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Cloning of the α_1 subunit of a voltage-dependent calcium channel expressed in pancreatic β cells

(cDNA/insulin secretion/gene family/*in situ* hybridization/polymerase chain reaction)

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ABSTRACT **The isoforms of the α_1 subunits of voltage-dependent Ca^{2+} channels expressed in human pancreatic islets were identified by using a pair of degenerate oligonucleotide primers and the polymerase chain reaction (PCR) to amplify mRNAs encoding α_1 subunit-like sequences. The sequences of the PCR products indicate that islets express the heart-type α_1 subunit as well as a second isoform whose complete sequence has not been previously reported. The sequences of cloned cDNAs encoding the human β-cell, or neuroendocrine-type, α_1 subunit indicate that it is composed of 2181 amino acids. It shares 68%, 64%, and 41% identity with the sequences of the α_1 subunits of rabbit heart, skeletal muscle, and brain, respectively, and is predicted to have a similar structure including four homologous domains composed of six membrane-spanning segments each. RNA blotting studies indicate that the β-cell-type α_1 subunit is also expressed in brain as well as in the insulin-producing cell lines RINm5F and βTC-3; however, it could not be detected by RNA blotting in a third cell line, HIT-T15. *In situ* hybridization studies revealed expression of β-cell-type α_1 subunit mRNA in β cells of rat pancreatic islets, implying that this protein may play a role in the regulation of insulin secretion.**

Intracellular Ca^{2+} levels are the primary signal that regulates insulin secretion from pancreatic β cells (1, 2). The main pathway causing an increase in cytosolic free Ca^{2+} in β cells is the influx of Ca^{2+} across the β-cell membrane through voltage-dependent Ca^{2+} channels (VDCCs). The VDCCs are multisubunit proteins (3) and the primary structures of the α_1, α_2/δ, β, and γ subunits of the dihydropyridine-sensitive, L-type VDCC of skeletal muscle have been determined by cDNA cloning (4–7). More recently the sequences of the α_1 subunits of the VDCCs expressed in heart and brain have been described (8, 9). A partial sequence of a protein expressed in brain and neuroendocrine tissues has also been reported (10, 11) and the complete sequence of the subtype expressed in rat brain has been recently described (12). Heterologous expression studies have shown that the α_1 subunit alone is sufficient for generating voltage-sensitive Ca^{2+} channel activity (8, 13) and that coexpression of the other subunits increases activity and normalizes current kinetics (9, 14). Thus, tissue-specific expression of a family of α_1 subunits may contribute to the distinct Ca^{2+} channel characteristics of different types of cells (15, 16).

Dihydropyridine-sensitive, L-type VDCC activity has been demonstrated in pancreatic β cells (17, 18). Reasoning that there may be a specific α_1 subunit expressed in the β cells of the pancreatic islets, we used the PCR to amplify the mRNA encoding α_1 subunit-like sequences expressed in human pancreatic islets. The sequences of the PCR products showed that two different isoforms were expressed in human islets. The sequence of one showed 98% identity with that of the α_1 subunit of rabbit heart and thus was the corresponding human protein. The sequence of the other indicated that it represented a distinct member of the α_1-subunit family, which we have termed the β-cell or neuroendocrine type. Here, we report the sequence of the human β-cell-type α_1 subunit. We also show by *in situ* hybridization that mRNA encoding this protein is expressed in pancreatic β cells, which implies that the β-cell-type α_1 subunit participates in regulating insulin secretion.¶

MATERIALS AND METHODS

General Methods. Standard procedures were carried out as described (19, 20). Human islets were provided by D. W. Scharp and P. E. Lacy (Washington University School of Medicine, St. Louis). RNA was isolated by the guanidinium thiocyanate/cesium chloride procedure. DNA sequencing was done by the dideoxynucleotide chain-termination procedure (21) after appropriate DNA fragments were subcloned into M13mp18 or M13mp19. Both strands of at least two independently isolated clones were sequenced.

