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Identification of a Novel Human Voltage-Gated Sodium Channel α Subunit Gene, *SCN12A*

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We have cloned a cDNA encoding a novel human voltage-gated sodium channel α subunit gene, *SCN12A*, from human brain. Two alternative splicing variants for SCN12A have been identified. The longest open reading frame of *SCN12A* encodes 1791 amino acid residues. The deduced amino acid sequence of SCN12A shows 37–73% similarity with various other mammalian sodium channels. The presence of a serine residue (S360) in the SS2 segment of domain I suggests that SCN12A is resistant to tetrodotoxin (TTX), as in the cases of rat *Scn10a* (rPN3/SNS) and rat *Scn11a* (NaN/SNS2). *SCN12A* is expressed predominantly in olfactory bulb, hippocampus, cerebellar cortex, spinal cord, spleen, small intestine, and placenta. Although expression level could not be determined, *SCN12A* is also expressed in dorsal root ganglia (DRG). Both neurons and glial cells express SCN12A. *SCN12A* maps to human chromosome 3p23-p21.3. These results suggest that SCN12A is a tetrodotoxin-resistant (TTX-R) sodium channel expressed in the central nervous system and nonneural tissues.

The voltage-gated sodium channel is a membrane protein that plays a fundamental role in the rising phase of the action potential in most excitable cells such as neurons and muscle cells (1, 2). The channel is a heteromeric protein complex composed of one α subunit (~270 kDa) and one or two auxiliary subunits β1 (~38 kDa) and β2 (~33 kDa) (3, 4). Expression studies of sodium channels in *Xenopus* oocytes and mammalian cell lines have demonstrated that α subunit mediates voltage-dependent gating and ion conductance and the β subunits regulate the kinetic properties and facilitate membrane localization of sodium channels (5). The molecular diversity of α subunit has been described. So far, ten subtypes have been identified in rat (6); *Scn1a* (rBI/αI), *Scn2a* (rBII/αII), *Scn3a* (rBIII/αIII), *Scn4a* (rSkM1/μ1), *Scn5a* (rH1/rSkM2/μ2), *Scn7a* (Na-G/SCL-11), *Scn8a* (rPN4/NaCh6/CerIII), *Scn9a* (rPN1), *Scn10a* (rPN3/SNS), and *Scn11a* (NaN/SNS2). In human, nine α subunit subtypes have been reported; *SCN1A* (partial sequence, 7), *SCN2A* (8), *SCN3A* (partial sequence, 9), *SCN4A* (10), *SCN5A* (11), *SCN6A* (12), *SCN8A* (13), *SCN9A* (14), and *SCN10A* (15).

All α subunits contain four internal repeat domains (DI-DIV), two large interdomain cytoplasmic loops (L1 and L2) and short interdomain loop (L3). Four transmembrane domains and loop L3 are highly conserved. However, loops L1 and L2, N- and C-termini of the remaining regions, all of which are assigned to the cytoplasmic side of the membrane are less well conserved (2, 6). Each of the four internal domains contains six presumably α-helical transmembrane segments (S1–S6); S4 is positively charged and the others are hydrophobic. The region between S5 and S6 contains two short segments, SS1 and SS2. These may partly span the membrane as a hairpin. The SS2 segment is postulated to form part of the channel lining (16). Segment S4 and loop L3 are responsible for voltage sensor (17) and inactivation (18), respectively. All subtypes contain several potential *N*-glycosyla-

The HGN-approved nomenclature for voltage-gated sodium channel α subunit gene is *SCN12A*. Sequence data from this article have been deposited with the EMBL/GenBank Database Libraries under Accession Nos. AF109737 and AF150882.

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tion sites (19) and cAMP-dependent phosphorylation sites (20).

Voltage-gated sodium channels are classified pharmacologically into two large groups according to their sensitivity or resistance to block by tetrodotoxin (21). Most sodium channels are tetrodotoxin-sensitive (TTX-S), but Scn5a/SCN5A, Scn10a/SCN10A and Scn11a are tetrodotoxin-resistant (TTX-R) channels. These TTX-R channels contain cysteine or serine residues in the SS2 segment of domain I (22), whereas an aromatic residue is located in TTX-S channels. They confer the tetrodotoxin resistant phenotype (23). SCN5A is expressed specifically in cardiac muscle and brain and its mutation is responsible for the cardiac arrhythmia of Long-QT syndrome type 3 (24). *Scn10a/SCN10A* and *Scn11a* have been isolated recently from dorsal root ganglia (DRG) and are expressed specifically in primary sensory neurons (15, 22, 25, 26). In this study, we have identified a novel human TTX-R voltage-gated sodium channel α subunit gene *SCN12A* that is expressed in the central nervous system (CNS) and non-neural tissues. Here, we report molecular cloning, chromosomal localization and expression of the novel sodium channel.

