4 1 and fractionated by SDS-PAGE (7.5% gel). Lanes 1 and 2 are CHO cells in a separate experiment, and lanes 4 and 5 were from two independent cell lines. Western blots were probed with anti-mSTRPC4 NH₂-terminal antipeptide antisera. Lanes 1 and 3, nontransfected control. Lanes 7 and 8, antitag (anti-COOH-terminal tag) antisera.

with pEYFP alone. There was no detectable difference in responses between control cells expressing EYFP from those remaining untransfected. Cells were loaded with 5 $\mu\text{mol/l}$ fura-2/AM and 0.05% pluronic F-127 in extracellular solution in the dark at 37°C for 30 min, at 24–48 h after transfection. Fluorescent images of the cells were recorded and analyzed at room temperature using the Metafluor Imaging System (Universal Imaging) by alternating excitation at 340 nm and 380 nm at 5-s intervals and detecting emission at 510 nm. The extracellular solution contains HEPES (15 mmol/l), glucose (10 mmol/l), NaCl (140 mmol/l), KCl (1 mmol/l), and MgCl₂ (1 mmol/l), supplemented with 1.8 mmol/l CaCl₂, 1.8 mmol/l SrCl₂, or 100 $\mu\text{mol/l}$ EGTA (nominal calcium-free solution). All reagents were diluted to their final concentration in the extracellular solution and applied to cells by perfusion. Data from 10 to 30 cells per coverslip were collected per experiment and repeated to give the total number of cells.

LTRPC2 expression in human islets. RNA was extracted from human islets (kindly provided by B. Hering, University of Minnesota) using Trizol reagent. The cDNA was obtained with random primers or oligodT. For PCR analysis of LTRPC2 expression, we used three forward primers: *For1*, -TGTGCAGA ATTGTACAGTTGCG; *For22* -AAACCATGTCGCTGGCAGCTGG; and *For40* -CTGGTGTGGCG-GTGGAGACTT; and one reverse primer *Rev584* -GCTCT TGAAGAGGCTGTGTTG. The amplified fragment (584 bp) was purified and fully sequenced in both directions using *For22* and *Rev584*.

RESULTS

Cloning of mouse STRPC4 from a βTC3 cDNA library.

A partial mammalian TRPC sequence, originally termed “mTrp4” and previously identified by RT-PCR amplification from mouse brain (21), was employed to obtain the identical cDNA fragment from βTC3 cells. It was then used to screen a mouse βTC3 cell cDNA library. We identified five positive clones that contained mTrp4-related cDNAs. The longest was a 3.9-kb cDNA fragment that overlapped with the other four clones as determined by the restriction map. The sequence of this clone revealed a continuous single open reading frame encoding a protein of 974 amino acids (Fig. 1). The methionine initiating this open reading frame was in a Kozak (25) sequence context compatible with efficient translation and was preceded by an in-frame termination codon. The full-length cDNA clone was originally termed “mTrp4 1” (GenBank U50922). A 2.0-kb clone revealed a 252-bp in-frame deletion corresponding to amino acid 780–864 of mTrp4 1, a potential splice variant. The full-length alternatively spliced form of mTrp4 was

termed mTrp4S (U50921) and obtained by interchanging the *PstI*–*NotI* fragment with the mTrp4 1 clone.

Tissue distribution of mSTRPC4. To determine which mouse tissues express mSTRPC4, multiple tissue Northern blots (Clontech) containing poly(A)⁺ RNA prepared from various mouse tissues was hybridized with the same probe used to screen the library. We found that mSTRPC4 was highly expressed in brain as a mRNA doublet species that migrated at ~4 kb and 7.5 kb, respectively (Fig. 2). No signals were detected in spleen, liver, or kidney. A smear was detected in lung, testis (Fig. 2), and, only after a much longer exposure, skeletal muscle and heart (not shown). Reprobing with another cDNA fragment from a different region of mSTRPC4 resulted in the same hybridization pattern (not shown). Since the β -actin control probe gave sharp bands, it is possible that the smear observed could be due to low homology interactions or nonspecific binding, rather than RNA degradation.

