

Cloning and tissue-specific expression of the brain calcium channel β -subunit

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A cDNA clone encoding a protein with high homology to the β -subunit of the rabbit skeletal muscle dihydropyridine-sensitive calcium channel was isolated from a rat brain cDNA library. This rat brain β -subunit cDNA hybridizes to a 3.4 kb message that is expressed in high levels in the cerebral hemispheres and hippocampus but is significantly reduced in cerebellum. The open reading frame encodes 597 amino acids with a predicted mass of 65 679 Da which is 82% homologous with the skeletal muscle β -subunit. The brain cDNA encodes a unique 153 amino acid C-terminus and predicts the absence of a muscle-specific 50 amino acid internal segment. It also encodes numerous consensus phosphorylation sites suggesting a role in calcium channel regulation. The corresponding human β -subunit gene was localized to chromosome 17. Hence the encoded brain β -subunit, which has a primary structure highly similar to its isoform in skeletal muscle, may have a comparable role as an integral regulatory component of a neuronal calcium channel.

β -Subunit; Calcium channel; cDNA cloning; Brain

1. INTRODUCTION

Voltage-activated calcium channels mediate the entry of calcium into a wide variety of cell types. The elevation of internal calcium contributes to a range of physiological functions including muscle contraction and the secretion of neurotransmitters and hormones [1–4]. The best studied of these channels is the skeletal muscle dihydropyridine receptor (DHPR). The purified DHPR consists of four subunits (α_1 , α_2 , β and γ) which have all been biochemically characterized and cloned [1–11]. The β -subunit, a 52 000 Da peripheral membrane protein is believed to form a tight association with the cytoplasmic domain of the α_1 -subunit, the 170 000 Da channel-forming component that contains the binding site for the dihydropyridines [12]. The primary structure of the DHPR β -subunit contains numerous consensus phosphorylation sites [10] and the native protein is phosphorylated *in vitro* suggesting a calcium channel regulatory role [13,14]. Recent evidence of regulation was shown by the modification of channel kinetics upon cotransfection of the β -subunit with the α_1 -subunit in L-cells [15,16].

Neurons have been shown to possess at least four types of calcium channels (L, N, T and P) based on their kinetics and pharmacology [17,18]. The cDNA encoding the α_1 -subunit of the skeletal muscle dihydropyridine

receptor has been used to isolate related sequences in brain. These numerous partial clones were assigned to classes A, B, C or D based on their distinct hybridization patterns [19]. The recently cloned full-length brain α_1 -subunit BI [20], which corresponds most closely with type P in toxin sensitivity and kinetics has homology with sequences assigned to class A. Several lines of evidence suggest the presence of a β -subunit as a component of these brain calcium channels. The coexpression of skeletal muscle β - and α_2 -subunits with the brain α_1 -subunit, BI, was shown to be essential for the formation of functional channels in *Xenopus* oocytes. The inclusion of these two subunits resulted in a 200-fold increase in channel activity [20]. A monoclonal antibody against the β -subunit of skeletal muscle DHPR was recently shown to immunoprecipitate both neuronal dihydropyridine (L-type) and ω -conotoxin (N-type) receptors [21]. Finally by criterion of Northern blot analysis a transcript similar to the DHPR β -subunit has been detected in brain [9,10]. Here we report the cloning of a full-length cDNA encoding a rat brain β -subunit and localization of the corresponding human gene to chromosome 17. Though regionally divergent, the high homology it shares with the DHPR β -subunit suggests the encoded protein is an integral regulatory component of a neuronal calcium channel.