MATERIALS AND METHODS

cDNA cloning and sequencing. From frozen normal human brain tissue, total RNA was extracted using TRIZOL reagent (Gibco BRL) and mRNA was then purified using oligo(dT)-cellulose (NEB). cDNA was synthesized using SuperScript preamplification kit (Gibco BRL) with oligo(dT) and random primers and used as templates for PCR amplification. Nested PCR was performed with primers designed based on the sequences of the EST AA446997 and the consensus sequence of known sodium channel α subunits. The primer sequences were as follows: Est-f1, 5′-ATC GGC GAC TCT GTC GGA GCC CTT GGA-3′; Est-f2, 5′-TGA CTG TGG TCC TGG TCA TTG TGA-3′; Na-r1, 5′-TTC ATK GCR TTR TAG TAY TTC TTC TG-3′; and Na-r2, 5′-GTT GAA RTT RTC WAT GAT GAC RCC AAT-3′. The adapter-conjugated cDNA was constructed from the human brain mRNA using Marathon cDNA constructions kit (Clontech) and used as templates for the RACE reactions. The primer sequences for RACEs were as follows: for 5′-RACE; Race-5, 5′-TGC GGA ATA CCA CTA GGA AGG AGT GCC AG-3′ and for 3′-RACEs; Race-3a, 5′-GAG AGC AAT TCA CTC GGT TAC ATT TAC TTC GTA G-3′ and Race-3b, 5′-CTC CCT GGC ATA GCC ACA TCC TAC TTT GTC AGT TAC-3′. The adapter primer sequences were AP1, 5′-CCA TCC TAA TAC GAC TCA CTA TAG GGC-3′ and AP2, 5′-ACT CAC TAT AGG GCT CGA GCG GC-3′. To confirm the presence of a single open reading frame of SCN12A and SCN12A-s, long RT-PCR was performed with the primers as follows: for SCN12A; 12A-f, 5′-TGG CTT GTC TCT GTC CTG AGG GTG AAG-3′ and 12A-r, 5′-GGT AGG CGT GGA GGT GAG GGC TCA-3′ and for SCN12A-s; 12A-f and 12As-r, 5′-CTG GTT CTT AAA GTC CCT CTG ACT GCT GA-3′. The PCR and RACE products were subcloned into pBluescript II SK+ (Stratagene). The subclones were sequenced by a 377 automatic sequencer (Perkin-Elmer). Genetyx-Mac software (Software Development Co., Ltd.) was used for DNA sequence analysis.

Chromosome mapping. Fluorescence *in situ* hybridization (FISH) was carried out as described in the previous report (27). Three distinct cDNA clones, pRT, p5R and p3Rb, were used as probes independently (Fig. 1A).

Northern blot analysis. Commercial human multiple-tissue RNA blots (Clontech) containing about 2 μg of poly(A)$^+$ RNA in each lane were used for Northern blot analysis of SCN12A. The 656 bp *Cla*I/*Pvu*II fragment containing the region of L2 of SCN12A was used as a probe for hybridization (Fig. 1A). Human β-actin cDNA was used as a control probe. The probes were labeled with [α-^{32}P]dCTP (6000 Ci/mmol) using Ready-To-Go DNA labeling Beads (Amersham Pharmacia). Hybridization and washing were performed according to the manufacturer's instructions.

