Tissue-specific expression of splice variants of the mouse voltage-gated calcium channel α2/δ subunit

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Abstract Five different splice variants of mouse α2/δ subunit isoforms (α2a–e), which arose from various combinations of three alternatively spliced regions, were cloned with a combination of cDNA library screening and RT-PCR. Expression patterns and relative abundance of the various isoforms in mouse tissues were determined with an RNAse protection assay. Skeletal muscle and brain expressed single isoforms, α2a and α2b, respectively; however, the cardiovascular system expressed all five isoforms. Heart expressed mainly isoforms α2c and α2d while, in contrast to other species, aorta expressed predominantly α2a, the 'skeletal muscle' isoform. Smooth muscle-containing tissues expressed α2d and α2e. Thus, α2/δ isoforms are restricted in their tissue expression, suggesting an important functional role for the differentially spliced variants.

Key words: Calcium channel; Tissue-specific expression; Alternative splicing; RNAse protection assay

1. Introduction

Voltage-gated calcium channels have been purified from skeletal muscle, heart, and brain and consist of various combinations of α1, α2/δ, β, and γ subunits [1–3]. Molecular cloning studies have revealed the presence of multiple gene families encoding the various subunits (for review, see [4]). To date six genes encoding α1 subunits have been cloned, and this protein accounts for not only the ion channel pore but also contains determinants for binding of various drugs and toxins [5]. Beta subunits, which are important for proper assembly, membrane targeting, and modulation of calcium channel gating properties, are encoded by four different genes [4,6]. At present, a single γ subunit gene has been described and the encoded protein has only been found in skeletal muscle calcium channels [7].

The α2/δ subunit is a heavily glycosylated protein derived from proteolytic cleavage of a single gene product [8,9]. The α2 and δ subunits are linked by disulfide bonds, and it was recently shown that the δ subunit anchors the protein to the membrane via a single transmembrane domain and only five amino acids are intracellular [10,11]. Functional co-expression of various α1 and β subunit cDNAs with α2/δ subunit cDNA has revealed a role for this subunit in modulation of current amplitude and also possibly activation/deactivation kinetics [12–15]. The mechanisms responsible are not known, although it has been speculated that α2/δ subunits may stabilize incorporation of channel complexes in the membrane. Another functional role for the α2/δ subunit may be in regulating or mediating drug/toxin effects on voltage-gated calcium channels. It has been shown that co-expression of α2/δ with the N-type calcium channel α1B subunit, can increase the binding affinity for ω-conotoxin [16]. Also, it has been demonstrated that the α2/δ subunit contains a high-affinity binding site for the new anticonvulsant drug gabapentin, although no effect on calcium channel function has been seen [17,18]. Recent data suggested that α2/δ may also be important in regulating depolarization-induced neurosecretion in PC12 cells [19] and may be genetically linked to a subset of patients with malignant hyperthermia [20].

Although only one gene for α2/δ has been identified, cDNA cloning and PCR analysis has revealed different splice variants expressed in various species and tissues [16,21–23]. To further understand the role of the α2/δ subunit in calcium channel physiology, pharmacology, and development, we first examined this protein in the well-characterized model, the mouse. We initially cloned the different splice variants, investigated their tissue-specific expression, and then also determined their relative abundance in these various tissues. By using a combination of library screening, PCR cloning, and RNAse protection assays, we determined that like rat and human α2/δ, single splice variants were found in mouse brain and skeletal muscle. However, it was discovered that the mouse cardiovascular system expressed all five splice variants, including the 'skeletal muscle' α2a subunit. In addition, it was determined that the α2d and α2e subunits most likely constitute a variant of the α2/δ subunit predominant in tissues containing smooth muscle.

2. Materials and methods

Sequenase 2.0 and Thermosequenase were purchased from Amersham (UK). [^{32}P]UTP (800 Ci/mmol) was purchased from ICN Biomedical (UK). Restriction and modifying enzymes were purchased from either New England Biolabs (Germany) or Boehringer Mannheim (Germany).

2.1. Cloning and construction of a full-length cDNA

A mouse brain cDNA library (Uni-ZAP XR, Stratagene) was screened with a 684 bp PCR-generated probe, derived from the rabbit skeletal muscle α2/δ sequence (bp 103–787) [21], that had been radiolabeled by random priming [24]. Six unique clones obtained from library screening were subcloned into pBlueScript II SK to create plasmids pα2.1–2.4, 2.6, and 2.8. Subcloning was carried out with the lambda-ZAP procedure outlined by the manufacturer. Inserts and derivative subcloned fragments were sequenced manually using a Sequenase 2.0 kit and T3, T7, or sequence-specific primers or with an ALF automated sequencer (Pharmacia) and a Thermosequenase kit. A full-length cDNA encoding the α2e splice variant was constructed by subcloning an *Eco*RI–*Eco*RV fragment of pα2.4 (bp 1–1718) into similarly digested pα2.8 (bp 1719–3882). The integrity of the cloning junction was verified by manual sequencing. Protein sequence comparisons were done with the MULTALIGN program from the Heidelberg UNIX Sequence Analysis Resources (Heidelberg).