Expression of mSTRPC4 in HEK293 cells. After transfection with pCMV-mSTRPC4 1, or pCMV-mSTRPC4 1-cp fused with the COOH-terminal tag, homogenates displayed strong and specific signals on Western blots, at ~97 kD, and appropriately higher in the case of the fusion proteins (Fig. 3).

Calcium influx in transfected HEK293 cells. We attempted to demonstrate functional expression of mSTRPC4 1 and mSTRPC4S by measuring Ca²⁺ or Sr²⁺ influx. Sr²⁺ has been shown to be preferentially carried by endogenous SOC channels, but not by inositol 1,4,5-trisphosphate (IP₃)-activated channels (26). HEK293 cells express an endogenous muscarinic acetylcholine receptor coupled to G_q protein and phospholipase C. Activation by IP₃ release was achieved by treatment with carbachol. In the absence of extracellular Ca²⁺, application of 100 $\mu\text{mol/l}$ carbachol to EYFP transfected control cells induced a transient rise in [Ca²⁺]_i, due to activation of IP₃ receptors on the endoplasmic reticulum (Fig. 4A1). When cells were treated with 1 $\mu\text{mol/l}$ ionomycin in the absence of extracellular Ca²⁺, a transient rise in [Ca²⁺]_i was also

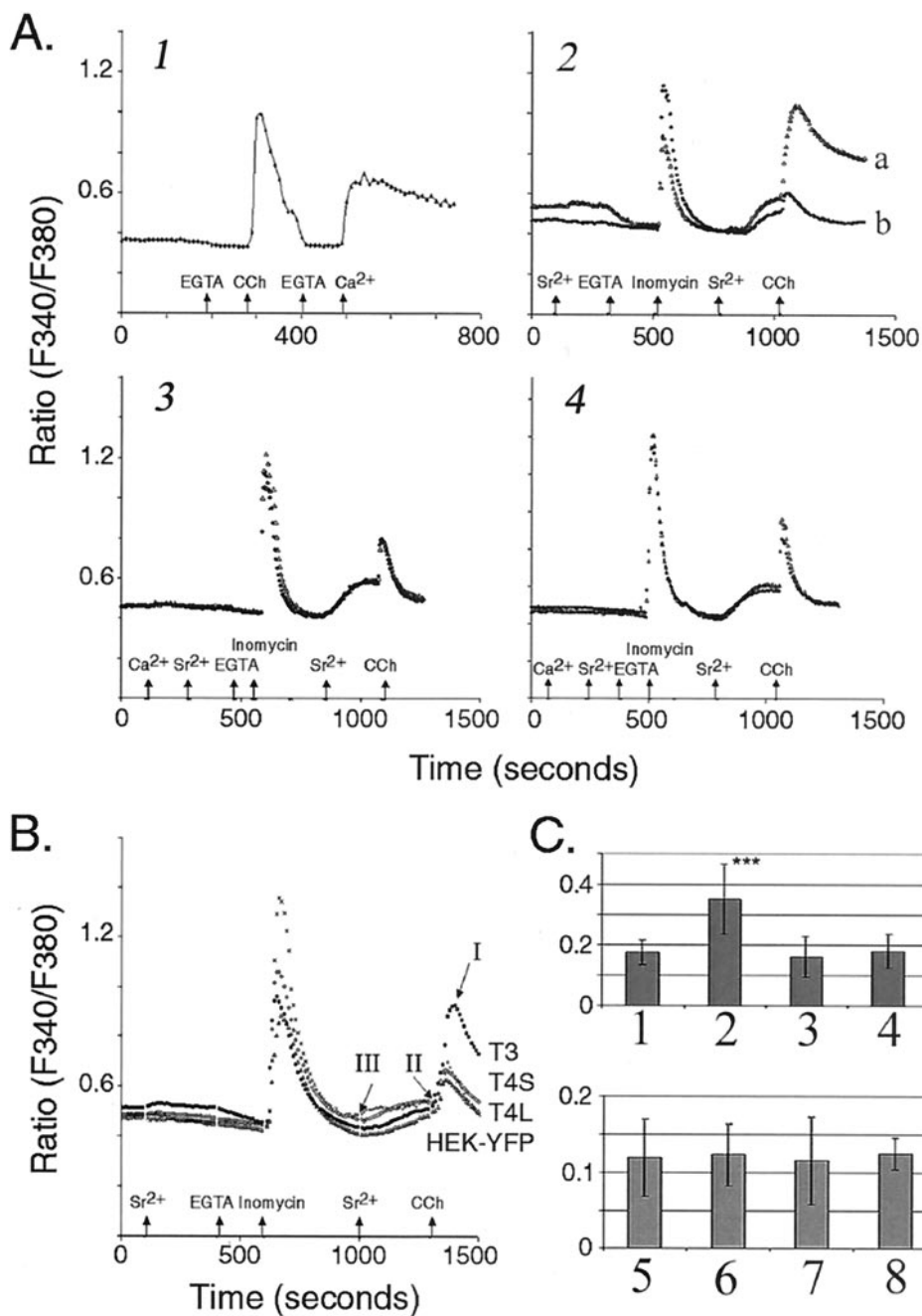


FIG. 4. Calcium influx in mSTRPC4 and hSTRPC3 transfected HEK293 cells. $[Sr^{2+}]_i$ and $[Ca^{2+}]_i$ were measured by ratiometric (F_{340}/F_{380}) fluorescence of fura-2 loaded cells as described in the text. Transiently transfected cells were identified by green fluorescence of EYFP cotransfected with the desired cDNA at a ratio of 1:9. Arrows indicate the addition of agents and changes of extracellular solutions containing Sr^{2+} , Ca^{2+} , or EGTA. **A1:** Ca^{2+} entry after carbachol stimulation in a