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Characterization of cDNA clones encoding two putative isoforms of the α_1 subunit of the dihydropyridine-sensitive voltage-dependent calcium channel isolated from rat brain and rat aorta

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cDNA clones encoding rat brain and rat aorta isoforms of the α_1 subunit of the dihydropyridine-sensitive, voltage-dependent calcium channel were isolated and sequenced. These tissue-specific cDNA clones share significant amino acid similarity with the rabbit skeletal muscle calcium channel α_1 subunit (75% and 66% amino acid identity for rat brain and rat aorta isoforms, respectively). Northern analysis revealed transcript sizes of 6.5 and 8.6 kb in aorta and 8.6 kb in brain.

Ca^{2+} channel, voltage-dependent; Subunit, α_1; mRNA; cDNA clone; Ca^{2+} antagonist

1. INTRODUCTION

Organic calcium antagonist drugs, such as 1,4-dihydropyridines (DHP), phenylalkylamines and benzothiazepines are important therapeutic compounds which exert their pharmacological effects by inhibiting calcium influx through voltage-dependent calcium channels (VDCCs) [1]. These drugs bind specifically to L-type VDCCs in a variety of tissues with high affinity [2]. The VDCC complex isolated from rabbit skeletal muscle T-tubules consists of 5 putative subunits: α_1, α_2, β, γ and δ [3]. The two largest subunits α_1 and α_2 have been cloned and sequenced [4,5]. The α_1 subunit shares significant sequence similarity with other known ion channels [4]. Photoaffinity labelling experiments have shown that the α_1 subunit is the receptor for the calcium antagonists [3,6]. These data suggest that α_1 may function as both a drug receptor and a calcium channel.

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Electrophysiological [7] and radioligand binding [8] studies strongly suggest the existence of tissue-specific isoforms of the L-type calcium channel. Functionally, the primary pharmacological target for the DHPs is vascular smooth muscle [1]. The existence of DHP-sensitive VDCCs in brain has been linked to neurotransmitter release [9]. Both DHP binding [10,11] and L-type VDCC activity [12,13] exist in vascular smooth muscle and neuronal tissues. We report here the isolation and characterization of partial cDNA clones encoding putative rat brain and rat aorta isoforms of the α_1 subunit of the DHP-sensitive VDCC.

2. MATERIALS AND METHODS

2.1. *Screening of cDNA libraries and sequencing of clones*

A rat aorta λgt11 cDNA library was purchased from Clontech (Palo Alto, CA). Approx. 10^6 recombinants were screened with rabbit skeletal muscle clone λSKmCaChα1.3 [5]. The same probe was used to screen 50000 colonies of a rat brain cDNA library as in [14]. The positive clones were identified and subcloned into M13 mp18/19 by conventional techniques and DNA sequencing was performed using the dideoxy chain termination method [5].

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2.2. *Northern blot analysis*

Total RNA was isolated from whole rat brain and rat aorta by a modification of a guanidinium thiocyanate/phenol procedure [15]. Poly(A^+) RNA was isolated using an oligo(dT)-cellulose column [5]. RNA was electrophoresed through 1% agarose/0.8% formaldehyde denaturing gels and transferred to nylon filters. The hybridization and washing conditions utilized have been described [5].

3. RESULTS AND DISCUSSION

The rat aorta cDNA clone (RA3) contains an open reading frame encoding 254 amino acids. The deduced sequence exhibits 66% amino acid identity with the rabbit skeletal muscle α_1 clone. The similarity is even greater among the proposed transmembrane segments (fig.1). Major sequence differences are observed in 2 regions: residues 1026–1042 and 1104–1120 (residues correspond to skeletal muscle α_1, fig.1).

The rat brain clone (RB19) has an open reading frame encoding 608 amino acids. There is 75% amino acid identity between the primary sequences of RB19 and the skeletal α_1 clone and 62% identity between RB19 and RA3. RB19 contains conserved positively charged residues in every third position in the S4 transmembrane domain. It has been proposed that the S4 domain is the voltage-sensor of Ca^{2+}, Na^+ and K^+ channels [4,5,16,17]. Signifi-

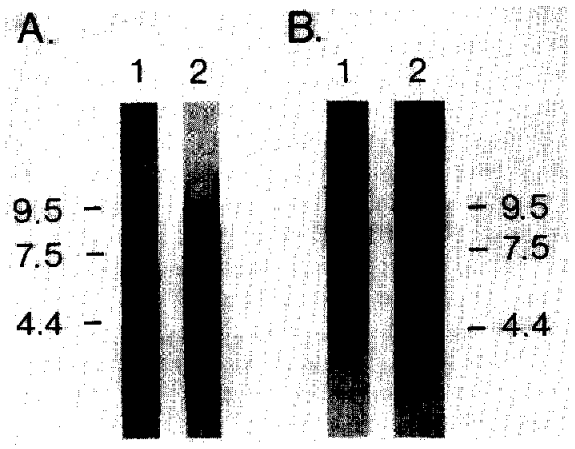

Fig.2. Northern blot analysis of tissue specific cDNA clones. (A) Lanes 1 and 2 contain rat aorta poly(A^+) RNA (3 μg); hybridization probes were rabbit skeletal muscle cDNA λSkmCaChα1.3 [5], and RA3 for lanes 1 and 2, respectively. (B) Lanes 1 and 2 contain rat brain poly(A^+) RNA (3.5 μg); hybridization probes were λSKmCaChα1.3 and RB19 for lanes 1 and 2, respectively.