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2.2. Total and poly(A) RNA isolation

Total RNA was obtained from 2–6-month-old male and female mice. RNA was isolated from aorta, brain, small intestine, heart, liver, lung, and skeletal muscle with a single step isolation procedure [25]. Poly(A) RNA was purified using a PolyATract III kit (Promega), according to the manufacturer's instructions.

2.3. Northern blot analysis

Poly(A) RNA samples (1–4 μg each) along with a RNA molecular weight ladder (Gibco) were fractionated through a gel of 1% agarose/6% formaldehyde/40 mM MOPS (pH 7.0) and then transferred to Hybond N (Amersham) using standard procedures [24]. The filter was prehybridized under stringent conditions at 60°C for 6 h in hybridization solution (5% SDS, 400 mM $NaPO_4$, 1 mM EDTA, 1 mg/mg BSA, 50% formamide). To generate an antisense riboprobe, a 549 bp *ClaI–XbaI* fragment of pα2.8 (bp 2940–3489) was subcloned in pBluescript KS II to create pα2.8D. Following digestion with *Asp*718, a labeled probe was synthesized with T7 RNA polymerase and [^{32}P]UTP using a MAXIScript kit (Ambion). Filter and probe (5.0×10^6 cpm/ml) were hybridized overnight in the above solution and then washed stringently at 70°C for 2 h in 0.1×SSC/0.1% SDS/1.0 mM EDTA, prior to exposure to Hyperfilm (Amersham).

2.4. RT-PCR amplification of splice variants

Approximately 1.0 μg of poly(A) RNA from various tissues was converted to single-stranded cDNA by priming with an oligo-dT primer, followed by a 20 μl reverse transcription reaction with 200 U of Superscript II (Gibco), and digestion with 3 U of RNAse H (Gibco) [24]. For RT-PCR amplification, 1.0 μl of cDNA was mixed with primers MA2RPA5 (5′-GGCCGGATCCGCAATTGATCCTAATGGC-3′, bp 1770–1787) and MA2SEQ8 (5′-GCACACGGATCCCTGATTGAAGTT-3′, bp 2654–2677) and amplified with 2.5 U *Pfu* polymerase (Stratagene) for 35 cycles. PCR products were digested with *Bam*HI (sites underlined in primers above), electrophoresed, eluted, and cloned into *Bam*HI digested pBluescript KS II (Stratagene). Individual clones were manually sequenced as above with T3, T7, and sequence specific primers.

2.5. RNAse protection assays

An antisense riboprobe containing both alternatively spliced regions was constructed for use in RNAse protection assays (RPA) (see Fig. 4). RT-PCR amplification of a 462 bp fragment of α2a (skeletal muscle cDNA) and 426 bp fragment of α2b (brain cDNA) was performed using primers that flanked the alternatively spliced regions: MA2RPA5 (see above) and MA2RPA3 (5′-GCGCGGTACCGCAGTATTCCCTTGGTG-3′, bp 2157–2174). The fragments were digested with *Bam*HI and *Asp*718 (site underlined in primer above), subcloned into similarly digested pBluescript II KS, to form plasmids pMA2a and pMA2b, respectively. In addition, a similar 405 bp fragment was amplified from pα2.8 (lacking both alternatively spliced regions A and C) and subcloned to form plasmid pMA2e. To construct the final RPA probe, pMA2a and pMA2b were cut with *Sty*I, and a 128 bp fragment from pMA2b (bp 2033–2160, containing alternatively spliced region C) was subcloned into pMA2a (containing alternatively spliced regions A and B) to form the plasmid pMA2RPA2. All plasmids were sequenced to confirm their identities. Radiolabeled antisense riboprobe was synthesized from *Bam*HI linearized pMA2RPA2 with T3 RNA polymerase and [^{32}P]UTP using a MAXIScript kit. RNAse protection assays of total RNA isolated from various tissues or positive control sense RNAs were performed using an RPA II kit (Ambion) according to the manufacturer's instructions, with the following modifications: 1×10^5 cpm of probe (200 pg RNA) was added per assay, RNAse T1 was used at a final dilution of 1 : 100, and digestions were allowed to proceed for 12 h at 37°C to ensure complete digestion of probe. Samples were analyzed by 5% PAGE/8 M urea gel, followed by drying of the gel and exposure to Hyperfilm or a FUJIFILM BAS 1500 Imaging System. A radiolabeled RNA molecular weight ladder was synthesized using T3 RNA polymerase and various other templates of known sizes. Sense RNA controls were synthesized from *Asp*718 linearized pMA2a, pMA2b, and pMA2e using T7 RNA polymerase in the presence of unlabeled deoxynucleotides, quantified on a spectrophotometer, and diluted to a final working concentration of 10 pg/μl.