2. MATERIALS AND METHODS

2.1. Isolation of rabbit skeletal muscle β -subunit cDNA

Skeletal muscle β -subunit specific antibodies were prepared from sheep polyclonal serum. The sheep was immunized subcutaneously

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| | human chromosome | human cells | hybrid 937 | hybrid 854 | hybrid 507 | hybrid 909 | hybrid 1006 | hybrid 811 | hamster cells |
|----|------------------|-------------|------------|------------|------------|------------|-------------|------------|---------------|
| 1 | + | + | | | | | | | |
| 2 | + | | + | | | | | | |
| 3 | + | | | + | | | | | |
| 4 | + | | | | | + | + | | |
| 5 | + | + | + | + | + | + | | | |
| 6 | + | | | | + | | | | |
| 7 | + | | | | | + | | | |
| 8 | + | | | | + | + | + | | |
| 9 | + | | | | | | | | |
| 10 | + | | | | | | | | |
| 11 | + | | | | | | | | |
| 12 | + | | | + | | | | | |
| 13 | + | | | | | + | | | |
| 14 | + | + | | + | + | | | | |
| 15 | + | + | | | | + | | | |
| 16 | + | | | | | | | | |
| 17 | + | + | | | | | | + | |
| 18 | + | | | | | | | + | |
| 19 | + | | | | | + | | | |
| 20 | + | | | + | | | | | |
| 21 | + | + | | | | + | | | |
| 22 | + | | | + | + | | | | |
| x | + | | | | | | | | |
| y | + | | | + | | | | | |

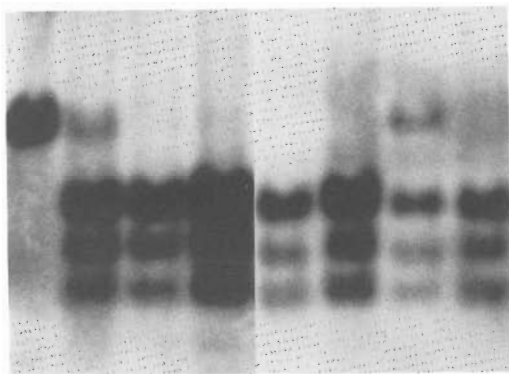


Fig. 4. Localization of the brain β -subunit gene to chromosome 17 using human/hamster somatic cell hybrids. Southern blot of genomic DNAs isolated from a panel of human/hamster cell hybrids (Bios Corp.) were hybridized with ^{32}P -labeled full-length rat brain β -subunit cDNA. Each lane contained 8 μg DNA from human cells, hybrid lines 937, 854, 507, 909, 1006, 811 and hamster cells. Hybrid lines shown represent only a portion (6 out of 25) of those screened; data from lines not shown were consistent with localization to chromosome 17. Human chromosomes present in cell lines are shown above the Southern blot.

cDNA encodes numerous consensus phosphorylation sites suggesting that they both share a channel regulatory role. The cAMP-dependent phosphorylation site at threonine²⁰⁵ and serine¹⁸² (not consensus) of the skeletal muscle protein was shown to be phosphorylated *in vitro* by the sequencing of tryptic phosphopeptides [10,27]. The conservation of these sites suggest that they may also be phosphorylated in the brain protein.

Analysis of DNA from panel of human/hamster

somatic cell hybrids [28] localized the human gene corresponding to the brain calcium channel β -subunit to chromosome 17 (Fig. 4).

The data presented here show that a mRNA with high homology to the β -subunit of the skeletal muscle dihydropyridine receptor is expressed in brain with regional specificity. The primary structure of the encoded brain calcium channel β -subunit predicts a unique C-terminus and the absence of a 50 amino acid internal segment specific to the skeletal muscle derived protein. The brain cDNA predicts a protein free of transmembrane domains with numerous consensus phosphorylation sites consistent with its skeletal muscle counterpart suggesting that they share a similar regulatory function. Together these data are consistent with the presence of a brain β -subunit which, similar to its highly homologous skeletal muscle isoform, may be an integral regulatory component of a brain calcium channel complex.