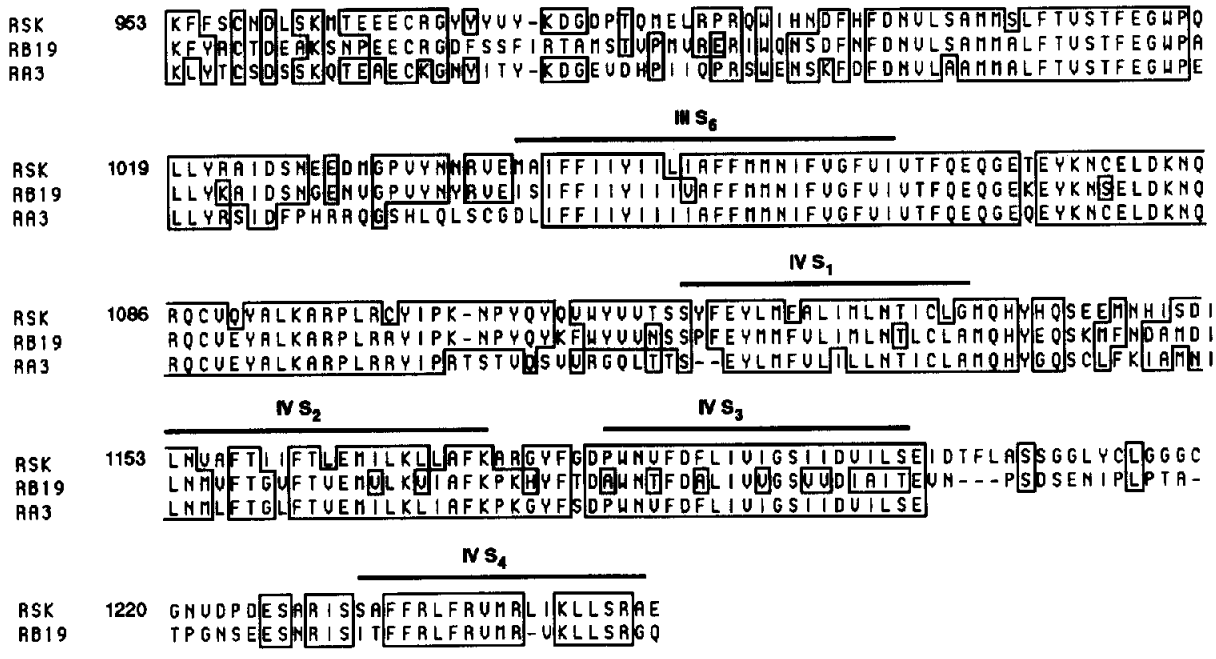

Fig.1. Comparison of the deduced amino acid sequences of the rat brain (RB19) and rat aorta (RA3) cDNAs with the rabbit skeletal muscle α_1 subunit (RSK). The amino acid sequence shown encodes parts of motif III and IV of RSK [4]. Putative transmembrane segments are indicated by solid lines and primary sequence identities between the 3 sequences are boxed.

cant sequence difference is observed in the region corresponding to skeletal muscle α_1 residues 1181–1225. This region contains the transmembrane S3 segment and the proposed cytoplasmic loop preceding the S4 domain of motif IV (fig.1). Differences among these primary sequences are not fully understood but could explain known tissue-specific differences in channel kinetics and ion selectivity; they may also be crucial determinants of specific binding affinities of the calcium antagonists in these tissues.

Tissue-specific transcript sizes were determined by Northern analysis of rat aorta and rat brain mRNA (fig.2). The rabbit skeletal muscle α_1 subunit has a single transcript size of 6.5 kb [4,5]. A cDNA clone from the skeletal muscle α_1 isoform hybridized to a single 6.5 kb transcript in rat aorta while 2 transcripts of 8.6 and 10.5 kb were observed in rat brain mRNA. RA3 hybridized to 6.5 and 8.6 kb transcripts in aortic mRNA. RB19 hybridized only to a 8.6 kb transcript in rat brain mRNA, indicating that the cDNA clone RB19 is probably derived from the 8.6 kb transcript.

The data are consistent with the existence of tissue-specific isoforms for the calcium antagonist receptor in brain, vascular smooth and skeletal muscle.

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