2.6. Miscellaneous

The nucleotide sequences of mouse α2a, α2b, α2c, α2d and α2e have been submitted to Genbank and can be found under accession numbers U73483, U73484, U73485, U73486, and U73487, respectively.

3. Results

3.1. cDNA sequence of mouse α2/δ subunit

Screening of more than 1×10^6 recombinants from a mouse brain cDNA library yielded four overlapping clones, α2.1–α2.4, which extended from the 5′-untranslated region to the middle of the open reading frame. Clone α2.1 was used to rescreen the library and two more unique clones were isolated, α2.6 and α2.8, which encoded the remaining carboxy terminus of the protein and the poly(A) tail. Together, clones α2.4 and α2.8 had an approximately 1300 bp overlap (bp 812–2104) and represented a full-length cDNA clone with their combined sequence encoding a contiguous open reading frame of 3252 bp (1084 amino acids). The complete cDNA sequence had a total of 3882 bp, with 230 bp of 5′ UTR and 400 bp of 3′ UTR including the poly(A) tail (data not shown). All clones were sequenced in their entirety and no cloning discrepancies were discovered between the various clones, except for splicing variations (see below).

When compared to other known forms of α2/δ from different species, the encoded protein was found to have 98.4%, 95.9%, and 95.4% amino acid homology with rat, human, and rabbit homologs, respectively. Most differences consisted of conservative amino acid changes (Fig. 1). A comparison of the deduced protein sequences from these four species revealed several areas of distinctions. The most notable difference was the absence of the alternatively spliced regions identified previously in α2/δ subunits from skeletal muscle and brain [21,23]. The 19 amino acid insertion seen in skeletal muscle would be found between amino acids 506 and 507 of the mouse α2/δ sequence, whereas the seven amino acid insertion seen in brain would be found between amino acids 601 and 602. Previously, an isoform of α2/δ subunit lacking these regions was been given the nomenclature α2e, whereas the isoforms cloned from skeletal muscle and brain were given the nomenclature α2a and α2b, respectively [16,22]. The α2e isoform of α2/δ had been previously obtained by RT-PCR amplification. However, a cDNA encoding this alternatively spliced region of the protein was found completely within clone α2.8 obtained from our library screening, thus this combination of alternate splicing/exon usage was found in a contiguous sequence.

Based on the known amino terminal sequence of the δ subunit [8], this subunit would arise from putative cleavage between ^{914}A and ^{915}V (Fig. 1). Though the δ subunit is identical between mouse and rat, except for the last amino acid, more divergence was seen within this subunit among mouse, human and rabbit sequences. This divergence was most concentrated within the extreme carboxy terminal 20 amino acids that form the transmembrane domain responsible for membrane anchoring of the α2/δ subunit [10].

3.2. Tissue distribution of α2/δ subunit

Tissue expression of the α2/δ subunit was determined by Northern blot hybridization. After probing with a riboprobe