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|---|------|
| TC TCCATGCGCTGAGGGCCCCGGCTGTGCCGGGAGGGCGCCGACCGGAGGATCCCTCTCCATGGTCCAGAAGAGCGGCATGTCCCGGGCCCTTACCCA | 102 |
| M V Q K S G M S R G P Y P | 13 |
| CCTTCCCAAGAGATCCCTATGGAGGTCTTCGACCCAGCCACAGGGCAAAACACAGAAGAGGAAAGGGCGTTCAAAGGTTCAGATGGGAGCACCTCTCA | 204 |
| P S Q E I P M E V F D F S P Q G K Y S K R K G R F K R S D G S T S S | 47 |
| GATACACATCCAACAGCTTTGTCGTCAGGGCTCAGCAGAGTCTACAGGAGCCGGCGTACAGTCCGACGTGTCCCTGGAGGAGGACCGGGAAGCCTTA | 306 |
| D T T S N S F V R Q G S A E S Y T S R P S D S D V S L E E D R E A L Q | 81 |
| AGGAAGGAGGAGCCAGCCAGGCTTAGCCAGCTCGAAGCAAGCAACCAAGCAGTGGCTTTGCTGTTCGGACAAATGTTGGCTACAACTCCGCTTCCA | 408 |
| R K E A E R Q A L A Q L E K A K T K P V A F A V R T N V G Y N P S P | 115 |
| GGAGATGAGGTGCCAGTGCAGGAGTGGCCATCACCTTTGAGCCCAAGGACTTCCGTCACATCAAGGAGAAATACAATAATGACTGGTGGATCGGGAGGCTG | 510 |
| G D E V P V Q G V A I T F E P K D F L H I K E K Y N N D W W I G R L | 149 |
| GTGAAGGAAGGCTGTGAGTGTGTTTCATCCCCAGCCCTGTCAAAGTGGACCTTCGCTGTCCAGGAACAGACGCTGCGCCAAAACCGCTCAGCTCC | 612 |
| V K E G C T E V G F I P S P V K L D S L T R L L Q E Q T L R Q N R L S S | 183 |
| AGCAAGTCAGGTGACAACCTCAGTTCAGTCTGGGAGATGTGGTACTGGCACCCTCCGACACCCCGCCAGTGCCAAACAGAGCAGAGAAGCTGACA | 714 |
| S K S G D N S S S L L G D V V T G T R R P T P P A S A K Q K Q K S T | 217 |
| JAGCAGTCCCGCTATGACGTGGTTCATGAGGCCATCTCTGGTGGACCTCAAGGGTATGAGGTAAGTACATGACATGACAGAGGCG | 816 |
| E H V P P Y D V V P S M R P I I C T L V G P S L K G Y E V T D M M Q K A | 251 |
| TTGTTGACTTCTGAAGCATCCGTTTGTATGGCAGGATTTCCATACCCGGTAAAGCGTGACATTTCCCTGGCAAGCGCTCGGTCTCAACAACCCAGC | 918 |
| L F D F L K H R F D G R I S I T R L A P I I V Y I K I T S P K V L N P L Q | 285 |
| AAACAGATTATCATCGACCTCAACACCCAGCCAGCTCTCTAAACCTTCGTCGGCCCATATTGTTTACATCAAGATCACATCCCPKAGVACTGCAG | 1020 |
| K H I I I E R S N T R S S L A E V Q S E I E R I F E L A R T L Q L V | 319 |
| GCCCTGGAGCCGACACCTCAACACCCAGCCAGCTCTCTAAACCTTCGTCGGCCCATATTGTTTACATCAAGATCACATCCCPKAGVACTGCAG | 1122 |
| A L D A D T I N H P A S K T S L A P I I V Y I K I T S P K V L N P L Q | 353 |
| AGGCTCATCAATCCCGAGGGAAGTCTCAATCCAACACCTCAATGTCCAATAGCAGCTCGGAGAAGTGGCACAGTGTCCCGGAAATGTTTGACATA | 1224 |
| R L I K S R G K S Q S K H L N V Q I A A S E K L A Q C P P E M F D I | 387 |
| ATCCTGGACGAGAACAATGGAAGATGCCTGGCAGCACCTGGCGGAGTACTTGGAAAGCTACTGGAAAGCCACACCCCGCTAGCAGTCCGACCCCAAT | 1326 |
| I L D E N Q L E D A C E H L A E Y L E A Y I W K A T H P P S R T P P N | 421 |