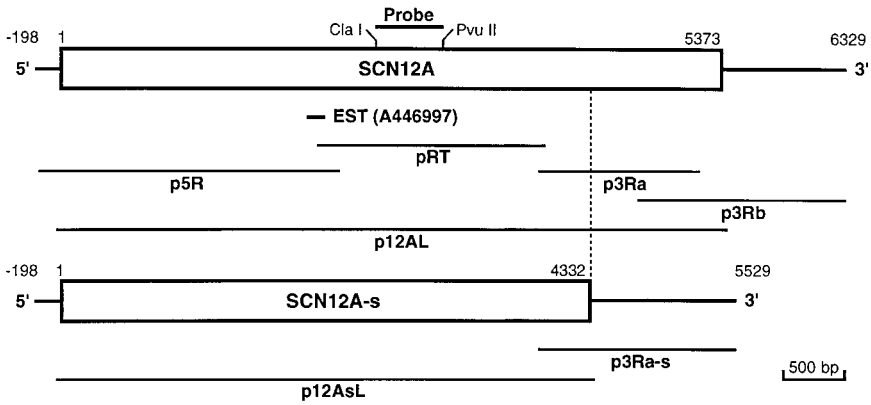
In situ hybridization. The DNA fragment used in Northern blot hybridization was subcloned into pBluescript II SK+. Digoxigenin (DIG)-labeled RNA probes (sense and antisense) were synthesized by *in vitro* transcription (Roche Diagnostics). Various tissues excised from the adult Wistar rats were immersed with paraformaldehyde, dehydrated, embedded in paraffin, and cut in 5 μm sections. The sections were treated with proteinase K, acetylated, and dehydrated according to the standard protocols. They were then hybridized with DIG-labeled RNA probes (4 μg per ml) for 16 hours at 55°C, treated with RNase (10 μg per ml), and washed to a final stringency of 0.2× SSC at 55°C. The sections were further reacted with anti-DIG antibody (Roche Diagnostics), washed, and developed by incubation in NBT and BCIP solution for 24 hours according to the manufacturer's instructions.

RT-PCR. Total RNAs from individual cell lines and tissues were treated with RNase-free DNase I (Gibco BRL) at room temperature for 15 min to avoid amplification of genomic DNA, denatured at 70°C for 10 min, and subsequently reverse transcribed by SuperScript II (Gibco BRL) with 0.5 μg of oligo(dT) primer in a volume of 20 μl according to the manufacturer's instructions. The primers used for PCR amplification of SCN12A were 5′-TTC TGT CAC AAG TGG TGC AGG AAG C-3′ and 5′-CAG GTA ACC ATC CAA AGC CAT CCT G-3′ and the primers for human β-actin were 5′-CCA AGG CCA ACC GCG AGA AGA TGA C-3′ and 5′-AGG GTA CAT GGT GGT GCC GCC AGA C-3′. The RT-PCR products were separated in 1.5% agarose gel, stained with 1/10,000 dilution of Vistra Green (Amersham Pharmacia) and then analyzed by a FluoroImager 875 (Molecular Dynamics).

RESULTS

Molecular cloning of SCN12A. We have recently cloned cDNA for *SCN8A* from a human brain (unpublished data). Compared with the EST sequence (GenBank Accession No. AA446997) that was registered as the human homologue of rat *Scn8a*, we found that the sequence of 160 bp of AA446997 was a partial sequence (domain II) for a novel human sodium channel α subunit. This novel channel was designated as *SCN12A*. To obtain a full-length cDNA clone for this novel sodium channel, we proceeded to use PCR-based cloning as follows. At first, we carried out nested RT-PCR using the primers, EST-f1/EST-f2 and Na-r1/Na-r2, designed based on the EST sequence and the consensus sequence sodium channel α subunits, respectively. Poly(A)$^+$ RNA used as a template was isolated from a normal brain of 59 years old male cadaver. The RT-PCR product pRT, 1.8 kb in length was obtained. Sequencing revealed that pRT was a partial cDNA clone for sodium channel α subunit (Fig. 1A). To obtain a full-length cDNA, we performed 5′-RACE and 3′-

A



B

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MDDRCYPVIFPDERNFRPFTSDSLAAIEKRIAIQKEKKKSKDQTGEVPQPRPQLDLKASRKLPKLYGDIP   70
RELIGKPLEDLDPFYRNHKTFMVLNRKRTIYRFSAKHALFIFGPFNSIRSLAIRVSVHSLFSMFIIGTVI  140
                                                          DI-S1

INCVFMATGPAKNSNSNNTDIAECVFTGIYIFEALIKILARGFILDEFSFLRDPWNWLDSIVIGIAIVSY  210
                  DI-S2                                   DI-S3