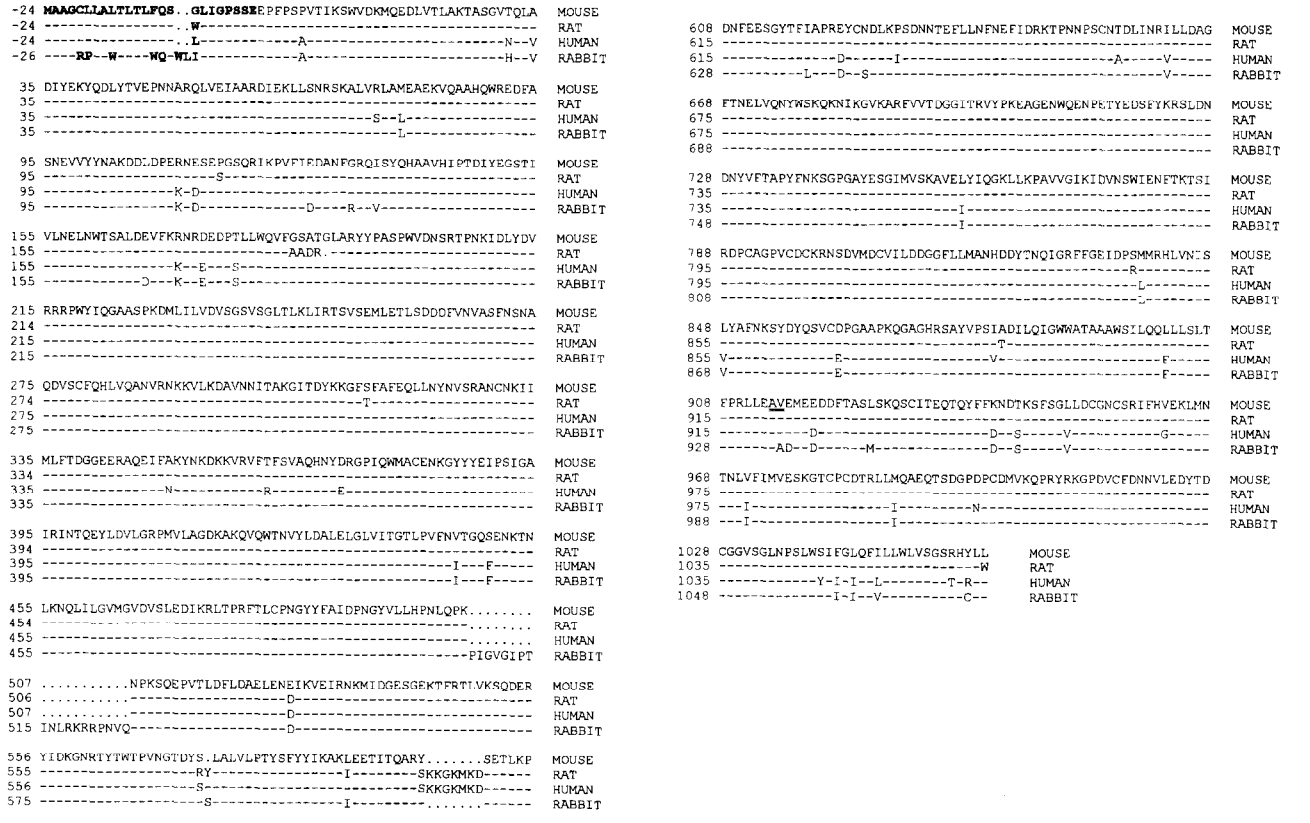

Fig. 1. Amino acid alignment of α2/δ subunits. Mouse α2e isoform was aligned with rat α2b (Genbank M86621), human α2b (Genbank L12887), and rabbit α2a (Genbank M21948) sequences using the MULTALIGN program from HUSAR. Introduced gaps are signified by a period (.) and identical amino acids are indicated with a dashed line (–). The signal peptide is shown in bold and amino acid numbering of the mature polypeptide starts with +1. The proposed site of cleavage which separates the α2 and δ subunits is underlined (^{914}A–^{915}V).

complimentary to the 3′ coding region, a predominant full-length message of 8.5 kb was seen in brain, heart, and skeletal muscle. No message was observed in liver (Fig. 2). In addition, overexposure of the blot showed similarly sized messages in aorta, small intestine, and lung (data not shown). The weak signal in aorta most likely resulted from the smaller quantity of poly(A) RNA loaded (1 μg), which was necessitated by the lower amounts of RNA isolated from this small tissue, whereas the weak signal in other tissues most likely reflected their relative abundance. Despite the weak signals ins some tissues, the relative abundance of α2/δ mRNA is consistent with that seen by RNAse protection assay (see below).

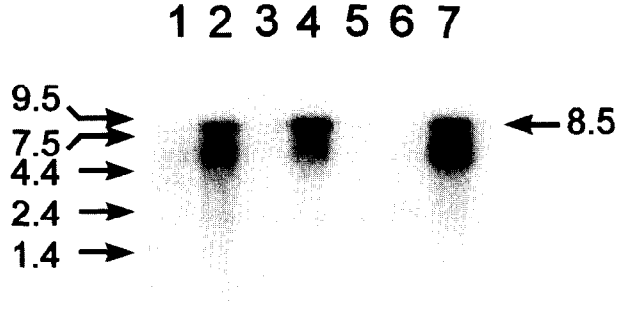

Fig. 2. Northern blot analysis. Various amounts of poly(A) RNA were analyzed after electrophoresis through a formaldehyde agarose gel as described in Section 2. Lanes (1) aorta; (2) brain; (3) small intestine; (4) heart; (5) liver; (6) lung; (7) skeletal muscle. One microgram (aorta, skeletal muscle) or 4 μg (all other tissues) were analyzed. RNA molecular weight standards are indicated on left. The blot was exposed to Hyperfilm for 18 h. Overexposure revealed specific 8.5 kb bands in lanes 1, 3, and 6 (data not shown).