representative control HEK293 cell. The EGTA solution is a nominal calcium-free solution, followed by carbachol (CCh) to discharge the intracellular inositol 1,4,5-trisphosphate (IP_3)-sensitive pools. The cells are then returned to calcium-free solution, and calcium-containing solution is added to reveal capacitative calcium entry. **A2:** Sr^{2+} entry in control HEK293 cell (b) compared with a cell transiently transfected with hSTRPC3 (a). Note that "a" indicates hSTRPC3 transfected cells and only these cells have a pronounced carbachol response greater than controls. **A3** and **A4:** Sr^{2+} entry induced by carbachol in HEK293 cells is indistinguishable between cells transfected with mSTRPC41 (3) and mSTRPC4S (4) or EYFP controls. **B:** Comparison of Sr^{2+} entry in HEK293 control cells (HEK-YFP) and transiently transfected with hSTRPC3 (T3) and mSTRPC4 (T4S and T41). The ratio difference between I and II is defined as receptor-activated calcium entry (RACE), whereas that between II and III is defined as capacitative calcium entry (CCE). The protocol is similar to that in 4A2. **C:** Summary of CCE and RACE evoked in transfected and control HEK293 cells. Experiments were conducted as in B. Columns 1-4 indicate the average change between points I and II, whereas 5 to 8 indicate the average change between points II and III. 1 and 5, control HEK293-YFP cells; 2 and 6, hSTRPC3 transfectants; 3 and 7, mSTRPC4CL; and 4 and 8, mSTRPC4CS. ***Significant difference ($P < 0.01$, Student's *t* test). Data represent multiple experiments from at least two separate transfections. Numbers of cells examined are as follows: HEK293, 55; hSTRPC3, 27; mSTRPC4 1, 34; and mSTRPC4CS, 56.