Cloning of cDNAs Encoding α_1 Subunits of VDCCs Expressed in Human Islets. First-strand cDNA was prepared using 10 μg of total human islet RNA, avian myeloblastosis virus reverse transcriptase (Molecular Genetic Resources, Tampa, FL), and the degenerate oligonucleotide primer CaCh-B [5′-GC(C/T)TT(G/A)AA(C/T)TC(G/A)TC(G/A/T/C)AG(G/A)TG-3′], whose sequence was selected using the cDNA sequence of the α_1 subunit of the rabbit skeletal muscle VDCC (4) as a guide. Since the cDNA sequences of the α_1 subunits expressed in heart and brain had not been reported when the primers for cDNA synthesis and PCR amplification were prepared, the primer sequences were selected from a region of homology between the α_1 subunit and the Na^+ channel that includes segments S3–S6 of repeat IV and part of the intracellular C-terminal region (4). The α_1 subunit-related sequences were amplified by PCR (22) using the sense and antisense primers, CaCh-A [5′-GA(C/T)CC(G/A/T/C)TGGAA(T/C)GT(G/A/T/C)TT(C/T)GA(C/T)T-3′] and CaCh-C [5′-GT(G/A/T/C)AG(G/A)TA(G/A)TC(G/A)AA(G/A)TT(G/A)TCCAT-3′], respectively. Primers CaCh-A, CaCh-B, and CaCh-C correspond to amino acid residues 1180–1187, 1398–1404, and 1381–1388, respectively, of the α_1 subunit of the rabbit skeletal muscle VDCC (4). The PCR was performed as previously described (23), using the

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Abbreviation: VDCC, voltage-dependent Ca^{2+} channel.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M83566).

following cycle conditions: denaturation for 1 min at 94°C, annealing for 1.5 min, and extension for 3 min at 72°C. The annealing temperatures of cycles 1–2, 3–4, and 5–40 were 37°C, 45°C, and 55°C, respectively. PCR products were separated by electrophoresis in a 1% low-melting-point agarose gel. DNA fragments of about 600 base pairs were eluted from the agarose, ligated into the *Hin*cII site of M13mp18, and sequenced. cDNAs encoding the human β-cell-type α_1 subunit were isolated by PCR amplification of RNA prepared from human islets, using specific and degenerate oligonucleotide primers based on the sequences of the human β-cell and rabbit skeletal muscle and heart isoforms (8), respectively, and by hybridization from a human insulinoma cDNA library provided by S. Smeekens and D. F. Steiner (24). The regions corresponding to the 5′ and 3′ ends of the cDNA sequence were amplified using the rapid amplification of cDNA ends (RACE) procedure (25).

The composite human β-cell α_1-subunit cDNA sequence was obtained by sequencing multiple clones of which the following are representative: phCaCh1, nucleotides 3999–4567 (this clone was originally obtained by amplification of cDNA prepared from human islet RNA using primers CaCh-A and CaCh-C as described above); λhCaCH1, nucleotides 3564–4968 (this clone was obtained by screening a human insulinoma cDNA library); phCaCH2, nucleotides 3293–3760; phCaCH3, nucleotides 1382–3394; phCaCH4, nucleotides 479–1463; phCaCH5, nucleotides 1–585; phCaCH6, nucleotides 4889–5295; phCaCH7, nucleotides 5232–6089; and phCaCH8, nucleotides 6040–7193 (the phCaCH series of clones were generated from human islet RNA).

***In Situ* Hybridization.** Adjacent frozen sections of fixed Wistar rat pancreas were hybridized to ^{35}S-labeled antisense β-cell-type α_1-subunit and insulin II RNA probes, as described previously (26). After hybridization, the slides were treated with RNase, washed under stringent conditions, dipped in NTB-3 emulsion, and stored in the dark at 4°C for 6 weeks for β-cell-type α_1-subunit mRNA and 6 days for insulin mRNA. Finally the slides were developed, fixed, and counterstained for observation. To prepare a specific antisense RNA probe for the β-cell-type α_1 subunit, we isolated a partial rat β-cell α_1-subunit cDNA, prCaCH4a1-1, encoding a region corresponding to nucleotides 2426–2743 (amino acids 770–875) of the human protein. This region includes the intracellular loop connecting repeats II and III, which is one of the most divergent regions among α_1-subunit isoforms (Fig. 1). There is 99% and 91% amino acid and nucleotide sequence identity, respectively, between the human and the rat sequences in this region of the β-cell α_1 subunit; however, the codon for Val-845 was deleted from the rat cDNA. The 315-base-pair rat β-cell α_1-subunit cDNA was cloned into the *Sma* I site pGEM-7Zf(+) (Promega). ^{35}S-labeled antisense RNA was prepared by transcription of *Bam*HI-digested prCaCH4a1-1 with T7 RNA polymerase.

RESULTS

Identification of α_1 Subunits Expressed in Human Islets. Partial cDNAs encoding the S3–S6 region of repeat IV of two structurally related α_1 subunits were isolated and sequenced after PCR amplification of α_1 subunit-encoding mRNAs expressed in human islets using primers CaCh-A and -C (see *Materials and Methods*). The predicted amino acid sequence of one group of partial-length cDNA clones showed 98% identity with the α_1 subunit of the rabbit heart VDCC and thus corresponded to the human heart isoform. The second showed 78% and 81% amino acid identity with the sequences of the rabbit skeletal muscle and heart α_1 subunits, respectively (the sequence of the brain isoforms had not yet been reported). This second sequence was termed the β-cell or neuroendocrine isoform of α_1.