3.3. *Cloning of other alternatively spliced α2/δ isoforms*

A full-length cDNA encoding the α2e isoform was constructed by combining clones α2.4 and α2.8 (see Section 2). However, the previously identified brain isoform, α2b, could not be constructed with the clones isolated from the cDNA library screening, because no clones were obtained that contained the complete alternatively spliced region C (see Section 4). Therefore we utilized a RT-PCR cloning strategy to isolate this isoform and to also determine if other isoforms of α2/δ that were not isolated by library screening were expressed in mouse tissues. RT-PCR analysis of poly(A) RNA isolated from mouse aorta, brain, heart, small intestine, lung, and skeletal muscle was performed. Initial polyacrylamide gel analysis of the RT-PCR products showed the presence of single PCR products in brain and skeletal muscle, but multiple products in other tissues examined (data not shown).

PCR products from brain, skeletal muscle, and heart tissue were then subcloned and sequenced. cDNA and deduced amino acid sequences for the various isoforms amplified were compared. Together, four different isoforms of α2/δ (α2a–d) were seen in these tissues, not including the original α2e isoform. These five isoforms were generated by alternative splic-

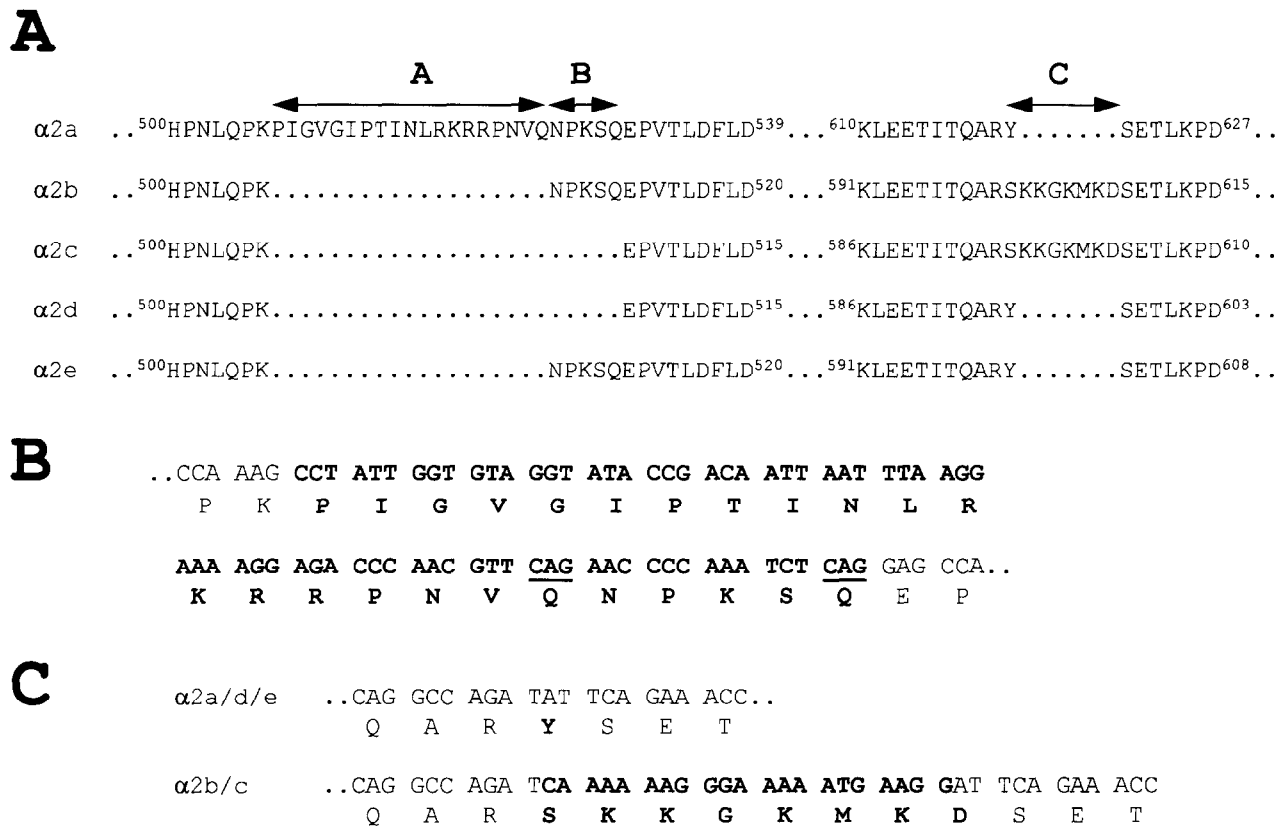

Fig. 3. Comparison of mouse α2/δ splice isoforms. A: Amino acid comparison of five splice variants obtained from RT-PCR and library screening. Alternatively spliced regions (A, B, and C) are indicated above isoform α2a. B: cDNA sequence and encoded amino acids of regions A and B. Alternate splice acceptor sites (CAG) are underlined. C: cDNA sequence and encoded amino acids of region C. Alternatively spliced sequences and encoded amino acids are indicated in bold face type.

ing of three different regions (A, B, and C) between ^{500}H and ^{608}D of α2e (Fig. 3A). Initial analysis of the subcloned RT-PCR fragments showed that skeletal muscle and brain expressed only single isoforms of α2/δ, α2a and α2b, respectively, whereas heart was found to express α2c and α2d in approximately a 9 : 1 ratio respectively (data not shown). Sequencing of several clones from RT-PCR amplification of brain, heart, and skeletal muscle did not reveal the α2e isoform, suggesting it may be a rare message (data not shown).