IPGITIKLLPLRTFRVFRALKAISVVSRLKVIVGALLRSVKKLVNVIILTFFCLSIFALVGQQLFMGSLN  280
            DI-S4                                 DI-S5

LKCISRDCKNISNPEAYDHCFEKKENSPEFKMCGIWMGNSACSIQYECKHTKINPDYNYTNFDNFGWSFL  350
                                                                DI-SS1

AMFRLMTQDSWEKLYQQTLRTTGLYSVFFFIVVIFLGSFYLINLTLAVVTMAYEEQNKNVAAEIEAKEKM  420
        DI-SS2                  DI-S6

FQEAQQLLKEEKEALVAMGIDRSSLTSLETSYFTPKKRKLFGNKKRKSFFLRESGKDQPPGSDSDEDCQK  490
KPQLLEQTKRLSQNLSLDHFDEHGDPLQRQRALSAVSILTITMKEQEKSQEPCLPCGENLASKYLVWNCC  560
PQWLCVKKVLRTVMTDPFTELAITICIIINTVFLAMEHHKMEASFEKMLNIGNLVFTSIFIAEMCLKIIA  630
                  DII-S1                                  DII-S2

LDPYHYFRRGWNIFDSIVALLSFADVMNCVLQKRSWPFLRSFRVLRVFKLAKSWPTLNTLIKIIGNSVGA  700
            DII-S3                          DII-S4

LGNLTVVLVIVIFIFSVVGMQLFGRSFNSQKSPKLCNPTGPTVSCLRHWHMGDFWHSFLVVFRILCGEWI  770
          DII-S5                                  DII-SS1   DII-SS2

ENMWECMQEANASSSLCVIVFILITVIGKLVVLNLFIALLLNSFSNEERNGNLEGEARKTKVQLALDRFR  840
                  DII-S6

RAFCFVRHTLEHFCHKWCRKQNLPQQKEVAGGCAAQSKDIIPLVMEMKRGSETQEELGILTSVPKTLGVR  910
HDWTWLAPLAEEEDDVEFSGEDNAQRITQPEPEQQAYELHQENKKPTSQRVQSVEIDMFSEDEPHLTIQD  980
PRKKSDVTSILSECSTIDLQDGFGWLPEMVPKKQPERCLPKGFGCCFPCCSVDKRKPPWVIWWNLRKTCY 1050
QIVKHSWFESFIIFVILLSSGALIFEDVHLENQPKIQELLNCTDIIFTHIFILEMVLKWVAFGFGKYFTS 1120
          DIII-S1                         DIII-S2

AWCCLDFIIVIVSVTTLINLMELKSFRTLRALRPLRALSQFEGMKVVVNALIGAIPAILNVLLVCLIFWL 1190
        DIII-S3                 DIII-S4                         DIII-S5

VFCILGVYFFSGKFGKCINGTDSVINYTIITNKSQCESGNFSWINQKVNFDNVGNAYLALLQVATFKGWM 1260
                                                    DIII-SS1   DIII-SS2

DIIYAAVDSTEKEQQPEFESNSLGYIYFVVFIIFGSFFTLNLFIGVIIDNFNQQQKKLGGQDIFMTEEQK 1330
                                DIII-S6

KYYNAMKKLGSKKPQKPIPRPLNKCQGLVFDIVTSQIFDIIIISLIILNMISMMAESYNQPKAMKSILDH 1400
                                DIV-S1

LNWVFVVIFTLECLIKIFALRQYYFTNGWNLFDCVVVLLSIVSTMISTLENQEHIPFPPTLFRIVRLARI 1470
          DIV-S2                DIV-S3    K 1444

GRILRLVRAARGIRTLLFALMMSLPSLFNIGLLLFLIMFIYAILGMNWFSKVNPESGIDDIFNFKTFASS 1540
      DIV-S4                        DIV-S5                    DIV-SS1

MLCLFQISTSAGWDSLLSPMLRSKESCNSSSENCHLPGIATSYFVSYIIISFLIVVNMYIAVILENFNTA 1610
          DIV-SS2                       DIV-S6