| CCGCTGTGAACCGACCATGGCTACCGCGCTTGGCTGTGAGCCCTCCCGCTCTCAACCTCCAGGACCCCTACCTTGTTCGCGGGACCGCGCTG | 1428 |
| P L L N R T M A T A A L A V S P A P V S N L Q G P Y L V S G D Q P L | 455 |
| GACCGGCACTGGGAGCATGCCAGTGTGCAGGATACCCGGGAGCTGGCCAGCCCGAGCCCTTACCCAGCAACCACCCACTGGCCGGCAGGGC | 1530 |
| D R A T G E H A S V H E Y P G H E L Y P S N H P P P R A G | 489 |
| ACCCTGTGGCGCTATCCCGCAAGCACCTTTGATGCTGACACCCCGGGAGCCGAAACTCTGTCTACACGGAGCCAGGAGACTCGTGTGTGGACATGGAG | 1632 |
| T L W A L S R Q D T F D A D T P G S R N S V Y T E P G D S C V D M E | 523 |
| ACAGCCCTCAGAGGCCCAGGCGCTGGAGACCTGCAGGGGAGGTACACCACAGCTCCGAGGGCTCCTGGGAAGAGGAGGAAATTATGAGGAGGAG | 1734 |
| T D P S E G P F G D P A F G G T T P P A R Q G S W E E E Y E E | 557 |
| ATGACCGACAACAGGAACCGGGCCGGAATAAGGCCCGCTACTGTGCGGAGGTGGTGGCCGGTTCGCGGCAATAAGAATGAGCTGGAGGGCTGGGGA | 1836 |
| M T D N R N R G R N K A R Y C A E G G G P V L G R N K N E L E G W G | 591 |
| CAAGCGTCTACATCCGCTGAGAGGCAGGGCAGTTCCTGGGAGAGAAGAGGCTCTGAACTCGGGGAGGGGAGGGCAGAGGGGCGCACCAAGATGTGTC | 1938 |
| Q G V Y I R | 597 |
| TTGCCTCAGGGGCGCTTTGTCCTCATTTTTCAGATGCCTTTGCTCAAAGCTTGGGGTTCTTTGGTGGTTATCATTCCCAACTCCTGGGAGTCTTAAT | 2040 |
| CCCAGCTGTAGGTTTTTCCCTAAACTGTGTGGTGGATGGGGGAATACCTACTTCTGAAAGTCCCGCTTCCCATCTTTGGGGTCTTCCCTCCAG | 2142 |
| AGAAAAGGTGCACTTCTTAACTTTTACTCGGGCTGTAAGCGGAGGTCTAATTTGGGGTGGGGTGGAGGGTCTTCTTACCAACCGAGGGTACTTGGG | 2244 |
| AAAGGTTGGGTGCTTTTCTGAAGAGAACCCGCTGAATCTTCCACAAACCCAGACCCGGATCCCAACCATATCCATTCCATAACCTAGTTTCTAGCAG | 2346 |
| CATTCTCTGGTGGTGAAGCCACAGCTTGGAAACCCATGAAGTCTTGGTTGGGGAGCAGAGCTGGGTCAAACCTGACGGGCATCAGTGTGTGCTCTCTG | 2448 |
| CCCTCCATGTCATGGCAAGAACAGGGCTTAGAACTTGAAGAGTGAAGTCAAGATGCTGGCCCCGCAACCCCTTCCAGCCCTTGTATCCCCCGG | 2550 |
| GAATGAGCTTTGTCTACAGCATACCCCTGCCTGCTGGCATCAGAAGAGAGGGGCCCTTTCATCTGAGCCCCACATCTCCGTGCCCTGGGTGGGAGACCA | 2652 |
| TGAGACCATGAATAGTTGGTCCACCTCATTTAAACCAAAAACCTCTCTTCACTTACCCCGAGGTCCAGGGAACAGGCTCATGTGTGGTGGCC | 2754 |
| CATGAGGTGACCGCAATTCAGGGCTTCTGAGGACCTTGGATCTCCCTAGGGTCTTGGTTGCTGCTTCTCTGTATTGCTCTTGTGATCCTGTCC | 2856 |
| TTCCCGTGTCCACTTTCCCAAGAGAGGCTCGGTAGCCCTCACCCCTGGGGTCCCTTAGCCTCGGCATCCCTGTAGTCCAGCAGCCCTGCCCTCCCC | 2958 |
| AGCATCACAGTGGTCCAGAGAGCCGATTTGGCAACGAGGACTGGGCCCTGCCAGCTGCCACCTCAGGGATGGGACCTCATGCCTGTCTGCCACC | 3060 |
| TCTGTGCCAATGGTGGGGTGGGGCAGCTTCCCTCAACAGTGTAGAACATAGCACTAGCACTGCCAACCTTTCGTCGCCCTCCCATCT | 3162 |
| ACTGTCTCGTCCCATCTGTCTGTCTGTCTGTACTCTCTAGGAGATATTTTCCACAAATAAACCCGAGTCTGTCTTTGCGGCCACCTCTGT | 3264 |
| CAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | 3293 |