observed as a result of the mobilization of the intracellular Ca^{2+} stores. Ca^{2+} influx induced by the addition of extracellular Ca^{2+} indicated activation of the endogenous SOC channels (e.g., Fig. 4A1), which was substantially decreased if Sr^{2+} was used instead of Ca^{2+} (not shown). A significant increase in carbachol-activated Sr^{2+} influx was detected in the HEK293 cells expressing hSTRPC3 protein (Fig. 4A2), confirming the previous reports of hSTRPC3 functioning as an IP_3 -activated channel. Under the same experimental conditions, the level of Sr^{2+} influx after carbachol stimulation was similar in both mSTRPC4 transfected cells and mock-transfected HEK293 cells (Fig. 4A3 and 4A4). Contrary to a previous report (27), mSTRPC4 transfected cells did not display an increased level of Sr^{2+} influx after intracellular stores were depleted by ionomycin or when they were stimulated by carbachol (Fig. 4B

and C). A direct comparison of receptor-activated calcium entry (RACE) and CCE in hSTRPC3, mSTRPC4 1, and mSTRPC4S in transfected HEK293 cells was also made. This showed that only the RACE pathway stimulated by carbachol in the hSTRPC3 cells was significantly increased over the controls or other transfectants (Fig. 4C).

LTRPC2 (TRP7) is expressed in human islets. As described above, the sequence originally termed TRP7 by Okada et al. (28) has recently been found to be a channel that expresses ADPR pyrophosphatase activity, and now is termed LTRPC2 by virtue of its homology to the long (L) TRP-related family (20). We found that human islets have readily detectable levels of expression of the LTRPC2 mRNA. Three of three NH_2 -terminal oligonucleotide primers gave the expected bands at 584, 562, and 544 bp (Fig. 5, lanes 2-4). The largest amplicon was purified and

sequenced using internal primers, and the sequence was found to be identical to LTRPC2.

DISCUSSION

We have cloned and characterized two splice variants of the mouse STRPC4 homolog, originally termed mTrp4 1 and mTrp4S. The sequence similarity among STRPC genes is highest in the regions encoding the putative transmembrane domains, whereas the lowest degree of conservation is in the 3' end. The mTRPC4 protein described here was 41, 42, and 48% identical and 62, 62, and 68% similar to the deduced sequences of *Drosophila* TRP, TRPL, and human STRPC1, respectively.

In contrast to some of the other TRPCs that are clearly capable of expressing channel activity, expression of STRPC4 has been problematic. Bovine Trp4 was originally described to be an ICRAC-like store-operated channel (29). However, both mouse STRPC4 and STRPC5 have also been reported to function as receptor-activated channels, possibly via phosphorylation signaling rather than via IP₃ (30). Our own studies, using exactly the same sequence and similar expression systems, did not show receptor activation. The mSTRPC4 protein studied here was recognized by NH₂-terminal antipeptide antisera, in both Western blot and immunohistochemical studies on the expressed protein, confirming the sequence of both cDNAs and the deduced mass of the expressed protein. However, recombinant expression of the mSTRPC4 cDNAs in HEK293 cells did not increase the calcium influx induced by either carbachol stimulation or intracellular calcium store depletion. Under the same conditions, the hSTRPC3 channel increased calcium influx evoked by carbachol stimulation and had no effect on endogenous store operated Ca²⁺ influx. The reasons for these conflicting results for STRPC4 function are not obvious, but several explanations are plausible. For example, HEK293 cells have endogenous TRPC protein expression, including human STRPC4 (31). Since it is known that HEK293 cells can vary in their expression of ion channels, it is possible that the particular subline of HEK293 cells expresses a critical subunit that is necessary for STRPC4 expression. This possibility is supported by the detection of nonselective cation channels when STRPC4 was coexpressed with STRPC1 (32).