TEESEDPLGEDDFDIFYEVWEKFDPEATQFIKYSALSDFADALPEPLRVAKPNKYQFLVMDLPMVSEDRL 1680
HCMDILFAFTARVLGGSDGLDSMKAMMEEKFMEANPLKKLYEPIVTTTKRKEEERGAAIIQKAFRKYMMK 1750
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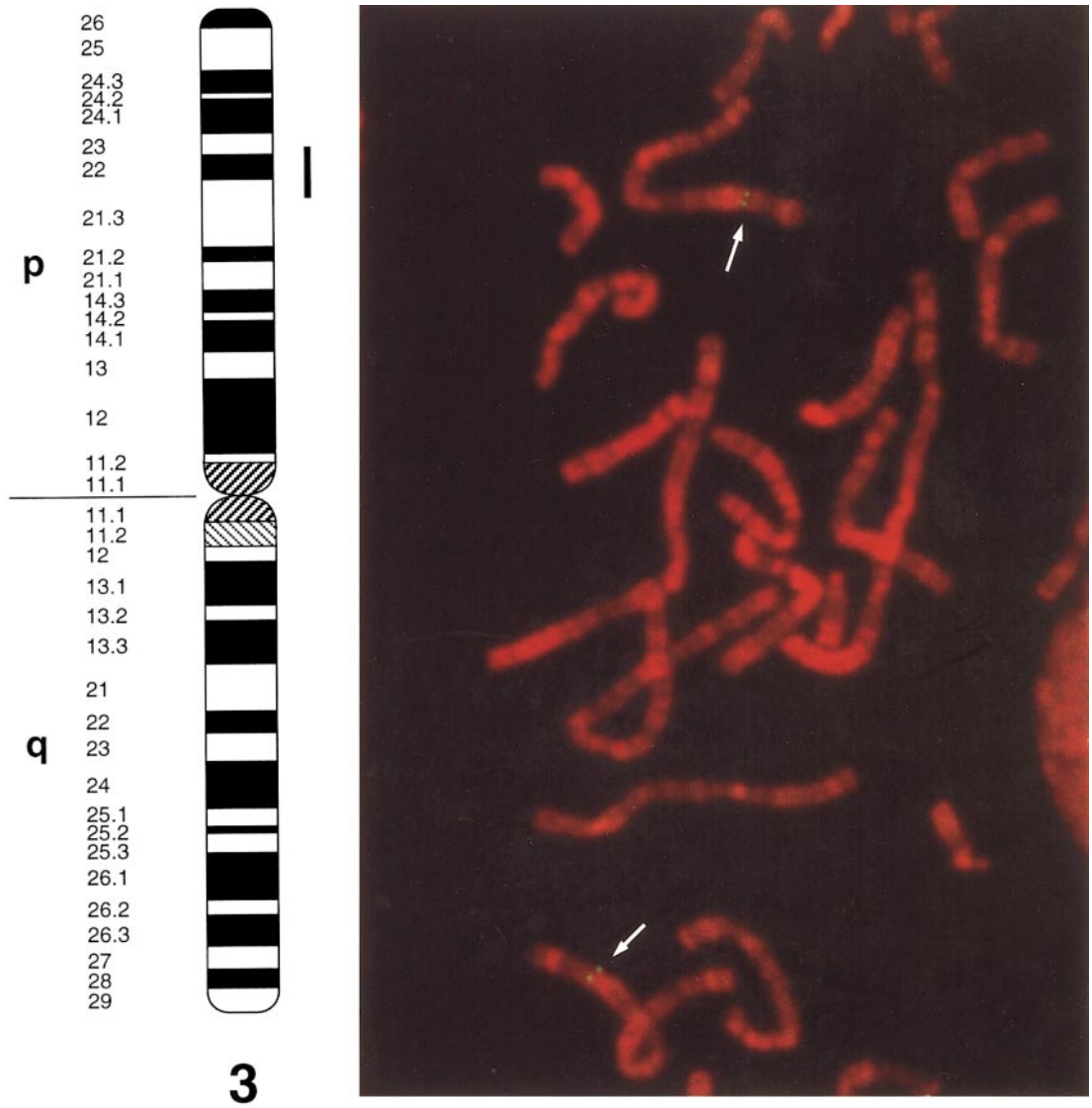

FIG. 2. Chromosomal location of *SCN12A*. Metaphase plate shows hybridization signal of *SCN12A* on human chromosome 3p23-p21.3 (arrows). Ideogram of chromosome 3 shows the localization of *SCN12A* (bar on the right side).