Fig. 2. Nucleotide sequence of cloned cDNA encoding the rat brain β -subunit and the deduced amino acid sequence. The deduced amino acid sequence of the encoded polypeptide is indicated in the single letter code below the nucleotide sequence. Nucleotide residues are numbered in the 5' to 3' direction. Numbers of the nucleotide and amino acid residues are given at the right hand end of the individual lines. In the cDNA sequence the stretch of 28 dA residues at the extreme 3' end may represent the poly(A) tail.

3. RESULTS AND DISCUSSION

A 1774 nucleotide cDNA with sequence identical to that encoding the β -subunit of the dihydropyridine-sensitive calcium channel in rabbit skeletal muscle [10] was isolated from a randomly primed rabbit skeletal muscle cDNA library in λ gt11 by a β -subunit specific component of a polyclonal antibody raised against the purified dihydropyridine receptor complex. This polyclonal antibody, raised in sheep, was affinity-purified

against immobilized β -subunit following the method of Sharp and Campbell [23].

This full-length cDNA encoding the β -subunit of the rabbit skeletal muscle dihydropyridine receptor (DHPR) hybridizes with high stringency to a 3.4 kb message in both rabbit and rat brain. To isolate this message a rat brain library constructed into the LambdaZapII phage vector was screened with the skeletal muscle derived cDNA. The largest clone isolated was 3.4 kb in size corresponding to the size of the message

| | | |
|--------|---|-----|
| Brain | MVQKSGMSRGPYPFSQEI P MEVFDPSPOGKYSKRGRFRKSDGSTSSDTSNSFVROGSAESYTSRPSDSVLSLEEDREALRKEAERQALAOLEKAKTKP | 100 |
| Muscle | MVQKTSMSRGPYPFSQEI P MEVFDPSPOGKYSKRGRFRKSDGSTSSDTSNSFVROGSAESYTSRPSDSVLSLEEDREALRKEAERQALAOLEKAKTKP | 100 |
| Brain | VAFVARTNVGYNPSPGDEVPPQGVVAITFEPKDFLHIKEKYNDWIGRLVKEGCEVGFIPSPVKLDSLRLLEQQLRQNRSSKSGDNSSSLGDVVVTG | 200 |
| Muscle | VAFVARTNVGYNPSPGDEVPPQGVVAITFEPKDFLHIKEKYNDWIGRLVKEGCEVGFIPSPVKLDSLRLLEQQLRQNRSSKSGDNSSSLGDVVVTG | 200 |
| Brain | TRRPTPPASAKQKQ-----KSTEHVPPYDVVPSMRPIILVGFSLKGYEVTDMQKALFDF | 255 |
| Muscle | TRRPTPPASGNEMTNLAFELEPLDLEDEAELEGEQSGSAKTSVSSVTPPPHGRIPFFKTEHVPPYDVVPSMRPIILVGFSLKGYEVTDMQKALFDF | 300 |
| Brain | LKHRFDGRISITRVTADISLAKRSVLNPNPSKHIIERSNTRSSLAEQSEIERIFELARTLQVALDADTINHPAQLSKTSLAPIIVYIKITSPKVQLRL | 355 |
| Muscle | LKHRFDGRISITRVTADISLAKRSVLNPNPSKHIIERSNTRSSLAEQSEIERIFELARTLQVALDADTINHPAQLSKTSLAPIIVYIKITSPKVQLRL | 400 |
| Brain | IKSRGKSQSKHLNVQIAASEKLAQCPPEMFDIILDENQLEDACEHLAEYLEAYWKATHPPSRTPPNPLLNRTMATAALAVSPAPVSNLQGPYLVSGDQPL | 455 |
| Muscle | IKSRGKSQSKHLNVQIAASEKLAQCPPEMFDIILDENQLEDACEHLAEYLEAYWKATHPPSSRTPPNPLLNRTMATAALAVSPAPVSNLQVQVLTSLRRNL | 500 |
| Brain | DRATGEHASVHEYPGELGQPPGLYPSNHPPGRAGTLWALSQRQTFDADTPGSRNSVYTEPGDSCVDMETDPESEGGPGDPAGGGTTPPARQGSWEEDDYE | 555 |
| Muscle | SFWGGLETSQRGGGAVPQQQEHAM 524 | |
| Brain | EEMTDNRNRGRNKARYCAEGGGPVLGRNKNELEGWGGVYIR 597 | |