Sequence of the Human β-Cell-Type α_1 Subunit. Overlapping cDNA fragments spanning 7193 base pairs and encoding the human β-cell isoform of the α_1 subunit were obtained by screening a human insulinoma cDNA library and by PCR-based strategies. The composite cDNA sequence contained a single long open reading frame beginning with the second ATG (nucleotides 119–121) in the cDNA sequence [there is an in-frame termination codon (nucleotides 95–97) upstream of this ATG] that predicted the sequence of a 2181-amino acid protein (M_r 247,641) (Fig. 1). The short open reading frame beginning with the first ATG (nucleotides 109–111) is followed by a termination codon, TGA (nucleotides 226–228). The N terminus of the β-cell-type α_1 subunit is characterized by a stretch of seven consecutive methionine residues, a feature not present in other α_1 subunit isoforms (Fig. 1). Its significance is unclear but it may indicate that translational regulation plays a role in regulating the expression of this protein.

The sequence of the human β-cell-type α_1 subunit is related to those of the α_1 subunits of rabbit heart, skeletal muscle, and brain, having 68%, 64%, and 41% overall amino acid identity with the sequences of these other proteins, respectively (Fig. 1) (4, 8, 9). Computer analysis of the sequence of the β-cell-type α_1 subunit predicts that it also has a structure similar to that originally proposed for the α_1 subunit of the skeletal muscle VDCC (4), including four intramolecular homologous domains (I–IV) with each repeat having six putative membrane-spanning regions (S1–S6). As shown in Fig. 1, the sequences of the α_1 subunits in the regions corresponding to the four internal repeats (I–IV) are well conserved, especially the fourth transmembrane segment of each repeat (S4), which has positively charged amino acid residues, arginine or lysine, at every third position and is believed to function as a voltage sensor (3). By contrast, the sequences of the N and C termini and the intracellular loop connecting repeats I and II and repeats II and III are divergent among different isoforms, implying that these regions may contribute to the isoform-specific electrophysiological and pharmacological properties (9, 27).

There are three potential N-linked glycosylation sites in the β-cell α_1 subunit at Asn-155, Asn-215, and Asn-329, located in regions of the protein that are predicted to be external to the plasma membrane; however, only one of the potential glycosylation sites (Asn-329) is present in all four members of the α_1-subunit family. There are 11 potential cAMP-dependent protein kinase phosphorylation sites (serine or threonine residues at 464, 465, 802, 869, 1510, 1679, 1720, 1793, 1820, 1942, and 1952), nine potential cGMP-dependent protein kinase phosphorylation sites (serine or threonine at 30, 100, 522, 1491, 1824, 1881, 1889, 1922, and 1998), and a protein kinase C-dependent phosphorylation site (Ser-1605) (28) in regions of the β-cell-type α_1 subunit that are predicted to be cytoplasmically located. The presence of these putative phosphorylation sites suggests that β-cell VDCC activity may be regulated by cAMP, cGMP, and/or activators of protein kinase C, as has been reported for L-type VDCCs in other cells (29–32). In fact, Henquin and Meissner (33) have suggested that cAMP facilitates Ca^{2+} influx into β cells by modulating the gating properties of the β-cell VDCC.

β-Cell-Type α_1 Subunit Is Expressed in Pancreatic Islets and Brain. A human β-cell-type α_1 subunit cDNA probe hybridized to a single 11-kilobase transcript present in rat islets and brain (Fig. 2). There was no detectable hybridization to blots containing 20 μg of total RNA from a number of different human and monkey tissues including skeletal muscle, heart, kidney, spleen, liver, jejunum, and colon (data not shown). Of the insulin-producing cell lines tested, the highest levels of β-cell-type α_1-subunit mRNA were found in RINm5F cells and low levels of hybridization were noted in βTC-3 cells (Fig. 2). There was no observable signal in HIT-T15 cells;