RACE using the primer sets (AP1/Ap2 and Race-5) and (AP1/Ap2 and Race-3a/Race-3b), respectively. We reconstructed the full-length cDNA based on the cDNA clones, pRT, p5R, p3Ra, p3Ra-s and p3Rb, obtained by RT-PCR and RACEs. There were at least two types of transcripts for the channel (Fig. 1A). It is presumed that they are produced by alternative splicing using different 3′-end exons. The larger clone, designated as SCN12A, consists of 6,527 bp including 5,373 bp of ORF, 198 bp of 5′-untranslated regions (UTR), and 956 bp of 3′-UTR. The 3′-UTR region includes a poly-adenylation signal (AATAAA), followed by a ploy(A)

FIG. 1. Schematic structures and predicted amino acid sequences of cDNA clones for SCN12A and SCN12A-s. (A) Schematic structures of the cDNA clones for SCN12A and SCN12A-s. An open box indicates the longest ORF. The cDNA fragments are labeled and represented by solid lines. The fragment used as a probe for Northern blot analysis and *in situ* hybridization is shown above the restriction enzyme sites. (B) Deduced amino acid sequences of cDNA clones for SCN12A and SCN12A-s. DI-DIV represent the four membrane spanning domains. S1–S6 and SS1–SS2 are underlined in individual domains indicating six putative α-helical transmembrane segments and two pore-lining segments, respectively. The serine residue (S360) in the SS2 of domain I, which is predicted to underlie the TTX-R phenotype, is indicated with a bold "S." The inactivation gate "IFM" residues in the loop L3 are shown in bold and are double underlined. The underlined lysine residue (K1444) indicates the C-terminal residue of SCN12A-s.

TABLE 1

Amino Acid Similarities between SCN12A and Other Sodium Channel α Subunits (%)

Subunits	Total	N	DI	L1	DII	L2	DIII	L3	DIV	C
Scn1a	47	49	48	22	60	24	61	90	59	44
SCN2A	47	51	48	24	60	24	63	90	58	47
Scn3a	49	50	48	27	61	27	63	90	59	46
SCN4A	49	49	48	28	60	24	64	89	59	44
SCN5A	50	52	50	28	61	28	67	92	61	45
SCN6A	40	39	40	24	46	23	51	54	42	41
Scn7a	37	40	35	22	40	21	48	54	40	41
SCN8A	47	49	48	22	60	25	62	92	58	47
SCN9A	48	45	53	24	60	25	64	90	59	46
SCN10A	51	55	55	28	60	28	63	94	62	44
Scn11a	73	84	70	77	73	52	77	94	77	78

Note. Percentage (%) amino acid similarities were calculated after aligning SCN12A with each sodium channel to maximize their respective similarities. DI-DIV and L1-L3 are depicted in Fig. 1B. N and C represent N- and C-termini, respectively. GenBank Accession Nos: rat Scn1a, X03638; human SCN2A, M94055; rat Scn3a, Y00766; human SCN4A, L01983; human SCN5A, M77235; human SCN6A, M91556; rat Scn7a, Y09164; human SCN8A, AF050736; human SCN9A, X82835; human SCN10A, AF117907; rat Scn11a, AF059030; human SCN12A, AF109737.

tail. The other one, designated as SCN12A-s, consists of 5,727 bp including a shorter ORF (4,332 bp) and a different 3′-UTR sequence from that of the larger SCN12A. The ORF of SCN12A encodes the complete α subunit and SCN12A-s encodes the shorter α subunit of which the domain IV and C-terminal region are truncated. To confirm the presence of these two transcripts, full-length ORFs of SCN12A (p12AL) and SCN12A-s (p12AsL) were generated by RT-PCR with primer sets (12A-f and 12A-r) and (12A-f and 12As-r), respectively (Fig. 1A).