Fig. 3. Homology of the deduced amino acid sequences of the rat brain β -subunit and the rabbit skeletal muscle DHPR β -subunit. Numbers of the amino acid residues are given at the right hand end of the individual lines. Sets of identical amino acid residues are indicated by vertical solid bars (|) with mismatches based on the evolutionary distance between amino acids as empirically measured by Dayhoff and normalized by Gribskov [29]. Decreasing relatedness is indicated by colons (:) and dots (·).

on Northern blots. As a probe this clone hybridized to a 1.6 kb band in rat skeletal muscle and a band at 3.4 kb in brain (Fig. 1A), a pattern identical to that derived with the rabbit DHPR β -subunit cDNA probe. No hybridizing species were detected in heart or lung at high stringency. A brain tissue distribution was performed with mRNAs isolated from the cerebral hemispheres, hippocampus, cerebellum, brainstem and spinal cord. High levels of the message were detected in the cerebral hemispheres and hippocampus whereas it is significantly reduced in cerebellum (Fig. 1B). The message levels in this tissue were found to be lower than those detected in the brainstem and spinal cord. The equal loading of mRNA was confirmed by the uniform levels of a 2.0 kb message in each lane when the blot was reprobbed with a β -actin cDNA (data not shown).

The distribution of the brain β -subunit message contrasts with the cerebellar localization of the recently cloned BI brain calcium channel [20]. This novel brain cDNA may therefore predict the distribution of yet another brain calcium channel, possibly the neuronal dihydropyridine or ω -conotoxin-sensitive channel. When hybridized with the neuronal β -subunit cDNA the 3.4 kb message was also detected in rat pancreatic islet cells, RIN cells [24] and β TC3 cells [25] (data not shown). The two latter cell lines were derived from rat islet cell tumors. The neuroendocrine properties of these cells suggest that the neuronal calcium channel may be important in the secretion of hormones and neurotransmitters.

The nucleotide and encoded amino acid sequence of this rat brain cDNA is shown in Fig. 2. Translation of its 3293 nucleotide sequence revealed an open reading frame of 597 amino acids which extends from nu-