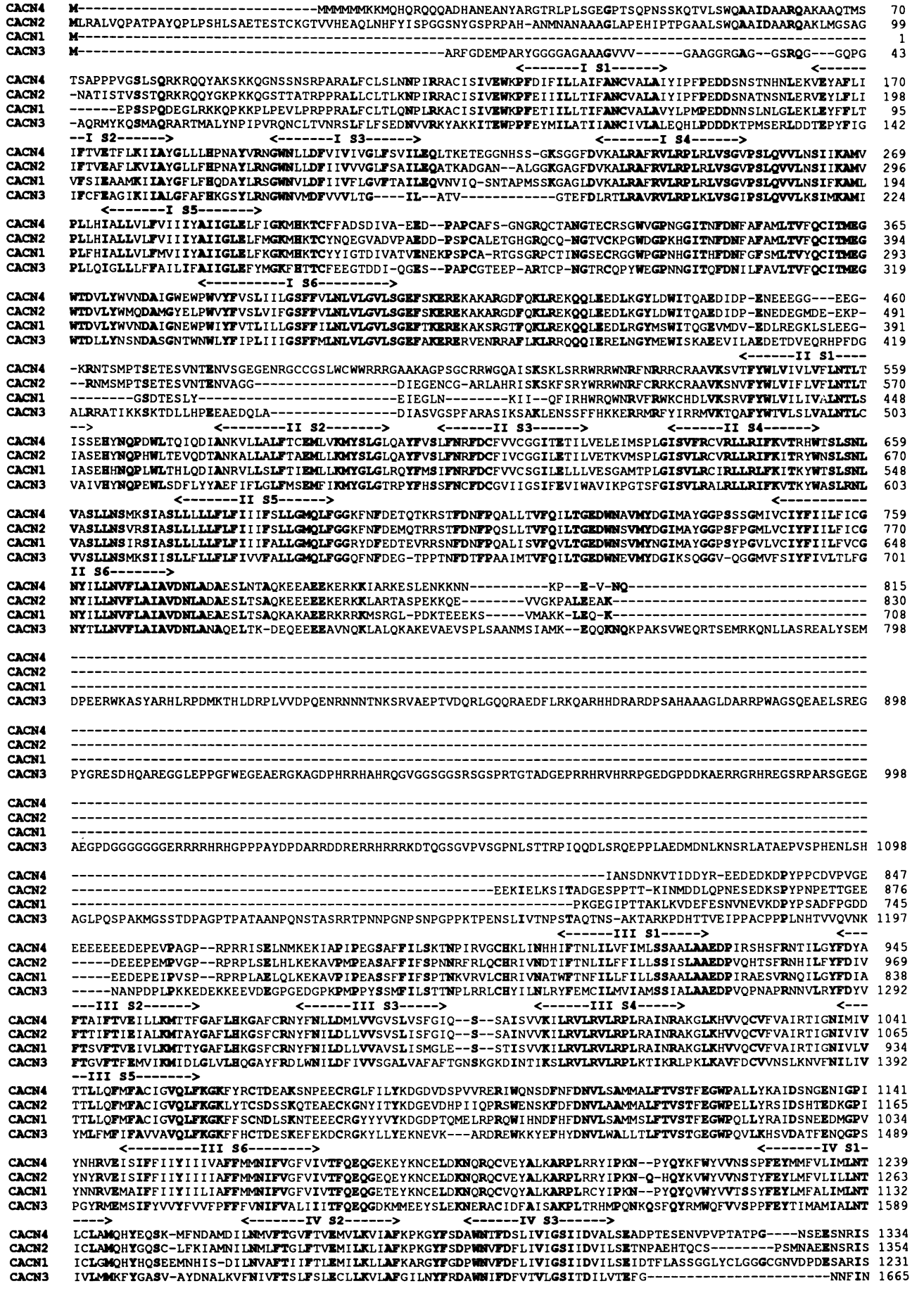

FIG. 1. (*Figure continues on the opposite page.*)

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          <------IV S4------>                    <-------IV S5------>
CACN4   ITFFRLFRVMRLVKLLSRGEGIRTLLWTFIKSFQALPYVALLIAMLFFIYAVIGMQMFGKVAM--------RDNNQINRNNNFQTFPQAVLLLFRCATGE 1426
CACN2   ITFFRLFRVMRLVKLLSRGEGIRTLLWTFIKSFQALPYVALLIVMLFFIYAVIGMQVFGKIAL--------NDTTEINRNNNFQTFPQAVLLLFRCATGE 1446
CACN1   SAFFRLFRVMRLIKLLSRAEGVRTLLWTFIKSFQALPYVALLIVMLFFIYAVIGMQMFGKIAL--------VDGTQINRNNNFQTFPQAVLLLFRCATGE 1323
CACN3   LSFLRLFRAARLIKLLRQGYTIRILLWTFVQSFKALPYVCLLIAMLFFIYAIIGMQVFGNIGIDMEDEDSDEDEFQITEHNNFRTFFQALMLLFRSATGE 1765
                                        <---------IV S6--------->
CACN4   AWQEIMLACLPGKLCDPESDY---NPGEEYTCGSNFAIVYFISFYMLCAFLIINLFVAVIMDNFDYLTRDWSILGPHHLDEFKRIWSEYDPEAKGRIKHL 1523
CACN2   AWQDIMLACMPGKKCAPESEPHNSTEGET-PCGSSFAVFYFISFYMLCAFLIINLFVAVIMDNFDYLTRDWSILGPHHLDEFKRIWAEYDPEAKGRIKHL 1545
CACN1   AWQEILLACSYGKLCDPESDY---APGEEYTCGTNFAYYYFISFYMLCAFLIINLFVAVIMDNFDYLTRDWSILGPHHLDEFKAIWAEYDPEAKGRIKHL 1420
CACN3   AWHNIMLSCLSGKPCDKNSGI--LTP-E---CGNEFAYFYFVSFIFLCSFLMLNLFVAVIMDNFEYLTRDSSILGPHHLDEYVRVWAEYDPAAWGRMLYR 1859