Structure of SCN12A and comparison with other sodium channels. The longest open reading frame of SCN12A encodes 1,791 amino acid residues with a calculated molecular mass of 205 kDa (Fig. 1B). The deduced amino acid sequence of SCN12A is aligned well with other known mammalian sodium channels, especially in transmembrane segments. SCN12A contains four internal repeat domains (I–IV), each containing six putative α-helical transmembrane segments (S1–S6) and two pore-lining segments (SS1 and SS2), as in other voltage-gated sodium channel α subunits. The positively charged residues in the voltage sensor (S4) and the inactivation gate (IFM residues) (28) in the L3 loop region are also conserved in SCN12A. Multiple putative sites for cAMP-dependent phosphorylation also exist in loop L1 and L2 regions of SCN12A. The serine residue (S360) in the SS2 of domain I confers the tetrodotoxin resistant phenotype (23) on SCN12A. As shown in Table 1, in overall comparison, rat Scn11a shows exceptionally high similarity to SCN12A (73%), while the others show significantly lower similarity (37–51%). The interdomain loops L1 and L2 of SCN12A show low sequence similarities to other channels; specifically, loop L2 shows the lowest similarity to rat Scn11a (52%). The remaining regions, N- and C-termini, four internal repeat domains (DI-DIV) and loop L3, show high similarity. The truncated form SCN12A-s encodes 1444 amino acid residues and lacks internal repeat domain IV and C-terminal domain (Fig. 1B). The stop codon is located in the transmembrane segment S3 of domain IV. Assuming that the exon-intron structure is conserved among the voltage-gated sodium channel α subunits, the border between the sequences of SCN12A-s and SCN12A is presumably an exon border.

Chromosomal localization of SCN12A. For chromosomal mapping of *SCN12A*, we performed fluorescence *in situ* hybridization (FISH) using three clones, pRT, p5R and p3Rb. These probes were used independently, each containing a different region of *SCN12A* (Fig. 1A). *SCN12A* was mapped to human chromosome 3p23-p21.3 (Fig. 2). Interestingly, two TTX-R sodium channel α subunit genes, *SCN5A* and *SCN10A* have been mapped to this region previously (6).

Expression of SCN12A. To investigate the expression of *SCN12A* mRNA transcript in human tissues, we performed Northern hybridization using human multiple-tissue Northern blots (Figs. 3A and 3B). To avoid cross-hybridization with other sodium channels, a specific probe for *SCN12A* containing the loop L2 region that showed the lowest similarity to other sodium channels (Table 1) was used (Fig. 1A). The channel was predominantly expressed in brain, spinal cord, spleen, small intestine and placenta (Fig. 3A). In the CNS, *SCN12A* was ubiquitously expressed in the areas examined (Fig. 3B). The size of the transcript is approximately 7.0 kb.

To examine the expression of *SCN12A* in detail, we performed *in situ* hybridization of rat tissues (Fig. 4). All previous rat and human sodium channel orthologs show greater than 83% homology. Because of this fact we were able to use an *SCN12A* probe on rat tissues. *SCN12A* was broadly expressed in the CNS. In the brain, the transcripts are abundant in gray matter rather than whiter matter. High levels of expression were observed in granule cells of dentate gyrus of hippocampal formation, pyramidal cells in CA1 to CA3 of hippocampus, the granular layer of cerebellum, and granule cells of olfactory bulb (Figs. 4A–4D). Moderate expression was also found in superior and inferior colliculi (data not shown). In the spinal cord, signals were found in ependymal cells lining the central canal and glial cells in the central gray matter (Figs. 4E and 4F). *SCN12A* was also expressed in non-neural tissues, germinal center cells of spleen and trophoblasts of pla-