cleotides 65 to 1855 with predicted mass of 65 679 Da. This is 7811 Da larger than the 524 amino acid 57 868 Da protein encoded by the rabbit skeletal muscle sequence. As shown in Fig. 3, the proteins encoded by both brain and skeletal muscle derived cDNAs share 98% homology extending from their common amino-terminus to residue 209. At this point a segment of 50 amino acids extending from muscle residue 210 to 260 is absent in brain. Beyond this point there is again high homology which extends to muscle residue 489. The remaining 35 amino acids of skeletal muscle and 153 amino acids of brain C-termini are completely different. The overall amino acid homology is 82%. This homology suggests that the immunoprecipitation of neuronal dihydropyridine and ω -conotoxin receptors [21] occurs by the cross-reactivity of monoclonal antibodies against the β -subunit of the skeletal muscle receptor with one or more neuronal β -subunit isoforms. By comparing the weight predicted by the cDNA encoding the skeletal muscle β subunit with its lower apparent weight on SDS-polyacrylamide gel electrophoresis the predicted weight of the neuronal isoform is most consistent with it being the smaller of the 78 000 and 58 000 Da brain proteins identified by immunoblot analysis with this antibody.

An analysis for local hydrophobicity within the brain derived sequence reveals the absence of typical membrane spanning domains (segments of at least 19 residues with an average hydrophobicity index greater than 1.6) [26]. This property is consistent with the skeletal muscle DHPR β -subunit which has been assigned to the cytoplasmic aspect of the membrane where it is believed to interact with an intracellular domain of the α_1 -subunit. Similar to its skeletal muscle counterpart the brain

with 500 μg of purified skeletal muscle DHPR [22] in Freund's Complete Adjuvant. After 8 weeks, the sheep was boosted intravenously with 500 μg of the receptor in Freund's Incomplete Adjuvant. The resulting antiserum was affinity purified using pure β -subunit that had been immobilized on polydivinylidene difluoride (PVDF) membranes following the method of Sharp and Campbell [23]. The affinity-purified antiserum was used to screen a $\lambda\text{gt}11$ random primed rabbit skeletal muscle cDNA library. Two of five positive clones were fully sequenced. These were 482 bp and 939 bp in length with a 167 bp overlap. The composite 1254 bp sequence had a single open reading frame but lacked both start and stop codons. To isolate a full-length clone duplicate filters were separately probed with the two ^{32}P -labeled cDNA clones. The largest of the four cDNAs isolated, when fully sequenced, contained 1774 nucleotides with an open reading frame of 1572 bp encoding 524 amino acids. The sequence of this full-length clone was identical to that encoding the β subunit of rabbit skeletal muscle dihydropyridine receptor [10] with the exception of sequence beyond position -93 of the untranslated region which represents the 5' extent of our cDNA.

2.2. Isolation of the rat brain β subunit cDNA

An oligo(dT) primed rat brain library in LambdaZapII (kindly provided by T.P. Snutch) was probed with the ^{32}P -labeled skeletal muscle DHPR β -subunit cDNA. Screening of 1.5×10^6 recombinants yielded 40 positives. Fifteen of these were isolated and characterized. The two largest inserts were sequenced either on an Applied Systems Automatic Sequencer or manually by the dideoxy chain termination

method. Sequences were analysed with Genetics Computer Group Inc. and PCGene Software.

2.3. Northern blot analysis

Total RNA was isolated by homogenization in RNazol (Cinna/Biotech, TX) followed by chloroform extraction. This was enriched for poly(A) by oligo(dT) cellulose chromatography. Poly(A) RNA (4 μg /lane) was resolved on 1.25% agarose gels containing 5% formaldehyde and transferred to Genescreen Nylon Membranes (NEN Research Products, MA). Prehybridization and hybridization with ^{32}P -labeled 3.4 kb rat brain cDNA probe were performed at 42°C in 50% formamide. Membranes were washed twice for 30 min at 62°C in $2 \times \text{SSC}$, 1% SDS then twice for 30 min at room temperature in $0.1 \times \text{SSC}$ according to the manufacturer's protocol. To control for equal loading of mRNA in the brain tissue distribution we reprobbed with the *Nco*-I-*Taq*I 770 bp cDNA fragment of chicken β -actin obtained from Oncor Inc. (Gaithersburg, MD). Equivalent amounts of 2.0 kb message were detected in each lane.

