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A GLIAL-SPECIFIC VOLTAGE-SENSITIVE NA CHANNEL GENE MAPS CLOSE TO CLUSTERED GENES FOR NEURONAL ISOFORMS ON MOUSE CHROMOSOME 2

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SUMMARY: A variety of glial cell types express saxitoxin (STX)-binding voltage-sensitive Na channels (1,2), although the possible role of impulse conduction in these cells is not understood. Gautron et al. (1992) recently identified a 7.5 kb species of mRNA in type 1 astrocytes cultured from rat brain cerebrum that hybridized with a "common" Na channel probe but not with brain isoform-specific cDNA probes. Sequence data from cloned cDNAs demonstrate that it encodes a structurally atypical Na channel isoform. We have prepared a cDNA probe specific for a portion of subunit domain IV of the glial channel and mapped the location of the corresponding gene (*Scn7a*) to mouse chromosome 2. The *Scn7a* gene mapped 0.9 (±0.9) cM distal to the *Gcg* locus; the location of the corresponding human gene (*SCN7A*) is predicted to be in the q36-q37 region of chromosome 2. This site lies just outside a cluster of genes for the brain-specific Na channel isoforms RI, RII and RIII which map proximal to *Gcg* (17). The presence of at least four genes from two distinct Na channel subfamilies suggests that multiple genetic defects for central and peripheral nervous system disorders ultimately may be linked to this area.

The familiar role of voltage-sensitive Na channels is to mediate the early inward currents of action potentials in nerve, muscle and related electrically excitable cells. Many types of central and peripheral glial cells, however, also express voltage-sensitive Na currents and Na channel neurotoxin binding sites (1,2,for review 3). Cloning and expression of these channels promises to reveal their structure and regulation and to provide information as to their normal functions in non-neuronal support cells. Cloning studies have previously demonstrated that the large α-subunits of nerve and muscle channels are members of a closely homologous multigene family; most strongly conserved are the sequences of four internally similar subunit domains which penetrate the bilayer and provide mechanisms for ion-selective conductance, activation gating and sites for drug and toxin binding (for review 4). Recently, an atypical α-subunit was cloned from human heart (5). This channel differed from other muscle and nerve channels in being shorter and having fewer charged residues in the regions associated with voltage-sensitive activation, residues which are perfectly conserved in other isoforms (Figure 1). Independently, Berwald-Netter and coworkers (6) have cloned a new Na channel isoform from astrocytes which closely resembles the atypical heart channel (Figure 1). These investigators found that glial-specific cDNA probes detected a 7.5 kb mRNA in rat skeletal muscle, spleen, intestine, uterus, pancreas, adrenals, lung and heart.

The distribution of the glial or glial-like isoforms among these diverse tissues, including nominally "non-electrically excitable" cells, may suggest roles for this Na channel subfamily other than conventional

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action potential propagation; this increases the possibility that these channels may be linked to unexpected genetically inherited diseases (cf.7). In this study we have used a cDNA probe for the glial isoform to map the chromosomal location of the glial α-subunit gene in mouse and predicted the location in the human genome.

MATERIALS AND METHODS

PCR: Glial Na channel probe (*JPg00*) was amplified from a λgt10 rat hippocampus library (λgt10RHIP) kindly provided by S. Heinemann (Salk Institute). Polymerase chain reaction (PCR) was performed as described (8) using primers synthesized on a 394 automated DNA synthesizer (Applied Biosystems, Inc.) following ABI procedures. The 30 base primer sequences used were AAAGGACCAAAGGTGTTCCATGATCTGATG and CCGCTCATCCTTTCCCATCACTCTTTTCGT, corresponding to nts 343-372 and 1035c-1006c of the published rat glial Na channel subunit sequence (6), encompassing 693 bps of the open reading frame (ORF). Reactions were performed in an Omnigene thermal cycler (Hybaid Limited, England) with the following cycle protocol: [(2'@94°C)x1][(30''@94°C/30''@55°C/105''@72°C)x40] [(4'@72°C)x1].
Sequencing: PCR product was subcloned into pBluescript II SK+ (Stratagene, La Jolla), amplified and the inserts sequenced with the fluorescent di-deoxy terminator method of cycle sequencing on a 373a automated sequencer (Applied Biosystems, Inc.) following ABI protocols (9,10).
Mapping: C3F/He-*gld* and *Mus spretus* (Spain) mice and [(C3F/He-*gld* x *Mus spretus*)F_1 x C3F/He-*gld*] interspecific backcross mice were bred and maintained (11). *Mus spretus* was chosen as the second parent in this cross because of the relative ease of detection of informative restriction fragment length variants (RFLVs) in comparison with crosses using conventional inbred laboratory strains. DNA isolated from mouse organs by standard techniques was digested with restriction endonucleases, and 10μg samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schull, Inc, Keene, NH), hybridized at 65°C and washed under stringent conditions, as described (8). Clones used as probes in the current study and RFLVs that detect the *Gcg* and *Thbs1* loci were as described (12). Gene linkage was determined by segregation analysis (13). The most likely gene order was determined by analyzing all haplotypes and minimizing crossover frequency between all genes determined to be within a linkage group (14).