LTRPC channels are the most recent members of the TRPC family to be described, and their functions are not fully understood. We have also now found that LTRPC2/TRPC7 is expressed in human islets. This channel was recently cloned by employing a Nudix motif homology strategy, a motif that functions as a specific ADPR pyrophosphatase (20). The LTRPC2 channel was shown both to express functional ADPR pyrophosphatases activity and to be a nonselective ion channel that is activated by low micromolar amounts of ADPR. A similar current was found to be present in RINm5f cells dialyzed with ADPR in the patch pipette (reference 33 and A. Flieg, personal communication). This suggests that ADPR, the breakdown product of cyclic ADPR (cADPR), could have a role as an intracellular signaling molecule in islet β -cells. This is the first suggested biological role for ADPR. cADPR has been proposed to be a signaling molecule in insulin secretion by stimulating Ca²⁺ release from ryanodine receptor-regu-

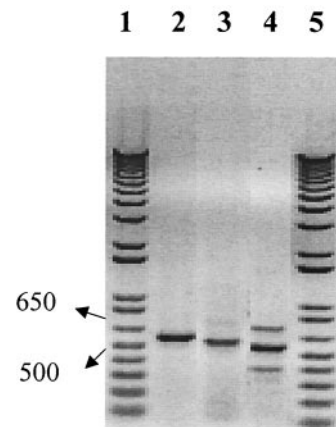


FIG. 5. LTRPC2 (TrpC7) is expressed in human islets. RNA was extracted from the human islets using Trizol reagent. The cDNA was obtained with random primers and oligodT primers (not shown). For PCR analysis of LTRPC2 expression, we used three forward primers and one reverse, as described in the text, to produce three bands of 584, 562, and 544 bp (lanes 2–4). The amplified fragment (lane 2, 584 bp) was purified and fully sequenced in both directions using *For22* and *Rev584*. The oligodT-directed cDNA did not yield any specific products. Lanes 1 and 5 are cDNA standards, with two of the bands indicated at 500 and 650 bp.

lated intracellular stores (34). In this hypothesis, the increased ATP concentration after glucose stimulation of islets leads to the accumulation of cADPR via the inhibition of the cADPR hydrolase activity of CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase). The demonstration of ADPR activation of LTRPC7 suggests that as cADPR is hydrolyzed to ADPR, activation of LTRPC7 could lead to further depolarization of the β -cell. In this way, LTRPC7 could contribute to the depolarization-induced Ca²⁺ entry via L-type, voltage-dependent calcium channels. The ADPR hydrolase activity of LTRPC7 would then make this a self-limiting process. This leads to the prediction that if intracellular concentrations of ADPR are in the micromolar range, inhibition or targeted disruption of the LTRPC7 gene should have specific inhibitory effects on regenerative calcium oscillations and second-phase insulin secretion. Note that, at this meeting, G. Rutter et al. presented evidence that cADPR can release Ca²⁺ from insulin secretory granules via ryanodine receptors, giving credence to the idea that there are separate IP₃- and ryanodine-sensitive pools in insulin-secreting cells. A scheme combining some of these ideas is shown in Fig. 6.

In summary, we report the cloning and expression of mouse STRPC4 cDNA and its alternatively spliced form from a mouse insulinoma cell library. We had previously reported that mSTRPC4 was the most readily detectable STRPC message in β TC3 cells and was also detectable in mouse pancreatic islets (12). When mSTRPC4 was expressed in HEK293 cells, it was not activated by calcium-store depletion or IP₃, despite expression of a full-length protein. We suggest that although STRPC4 is a highly conserved member of the STRPC family, additional subunits or regulatory proteins, such as STRPC1, are necessary for its function. These might be variably expressed in tumor cell lines, such as CHO and HEK293, which have been employed by others to determine STRPC4 function. The finding that LTRPC2 is also expressed in islets suggests that additional mechanisms related to nonselective