2.4. Chromosomal Localization

Prepared blots of human/hamster somatic cell hybrids were obtained from Bios Corp. (New Haven, CT) and were prehybridized and hybridized according to the manufacturer's protocol with the ^{32}P -labeled 3.4 kb rat brain cDNA probe at 65°C. Blots were washed twice for 10 min at room temperature in $2 \times \text{SSC}$, 0.5% SDS, once for 15 min at 65°C in $1 \times \text{SSC}$, 1% SDS and twice for 15 min at 65°C in $0.1 \times \text{SSC}$, 1% SDS.

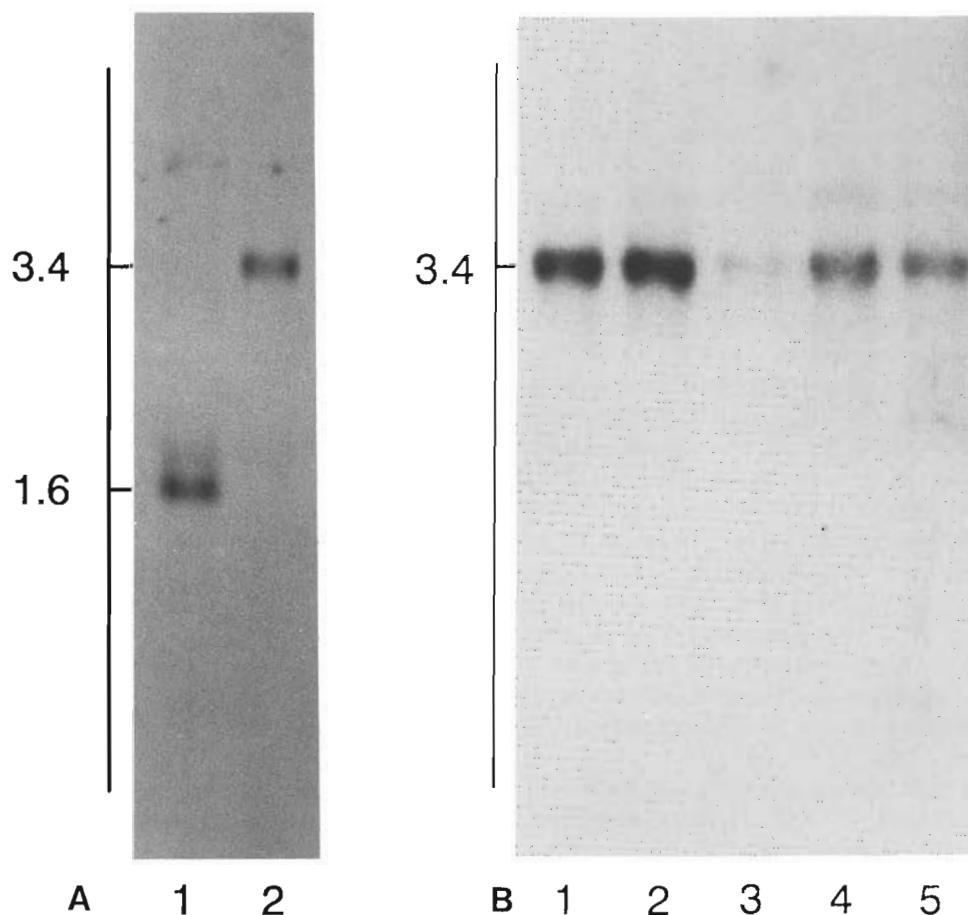


Fig. 1. Northern blot analysis with rat brain β -subunit cDNA probe. Samples of 4 μg poly(A) RNA were separated by 1.25% agarose gel electrophoresis transferred to Genescreen membranes and hybridized with radiolabeled full-length rat brain calcium channel β -subunit cDNA. (A) Rat skeletal muscle (lane 1) and brain (lane 2). (B) Rabbit cerebral hemispheres (lane 1), hippocampus (lane 2), cerebellum (lane 3), brainstem (lane 4) and spinal cord (lane 5). The sizes of the two messages in kilobases are shown on the left.

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