As seen previously, skeletal muscle expressed an isoform that contained a 19 amino acid insertion (region A), encoded by a 57 bp sequence (Fig. 3B). The next alternatively spliced region B was found adjacent to the carboxy terminal end of region A and encoded the five amino acid sequence NPKSQ. Based on the cDNA sequences surrounding regions A and B, it appeared that alternate usage of splice acceptor sites (trinucleotide CAG encoding ^{525}E and ^{530}E, of α2a) accounted for the differential splicing seen. Supporting this possibility is the presence of pyrimidine-rich regions immediately 5′ to the alternate splice acceptor sites mentioned above, which are know to be important determinants of mRNA splicing junctions [26].

Isoforms α2b and α2c differ from the other isoforms by the presence of region C, the last alternatively spliced site (Fig. 3C). No consensus splice donor or acceptor sequences are present in this 21 bp region, suggesting that it is encoded by a single exon [26]. Alternate splicing of this exon inserts seven amino acids into the protein and in addition, converts an eighth amino acid ^{601}Y to ^{601}S (based on α2b).

3.4. Relative distribution of α2/δ isoforms in various tissues

We next wanted to determine the distribution and relative abundance of the various isoforms in different tissues known to express the α2/δ subunit. Though RT-PCR can demonstrate the presence or absence of a particular message, it is only quantitative when done under appropriate conditions [24]. In contrast, Northern blot hybridization can show relative abundance of a message across various tissues (see above). However, the limitation of the latter technique is that it cannot show the relative abundance of a given isoform

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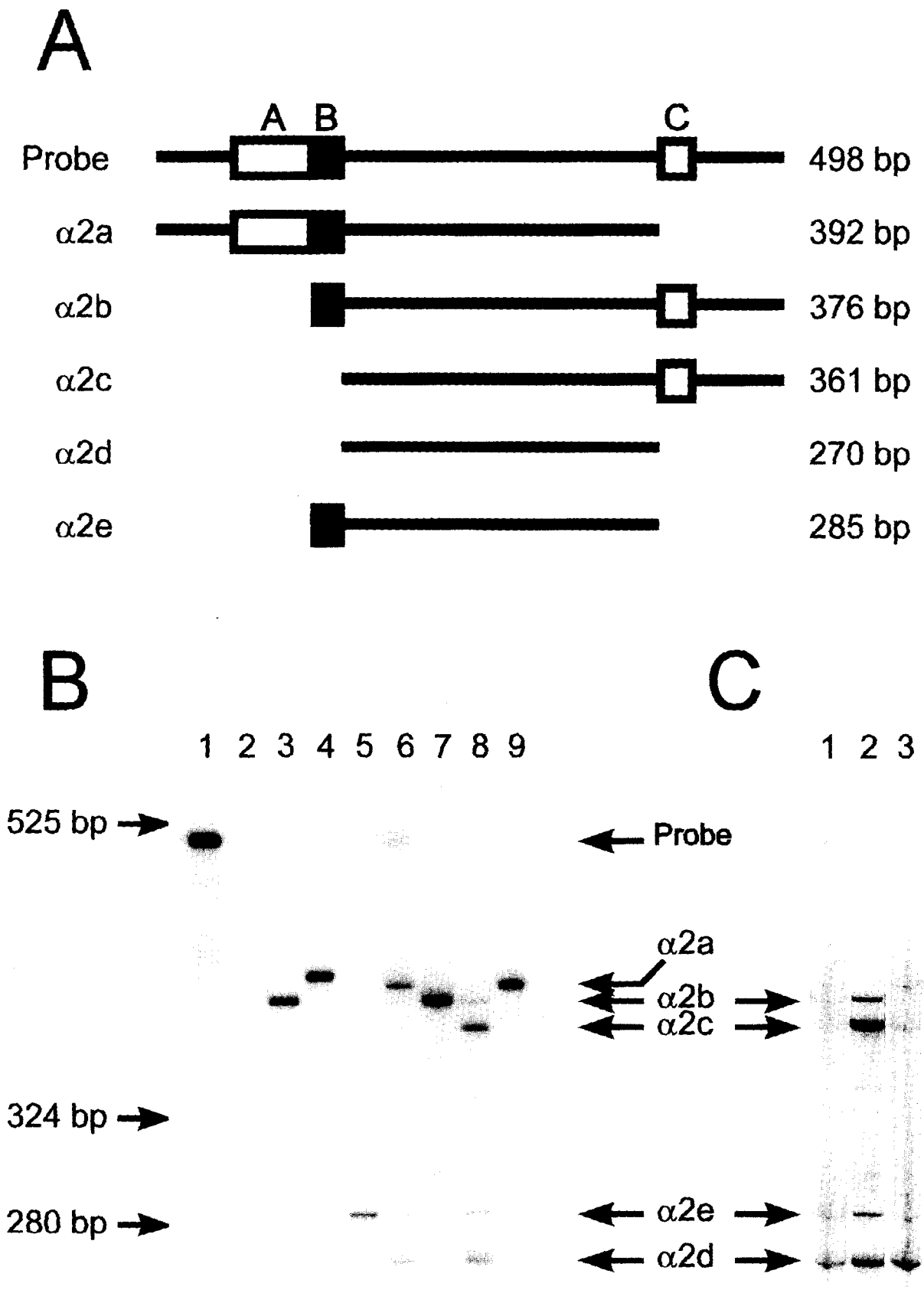
Fig. 4. RNAse protection assay (RPA) of α2/δ isoform expression. A: Schematic of probe used for RPA, showing alternatively spliced regions A, B, and C, followed by expected fragments (>200 bp) generated by RPA of each isoform. Size of probe or expected fragment is shown to right. B: RPA of total RNA from various tissues. Lanes (1) Probe (− RNAse); (2) Probe (+ RNAse); (3) α2b sense RNA; (4) α2a sense RNA; (5) α2e sense RNA; (6) 40 μg aorta; (7) 20 μg brain; (8) 20 μg heart; (9) 10 μg skeletal muscle RNA. Dried gel was exposed to Hyperfilm for 3 days. C: RPA of total RNA from small intestine and lung. Lanes (1) 50 μg small intestine; (2) 10 μg heart; (3) 50 μg lung RNA. Dried gel was exposed to Fuji imaging plate for 3 days. RNA molecular weight markers are indicated to left; identification of fragments are indicated between B and C. Full-length probe and protected fragments generated from sense RNA (B, lanes 3–5) are slightly larger than cDNA counterparts due to presence of complimentary vector sequences.