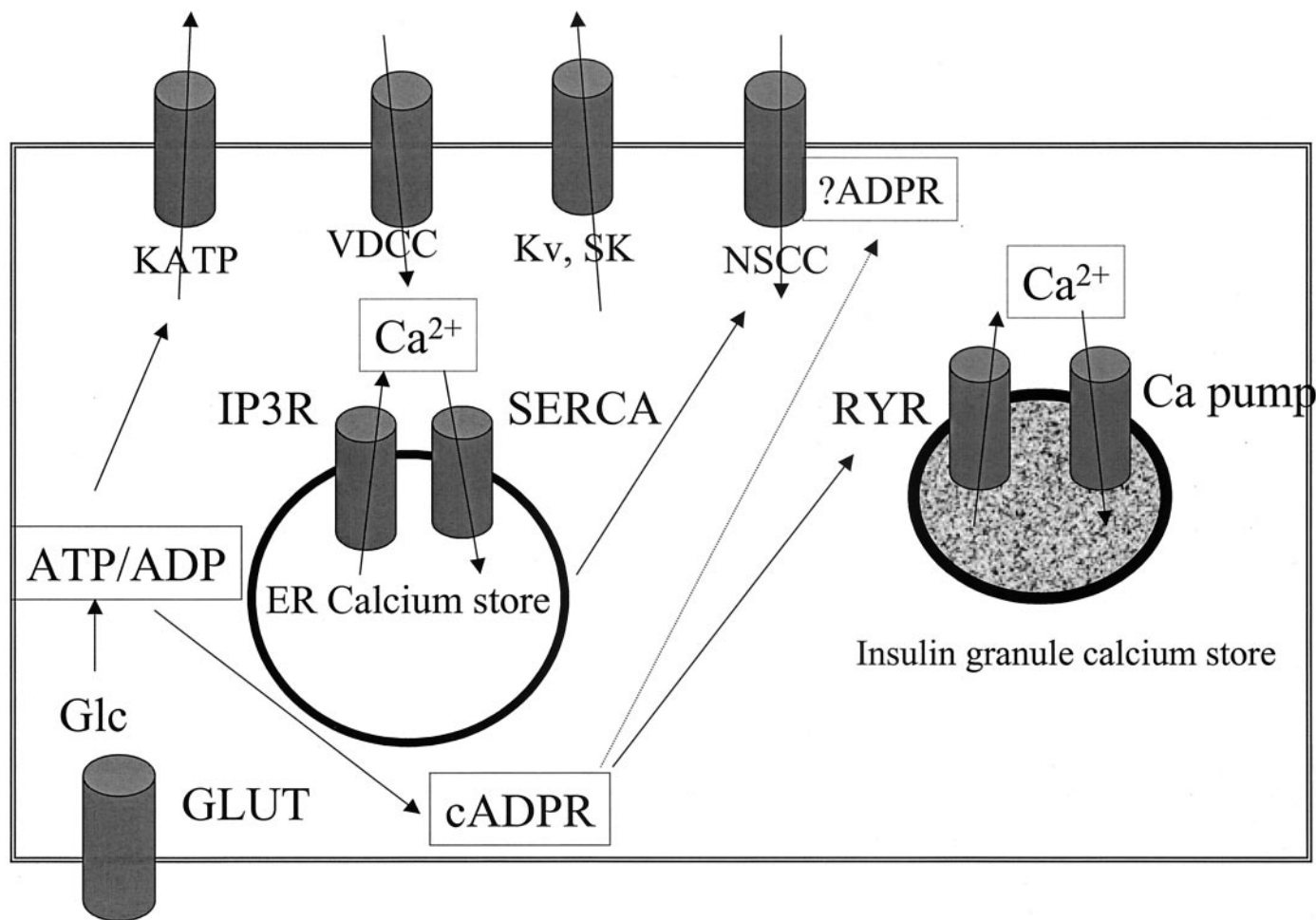


FIG. 6. Scheme: shown are the various major channels potentially involved in regenerative calcium oscillations in pancreatic β -cells. The nonselective cation channels (NSCC) are at the moment an unresolved combination of TRP-related genes, including the S (principally STRPC4 and others) and L family members (e.g., LTRPC2). The presence of O-TRPC genes is yet to be determined. KATP, ATP-sensitive K^+ channel; VDCC, voltage-dependent calcium channel; Kv, voltage-dependent delayed rectifier K^+ channel; SK, small conductance Ca-activated K^+ channel; IP3R, inositol 1,4,5-trisphosphate receptor; SERCA, sarco/endoplasmic reticulum calcium ATPase; Glc, glucose; RYR, ryanodine receptor; ER, endoplasmic reticulum.

cation channels in β -cells may be involved in regulating excitability and insulin secretion.

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