RESULTS

PCR of *JPg00*: PCR was performed on the library λgt10RHIP employing primers directed at the glial Na channel ORF (Figure 1), yielding a single product of ~700 bp as determined by gel electrophoresis. The PCR product was cloned into the plasmid vector Bluescript SK+ and sequenced in its entirety. The sequence was identical at every base with nt 343-1035 of the published partial clone of rat glial Na channel α subunit (6) and homologous to nt 4220-4912 in the fourth domain of the atypical heart channel (5). This cloned PCR product was designated *JPg00*.

Chromosomal mapping of the glial Na channel: To determine the chromosomal location of the gene encoding the glial Na channel α subunit in mice, we analyzed *JPg00* cDNA hybridization to a panel of DNA samples from an interspecific cross previously characterized for more than 500 genetic markers. The included genetic markers span between 50 and 80 centi-Morgans (cMs) on each mouse autosome and the X chromosome (cf.15, 16). Initially, DNA from the two parental mice C3H/HeJ-*gld* and (C3H/HeJ-*gld* x *Mus spretus*)F1 were digested with various restriction endonucleases and hybridized with *JPg00* cDNA probe to determine RFLVs for haplotype analyses. Informative RFLVs were detected with *TaqI* restricted DNAs: C3H/HeJ-*gld*, 700 bp; *Mus spretus*, 1000 bp.

Comparison of the haplotype distribution of the gene coding for *JPg00* (designated *Scn7a*) in the 114 meiotic events, shown in Figure 2, enabled us to determine best gene order for the markers *Gcg* and

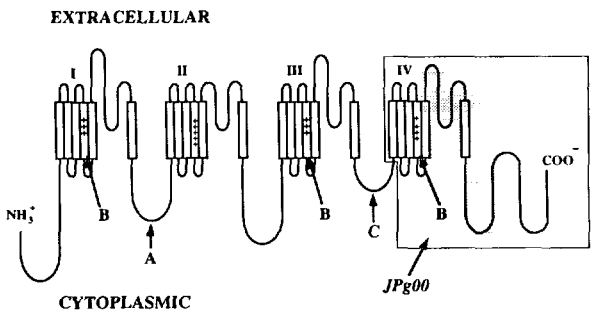

Figure 1. Distinct structural characteristics of the rat glial and human atypical heart Na channel isoforms. Shown is a topological map of the predicted orientation of the human atypical heart Na channel peptide (5) relative to the plasma membrane. The published sequence for cloned domain IV of the rat glial isoform (enclosed by solid line) corresponds to nt 3878-5188 of the cloned atypical heart isoform (nt similarity = 81.6%, aa similarity = 86.5%). The cDNA probe *JPg00* (shaded region) is identical to nt 343-1035 of the glial domain IV clone and corresponds to nt 4220-4912 of the atypical heart isoform clone (nt similarity = 87%). Arrows indicate some of the structural features that distinguish this sub-family of Na channels: **(A)** an abbreviated I-II inter-domain region, **(B)** a reduced number of total charges in the voltage-sensing S4 transmembrane spans and **(C)** a poorly conserved III-IV interdomain region, an area known to play an essential role in channel inactivation.

Thbs1 in mouse chromosome 2. The best gene order ± the standard deviation (13,14) indicated that *Scn7a* was located 0.9 (±0.9) cM distal to *Gcg* and 12.3 (±3.1) cM proximal to *Thbs1*.

DISCUSSION

As illustrated in Figure 3, the probe to *JPg00* hybridized to a region of chromosome 2 distal to the *Gcg* locus. Composite mapping data for mouse chromosome 2 (17) indicate that a cluster of three neuronal Na channel genes (*Scn1a, Scn2a, Scn3a*) (18) lies just proximal to *Gcg*. The resolution of these loci makes it unlikely that the signal results from cross hybridization with the neuronal isoforms; this conlusion is further supported by the facts that *JPg00* identified only one locus and there is no more than 70.9% nucleotide similarity between *JPg00* and homologous regions of the neuronal isoforms. Similarly there was no evidence for signals in chromosomes 11 and 9, the locations of *Scn4a* and *Scn5a* (19) which code for the muscle isoforms μ1 and μ2 (63.0 % and 62.0 % homologous to *JPg00*, respectively). The close