CACN4   DVVTLLRRIQPPLGFGKLCPHRVACKRLVAMNMPLNSDGTVMFNATLFALVRTALKI---KTEGNLEQANEELRAVIKKIWKKTSMKLLDQVVPPAGDDE 1620
CACN2   DVVTLLRRIQPPLGFGKLCPHRVACKRLVSMNMPLNSDGTVMFNATLFALVRTALRI---KTEGNLEQANEELRAIIKKIWKRTSMKLLDQVVPPAGDDE 1642
CACN1   DVVTLLRRIQPPLGFGKFCPHRVACKRLVGMNMPLNSDGTVTFNATLFALVRTALKI---KTEGNFEQANEELRAIIKKIWKRTSMKLLDQVIPPIGDDE 1517
CACN3   DMYAMLRHMPPPLGLGKNCPARVAYKRLLRMDLPVADDNTVHFNSTLMALIRTALDIKIAKGGADKQQMDAELRKEMMAIWPNLSQKTLDLLVTPHKSTD 1959
                                                                ↓
CACN4   VTVGKFYATFLIQDYFRKFKKRKEQGLVGKYPAKNTTIALQAGLRTLHD-IGPEIRRAISCDLQDDEPEETKREE------EDDVFKRNGALLGNHVNHV 1713
CACN2   VTVGKFYATFLIQEYFRKFKKRKEQGLVGKPSQRNALS-LQAGLRTLHD-IGPEIRRAISGDLTAEEELDKAMKEAVSAASEDDIFRRAGGLFGNHVSYY 1740
CACN1   VTVGKFYATFLIQEHFRKFMKRQEE-YYGYRPKKDTVQ-IQAGLRTIEEEAAPEIRRTISGDLTAEEELERAMVEA---AMEERIFRRTGGLFGQVDTFL 1612
CACN3   LTVGKIYAAMMIMEYYRQSKAKKLQ-AMREEQNRTPLM-FQRMEPPPDEGGA----------------------GQNALPSTQLDPAGGLMAHEDGLK 2033

CACN4   NSDRRDSLQQTNTTHRPLHVQRPSIPPA-----SDTEKPLFPPAGNSVCHNHHNHNSIGKQVPTSTNANLNNANMSKAAHGKRPSIGNLEHVSENGHHSS 1808
CACN2   QSDSRSAFPQTFTT------QRPLHISKAGNNQGDTESPSHEKLVDSTFTPSSYS-------STGSNANINNNAN-NTALGRLPRPAGYPSTVSTVEGHGS 1826
CACN1   E--------RTNSLPPVMANQRPLQFAEIEMEE--LESP----VFLEDFPQDART-------NPLARANTNNANANVAYGN--SNHSNNQMFSSVHCERE 1689
CACN3   DS----PSWVT---------QRAQEMFQKTGTWSPERAPPADMA-DSQPKPQS---------------------------------------------- 2072

CACN4   HKHDREPQRRSSVKRTRYYETYIRSDSGDEQLPTICREDPEIHGYFRDPHCLGEQEYFSSEECYEDDSSPTWSRQNYGYYSRYPGRNIDSERPRGYHHPQ 1908
CACN2   PLSPAVRAQEAAWKLSSKRCHSQESQIAMACQEGASQDDNYDVRIGEDAECCSEPSLLSTEMLSYQDDENRQLAPPEEEKRDIRLSPKKGFLRSASLGRR 1926
CACN1   FPGEAET---------------------------------------------------------------------------------------- 1696
CACN3   -----VEMREMSQDGYSDSEHCLPMEGQARAASMPRLPAENQRRRGRPRGSDLSTICDTSPMKRSASVLGPKASRRLDDYSLERVPPEENQRHHPRRRER 2167

CACN4   GFLEDDDSPVCYDSRRSPRRRLLPPTPASHRRSSFNFECLRRQSSQEEVPSSPIFPERTALPLHLMQQQIMAVAGLDSSKAQKYSPSHSTRSW-ATPPAT 2007
CACN2   ASFHLECLKRQKNQGGDISQKTVLPLHLVHHQAL---------------------------------------AVAGLSPLLQRSHSPT-SLPRPCATPPAT 1988
CACN1   ---------------------------------------------------------------PAAGKGAL-SHSHRALGPHSKPCAGKLNG 1724
CACN3   AHRTSERSLGRYTDVDTGLGTDLSMTTQSGDLPSREREQERGRPKDRKHRPHHHHHEHHHPGRGPGRVSPGVSARRRRRGPVARVRPARAPALAHARARA 2267