A
A B
C
Probe
498 bp
α2a
392 bp
α2b
376 bp
α2c
361 bp
α2d
270 bp
α2e
285 bp
B
1 2 3 4 5 6 7 8 9
525 bp
324 bp
280 bp
C
1 2 3
Probe
α2a
α2b
α2c
α2e
α2d

of a message within a tissue unless multiple blots are probed with isoform-specific probes. Thus, to more easily determine which α2/δ isoforms were expressed in which tissues and to determine their relative amounts, an RNAse protection assay (RPA) was utilized. To enhance the ability of the RPA to discriminate between the different isoforms, a probe template which contained all three alternatively spliced regions A, B, and C was created by subcloning various RT-PCR products. A schematic showing the constructed probe and the expected fragments generated by RPA is shown (Fig. 4A). Total RNA from various tissues was hybridized to [^{32}P]UTP labeled antisense riboprobe and digested with RNAse T1. As a positive control, in vitro transcribed sense RNAs for α2a, α2b, and α2e isoforms were also analyzed (Fig. 4B). The positive control RNAs gave protected fragments of the correct size. The identity of various fragments were confirmed by comparing the relative migration of each band to that of known molecular weight standards (data not shown). As expected from RT-PCR analysis, skeletal muscle expressed only a single isoform, α2a and brain expressed exclusively the α2b isoform. The original isoform cloned from the brain library, α2e, was not detectable in brain. The limit of detection as determined with sense control RNAs was 1 pg (data not shown). The two isoforms of α2/δ amplified from heart, α2c and α2d, were found in this tissue, with α2c being the most abundant. However, heart also expressed significant amounts of α2b and also a small amount of α2e. Most interesting was the discovery that the predominant isoform in aorta was not the previously identified isoforms seen in other species (α2d or α2e), but isoform α2a. This observation was repeated with a second RNA preparation from aorta and a similar result was seen on RT-PCR analysis of aorta poly(A) RNA (data not shown). RPA examination of α2/δ expression in small intestine and lung revealed that the predominant isoforms were α2d, with a small amount of α2e also being seen (Fig. 4C). No protected fragment of 484 bp, representing a possible sixth isoform containing all three alternatively spliced region A, B, and C was seen in any tissues tested.