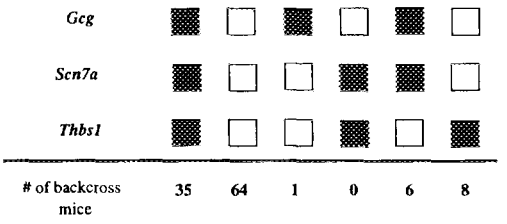

Figure 2. Haplotype distribution of *Scn7a*. The segregation of *Scn7a* among proximal mouse Chromosome 2 loci in [(C3H/HeJ-*gld* x *Mus spretus*)F1 x C3H/HeJ-*gld*] interspecific backcross mice is shown. The loci are listed from proximal to distal on the left side. Each column represents a possible haplotype and the number of mice observed with each haplotype is indicated at the bottom of the column. The boxes indicate whether the mice were typed as C3H/HeJ-*gld* homozygotes (filled) or F1 heterozygotes (open) for each locus. The RFLVs and haplotype distribution of *Gcg* and *Thbs1* in this cross were reported previously (12).

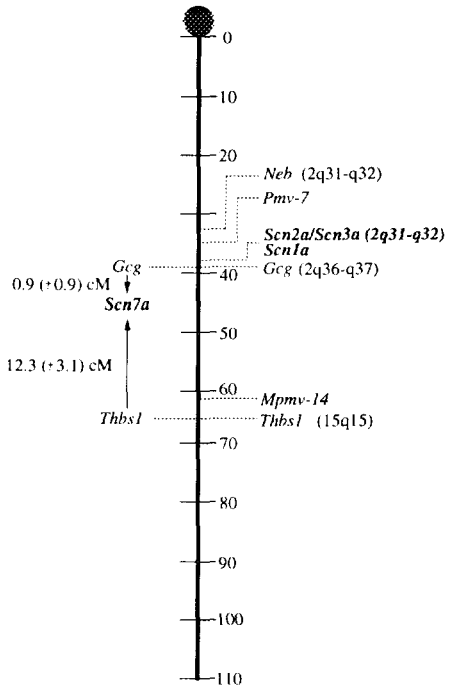

Figure 3. Linkage map of mouse chromosome 2. Shown on the right are relevant loci as reported in composite form (17). Dashed lines indicate the approximate location of the loci relative to the centromere (●). Human chromosomal locations of the loci are shown in parentheses (if known). Numbers along the side of the map indicate the distance from the centromere in cM. Shown on the left is the relative position of *Scn7a* from the loci *Gcg* and *Thbs1* as calculated from the mapping data presented in Figure 2.

similarity between the atypical heart channel (87% homologous to *JPg00*), however, leaves open the possibility that genes for these two similar forms could reside near one another on the same chromosome.

Mouse chromosome 2 contains 15 different genes that have been mapped to the long arm of human chromosome 2 (17), including *Scn2a* (18,20) which encodes the type II brain isoform. Because the human homologue of *Gcg* maps to 2q36-q37 (21) we predict that the glial Na channel gene will also map to this same region of human chromosome 2.

The tight linkage of *Scn1a*, *Scn2a* and *Scn3a* (18) has led to the speculation that these closely related sequences may have resulted from comparatively recent gene duplication events. There has been little reason, however, to predict that other Na channel genes would be clustered nearby. For example, the genes for skeletal muscle isoforms, μ1 and μ2, lie on mouse chromosomes 11 and 9, respectively (19) and, similarly, the related human cardiac and skeletal Ca^{2+} channel α_1-subunit genes also map to disparate chromosomes (22,23). It now appears that at least four Na channel genes from two distinct subfamilies lie near one another on mouse chromosome 2; it seems possible that still more Na channel genes may map to this area.

While many glial cell types express both Na currents and STX binding sites (1,2) the physiological roles of these channels and the action potentials they generate are not known. Two forms of Na current have been observed in glia. One is quantitatively similar to neuronal Na currents but the second is distinct from currents of other known Na channels and has been presumed to be mediated by a 'glial-specific'

isoform. This current has a more negative onset of activation and slower gating kinetics than other Na currents (2,24). The glial-specific Na current form has been observed in all white matter and cortical astrocytes and ependymal cells of the optic nerve (for review see 3). The relationship of the channel studied here to those currents may be established if these channels can be expressed functionally in stably or transiently transfected cells. The reduced charge in the S4 elements suggests that the gating of these channels may be distinctive. Their functional characteristics may provide a clue to their roles in glia and other excitable and non-excitable cells.

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