CACN4   PP-YRDWT-PCYTP-LIQVEQSEALDQVN--------GSLPSLHRSSWYT-DEP------DIS-YRTFTPASLTVPSSFRNKNSDKQRSADSLVEAVLIS 2088
CACN2   P-GSRGWP-PQPIPT-LRLEGADSSEK--------LNSSFPSIHCGSWSGENSPCRG---DSSAARRARPVSLTVPSQAGAQGRQFHGSASSLVEAVLIS 2074
CACN1   QLVQPGMPINQAPPAPCQQPSTDPPER----------GQRRTSLTGSLQDEAPQRRSSEG--STPRRPAPATALLIQEA---------------------- 1791
CACN3   RAPARLLPELRLRRARRPRPRQRRRPRRRRGGGGRALRRAPGPREPLAQDSPGRGPSVCL-ARAARPAGPQRLLPGPRTGQAPRARLPQ----------- 2355

CACN4   EGLGRYARDPKFVSATK---HEIADACDLTIDEMESAASTLLNGNVRPRANGDVGPLSHRQDYELQDFGPGY---S-DEEPDPGRDEEDLADEMICITTL 2181
CACN2   EGLGQFAQDPKFIEVTT---QELADACDLTIEEMENAADDILSGGARQSPNGTLLPFVNRRDPGRDRAGQNEQDASGACAPGCGQSEEALADRRAGVSSL 2171
CACN1   LVRGGLDTLAADAGFVTATSQALADACQMEPEEVEVAATELLK--ARESVQGMASVPGSLSRRS--------SLGSLDQVQ--G-SQETLIPPRP      1873
CACN3   KPARSVQRERRGLVLSPPPPPP-----------GELAPRAHPARTPRPGP-GDSRSRRGGRRWT----------ASAGK---GGGGPRASA-PSP      2424
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FIG. 1. Comparison of the predicted amino acid sequence of the human β-cell-type α_1 subunit and other α_1-subunit isoforms. The designations for the isoforms are as follows: human β-cell, CACN4; rabbit skeletal muscle, CACN1; rabbit heart, CACN2; and rabbit brain (BI-2; ref. 9), CACN3. The single-letter abbreviations for the amino acids are shown. The domains of the α_1 subunit are presented above the sequence. Residues that are identical among all isoforms, excluding gaps, are shown in bold type, and gaps introduced to generate this alignment are shown as dashes. The number of the amino acid residue at the end of each line is noted. The arrow above amino acid 1667 of the human β-cell α_1 subunit, CACN4, indicates the location of the C terminus of the related rat brain protein, RBα1 (12).

however, Perez-Reyes *et al.* (11) have shown the presence of β-cell-type α_1 subunit mRNA in HIT cells by PCR amplification.

Localization of β-Cell-Type α_1-Subunit mRNA in β Cells by *in Situ* Hybridization. PCR amplification and RNA blotting studies indicate that the β-cell-type α_1 subunit is expressed in human and rat islets. The localization of β-cell α_1 subunit mRNA within the islet was determined by *in situ* hybridization. A rat β-cell α_1-subunit antisense RNA probe showed specific hybridization to rat islets (Fig. 3*A*). The pattern of hybridization was identical to that seen in an adjacent section that was hybridized with an insulin probe (Fig. 3*B*), indicating that β-cell-type α_1-subunit mRNA is expressed in β cells.

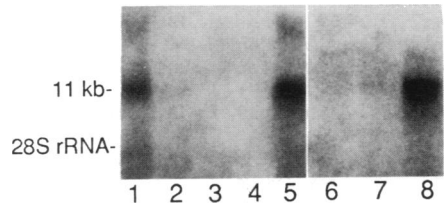

FIG. 2. Expression of β-cell-type α_1 subunit mRNA in adult rat tissues and insulin-producing cell lines. Lane 1, brain; lane 2, liver; lane 3, pancreas; lane 4, no sample; lane 5, pancreatic islets; lane 6, HIT T15 cells; lane 7, βTC-3 cells; lane 8, RINm5F cells. Twenty micrograms of total RNA was denatured with glyoxal, separated by agarose gel electrophoresis, and blotted onto a nylon membrane. The filter was hybridized with the nick-translated insert from phCaCH3 (described in *Materials and Methods*) under standard hybridization conditions and washed in 15 mM NaCl/1.5 mM sodium citrate, pH 7/0.1% SDS at 50°C and exposed to x-ray film with an intensifying screen at −80°C for 1 week. The size of the hybridizing transcript [11 kilobases (kb)] and the position of 28S rRNA are indicated. Lanes 1–5 and 6–8 are from two different RNA blots.