4. Discussion

A combination of library screening and RT-PCR revealed the existence of five isoforms of the mouse α2/δ subunit, arising from alternate splicing of three regions (A, B, C) of the mRNA. Splicing of the α2/δ subunit in mouse tissues appears to be similar to other species examined [16,21,23]. Usage of alternate splice acceptor sites in regions A and B and the presence of an alternatively spliced exon, region C, would account for the five different isoforms. All possible combinations of the three alternatively spliced regions A, B, and C were discovered, except one (region B cannot be spliced out without removal of region A). This excluded combination would be a subunit that contained all three regions. However, it was not seen by RT-PCR, sequencing, nor RPA of various mouse tissues and has not been described in other species. Therefore, it may exist in other tissues not tested or at extremely low levels.

Only two clones from the original mouse brain library screen extended over the alternatively spliced regions. Clone α2.8 was missing regions A and C thus classifying it as the α2e isoform. The other clone, α2.4, extended into the alternatively spliced region, but it was an aberrant clone that initiated from within region C due to false priming of the library (data not shown). However, this clone also lacked region A, thus a full-length clone would have been given the nomenclature of α2b. Therefore, two forms of α2/δ subunit were isolated from this brain library, α2b and α2e. However, the α2b and not the α2e isoform was expressed in mouse brain, as assessed by RPA, thus suggesting that the presence of the latter isoform in the cDNA library came from another tissue source, possibly vascular smooth muscle (see below).

Isoforms of α2/δ subunit have been identified previously by PCR analysis of rabbit, rat, and human tissues and cell lines [16,21,23,27]; however, only partial information about the α2/δ subunit from mouse cochlea was reported previously [28]. It had been speculated that these splice variants arose from tissue-specific splicing events, as the α2a isoform was only identified in skeletal muscle and α2b was only found in CNS tissues or derivative cell lines. However, our RPA analysis of various mouse tissues has revealed that the previously termed skeletal muscle isoform, α2a, is the predominant variant in aorta, and the brain specific isoform, α2b, is expressed to significant levels in heart. Initial RT-PCR studies by Green et al. [28], suggested that α2b may be expressed in mouse lung, however, that was based on PCR product size alone and was not confirmed by sequencing. The results of our RPA analysis suggested that this isoform was not the predominant form in mouse lung.

Previous Northern blot analysis of rabbit aorta, ileum, and lung revealed the presence of an α2/δ transcript, similar to our results with mouse tissues, though the exact isoform(s) expressed in these tissues was not determined [21,29]. Previous RT-PCR analysis of human aorta has revealed the presence of α2c, α2d, and α2e isoforms, but the relative abundance of these different isoforms was not mentioned [16]. A more quantitative RPA analysis of the tissues from mouse revealed that α2d was the most abundant isoform in small intestine and lung, with a lower expression level of α2e. A similar ratio of α2d to α2e was seen in mouse aorta and heart, though these were not the most abundant isoforms in these tissues. The finding of α2d and α2e isoforms in small intestine, aorta, and lung, tissues known to express the α1C subunit [4], suggests that these two isoforms may co-assemble to form calcium channels in vasculature and other tissues which contain smooth muscle. This result may explain the original cloning of the α2e isoform from a brain library, an organ known to have extensive vasculature. However, α2e was also amplified from HEK 293 and PC12 cells [16,27] and sequencing of an α2/δ fragment amplified from mouse cochlea suggested the presence of α2d, proving again that the different isoforms are not tissue specific.

The presence of all five isoforms in the mouse cardiovascular system (aorta and heart) suggests that the different splice variants may have specific, but yet unknown, functions. Mouse heart expressed predominantly α2c and α2d and was the only tissue tested that expressed the former isoform. No obvious functional domains could be identified in the three alternatively spliced regions to give insight into possible functions. Heart is not a homogeneous tissue and consists of contractile and specialized conducting tissues and also expresses both L- and T-type calcium channel currents [4,30]. Thus it would be of interest to use in situ hybridization or other techniques to determine the exact cellular location of these different isoforms.

By using a highly specific and sensitive RPA analysis, we were able to determine the relative level of expression of α2/δ subunit mRNA between various mouse tissues and also determine the relative abundance of any given isoform within a tissue. Our results demonstrate that the multiple splice variants of the α2/δ subunit have been conserved between mice and humans, though they differ with respect to tissue expression, suggesting a functional role for these different isoforms. Recent evidence that α2/δ can modulate calcium channel currents, ω-conotoxin binding, neurosecretion, and form a high-affinity binding site for the anticonvulsant gabapentin implies that the various isoforms may differ with respect to these effects [13,16,17,19,20]. Future experiments will need to be directed at examining these possibilities and these will be aided by having cloned the full-length cDNAs encoding all five mouse α2/δ subunit isoforms.

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