DISCUSSION

Electrical activity of the pancreatic β cell plays an important role in stimulus–secretion coupling. The metabolism of glucose by β cells leads to an increase in the ATP/ADP ratio resulting in the closing of ATP-sensitive K^+ channels and membrane depolarization (34, 35). This causes the opening of VDCCs, and the influx of Ca^{2+} leads to fusion of secretory granules with the plasma membrane and release of insulin (1). The present study indicates that pancreatic islets express two different VDCC α_1 subunits. One corresponds to the α_1 subunit first identified in heart and subsequently found in aorta (36) and lung (37). The other represents an α_1 subunit for which partial cDNA clones were described from brain (10) and HIT cells (11). Hui *et al.* (12) recently reported the sequence of cDNA clones encoding a rat brain VDCC α_1 subunit, RBα1. There is 98% identity between the amino acid sequences of RBα1 and the human β-cell/neuroendocrine-type α_1 subunit presented in this report. Although the amino acid identity between these two proteins is very striking, they differ significantly in size, and the intracellular C-terminal

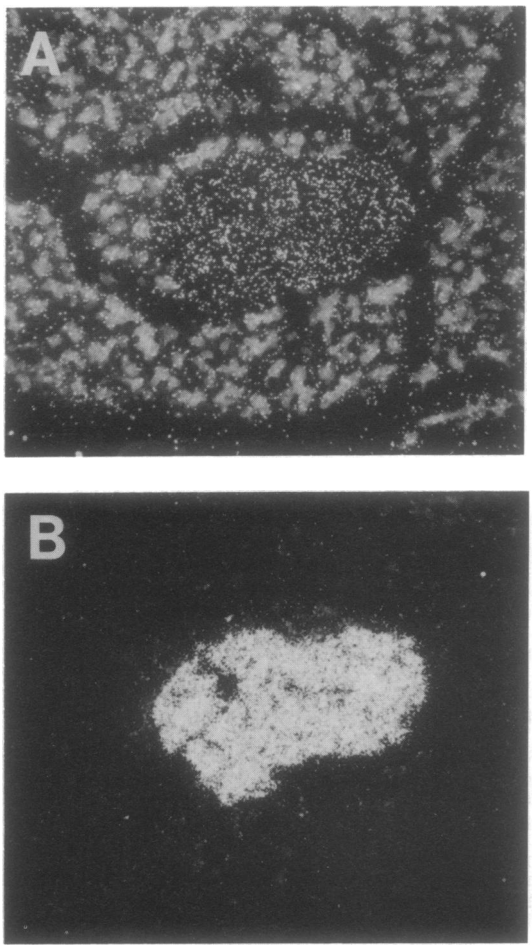

FIG. 3. Dark-field photomicrographs of adjacent sections of rat pancreas hybridized *in situ* with ^{35}S-labeled antisense RNA probes for rat β-cell-type α_1 subunit (*A*) and insulin (*B*). Bright areas due to deposition of silver grains indicate regions of hybridization to β-cell-type α_1-subunit (*A*) or insulin (*B*) mRNAs.

domain of RBα1 is 548 amino acids shorter than that of the human β-cell protein (Fig. 1). It is unknown whether this reflects tissue-specific splicing and the expression of α_1 subunits having C termini of different lengths in brain and β cells. The functional consequences of this size difference are unknown.

Electrophysiological studies indicate that there are two types of Ca^{2+} channels in β cells (17, 34, 35). The presence of mRNA encoding β-cell and heart-type α_1 subunits of VDCCs in human and rat islets (this paper; Y. Iwashima, K. S. Polonsky, G.I.B., and S.S., unpublished work) and in insulin-secreting HIT cells (11) provides a molecular explanation for the presence of different Ca^{2+} currents in β cells. Alternative splicing may also generate additional α_1-subunit diversity (11, 12), which could alter the electrical properties of β cells. Determination of the relative abundance of the β-cell and heart-type α_1 subunits in normal islets and characterization of their electrophysiological and pharmacological properties when expressed in heterologous systems will clarify their contributions to β-cell Ca^{2+} channel activity.

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1. Wollheim, C. B. & Sharp, G. W. G. (1981) *Physiol. Rev.* **61**, 914–973.
2. Prentki, M. & Matschinsky, F. (1987) *Physiol. Rev.* **67**, 1185–1248.
3. Catterall, W. A. (1988) *Science* **242**, 50–61.
4. Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. & Numa, S. (1987) *Nature (London)* **328**, 313–318.
5. Ellis, S. B., Williams, M. E., Ways, N. R., Brenner, R., Sharp, A. H., Leung, A. T., Campbell, K. P., McKenna, E., Koch, W. J., Hui, A., Schwartz, A. & Harpold, M. M. (1988) *Science* **241**, 1661–1664.
6. Ruth, P., Rohrkasten, A., Biel, M., Bosse, E., Regulla, S., Meyer, H. E., Flockerzi, V. & Hofmann, F. (1989) *Science* **245**, 1115–1117.
7. Jay, S., Ellis, S., McCue, A. F., Williams, M. E., Vedrick, T. S., Harpold, M. M. & Campbell, K. P. (1990) *Science* **248**, 490–492.
8. Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S. & Numa, S. (1989) *Nature (London)* **340**, 230–233.
9. Mori, Y., Firedrich, T., Kim, M.-S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furuichi, T., Mikoshiba, K., Imoto, K., Tanabe, T. & Numa, S. (1991) *Nature (London)* **350**, 398–402.
10. Snutch, T. P., Leonard, J. P., Gilbert, M. M., Lester, H. A. & Davidson, N. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3391–3395.
11. Perez-Reyes, E., Wei, X., Castellano, A. & Birnbaumer, L. (1990) *J. Biol. Chem.* **265**, 20430–20436.
12. Hui, A., Ellinor, P. T., Krizanova, O., Wang, J., Diebold, R. J. & Schwartz, A. (1991) *Neuron* **7**, 35–44.
13. Perez-Reyes, E., Kim, H. S., Lacerda, A. E., Horne, W., Wei, X., Rampe, D., Campbell, K. P., Brown, A. M. & Birnbaumer, L. (1989) *Nature (London)* **340**, 233–236.
14. Varadi, G., Lory, P., Schultz, D., Varadi, M. & Schwartz, A. (1991) *Nature (London)* **352**, 159–162.
15. Miller, R. (1987) *Science* **235**, 46–52.
16. Tsien, R. W., Lipscombe, D., Madison, D. V., Bley, D. R. & Fox, A. P. (1988) *Trends Neurosci.* **10**, 431–438.
17. Cook, D. L., Satin, L. S. & Hopkins, W. S. (1991) *Trends Neurosci.* **14**, 411–414.
18. Smith, P. A., Rorsman, P. & Aschroft, F. M. (1989) *Nature (London)* **342**, 550–553.
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Fukumoto, H., Seino, S., Imura, H., Seino, Y., Eddy, R. L., Fukushima, Y., Beyers, M. G., Shows, T. B. & Bell, G. I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5434–5438.
21. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161–178.
22. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
23. Seino, S., Seino, M. & Bell, G. I. (1990) *Diabetes* **39**, 123–128.
24. Smeekens, S. P. & Steiner, D. F. (1990) *J. Biol. Chem.* **265**, 2997–3000.
25. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8998–9002.
26. Chen, L., Alan, T., Johnson, J. H., Hughes, S., Newgard, C. B. & Unger, R. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4088–4092.
27. Tanabe, T., Beam, K. G., Adams, B. A., Niidome, T. & Numa, S. (1990) *Nature (London)* **346**, 567–569.
28. Kemp, B. E. & Pearson, R. B. (1990) *Trends Biochem. Sci.* **15**, 336–342.
29. Trautwein, W. & Hescheler, J. (1990) *Annu. Rev. Physiol.* **52**, 257–274.
30. Artalejo, C. R., Ariano, M. A., Perlman, R. L. & Fox, A. P. (1990) *Nature (London)* **348**, 239–242.
31. Mery, P.-F., Lohmann, S. M., Walter, U. & Fischmeister, R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1197–1201.
32. Dösemeci, A., Dhallan, R. S., Cohen, N. M., Lederer, W. J. & Rogers, T. B. (1988) *Circ. Res.* **62**, 347–357.
33. Henquin, J. C. & Meissner, H. P. (1984) *Endocrinology* **115**, 1125–1134.
34. Ashcroft, F. M. & Rorsman, P. (1989) *Prog. Biophys. Mol. Biol.* **54**, 87–143.
35. Rajan, A. S., Aguilar-Bryan, L., Nelson, D. A., Yaney, G. C., Hsu, W. H., Kunze, D. L. & Boyd, A. E. (1990) *Diabetes Care* **13**, 340–363.
36. Koch, W. J., Ellinor, P. T. & Schwartz, A. (1990) *J. Biol. Chem.* **265**, 17786–17791.
37. Biel, M., Ruth, P., Bosse, E., Hullin, R., Stühmer, W., Flockerzi, V. & Hofmann, F. (1990) *FEBS Lett.* **269**, 409–412.