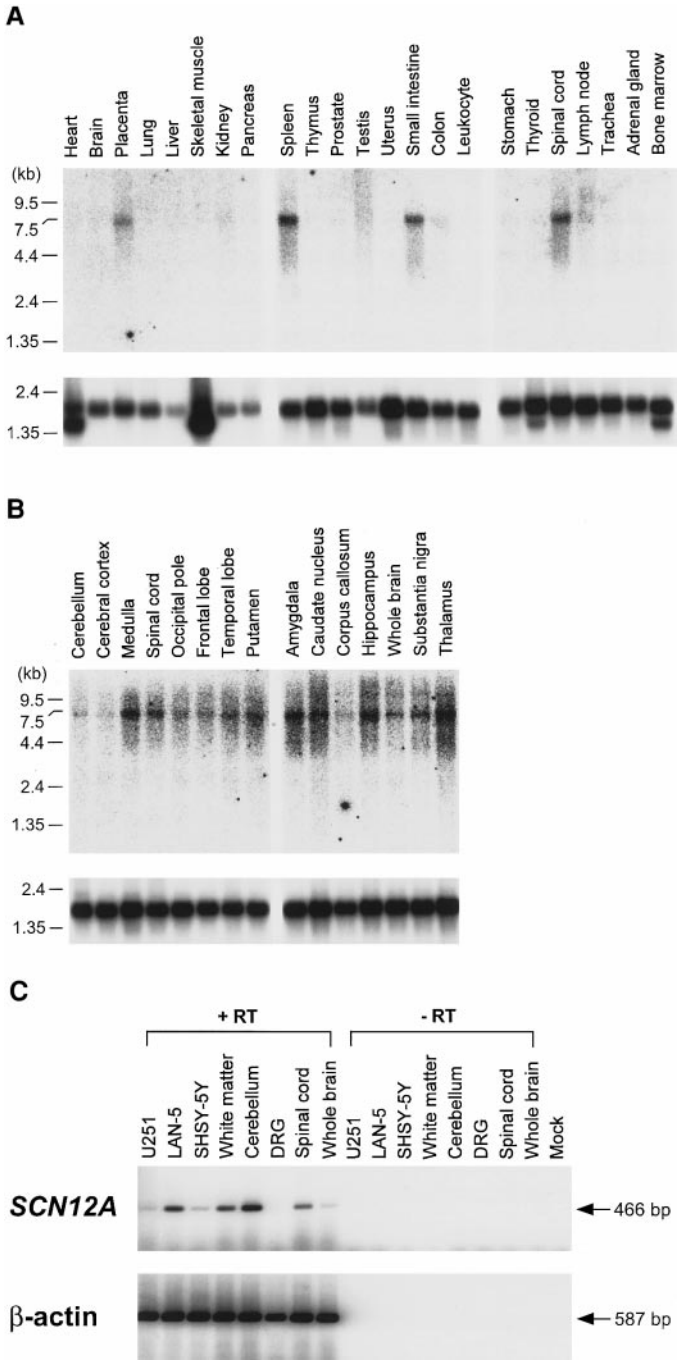

FIG. 3. Analysis of *SCN12A* expression in human tissues by Northern hybridization and RT-PCR. Northern blot analysis of various adult human tissues (A) and various regions of the CNS (B) using ^{32}P-labeled probes for *SCN12A* (top) and human β-actin (bottom). Each lane contains 2 μg of poly(A)$^+$ RNA. Sizes in kilobases are indicated along the left side. (C) RT-PCR analysis of *SCN12A* in human cell lines. RT-PCR of human cell lines, glioblastoma U251, neuroblastoma LAN-5 and SHSY-5Y, and whole brain, cerebral white matter, cerebellum, spinal cord, and DRG. RT-PCR showed the specific amplification of *SCN12A* and human β-action with 466 bp and 587 bp products, respectively.

centa (Figs. 4G and 4H). No hybridization signal was detected in all sections hybridized with sense RNA probe (data not shown). These results of rat are consistent with the results of Northern blot analysis of human tissues described above.

To elucidate what kind of cells express *SCN12A* in the nervous system, we performed RT-PCR analysis of several human cell lines and several areas of the nervous system (Fig. 3C). The analysis included glioblastoma cell line U251, neuroblastoma cell lines LAN-5 and SHSY-5Y, adult human whole brain, cerebral white matter, cerebellum, spinal cord, and DRG. LAN-5 is a differentiated neuroblastoma cell line while SHSY-5Y is undifferentiated. Molecular characteristics of these three cell lines were described previously (29). SCN12A mRNA was amplified by RT-PCR in all cell lines and tissues, except for DRG (Fig. 3C). We have cloned the cDNA fragments of *SCN12A* from the same DRG sample by high frequency nested RT-PCR (data not shown), indicating that *SCN12A* is expressed in DRG. Using this sample tissue, expression level could not be determined in DRG. Indeed, the low β-actin level implies that the lack of *SCN12A* transcript may be caused by mRNA degradation. The amounts of *SCN12A* RT-PCR product expressed in both U251 and SHSY-5Y cell lines were low. In contrast, the products were ten times or more abundant in LAN-5. The products in cerebellum were two or three times more abundant than in cerebral white matter. From these results *SCN12A* appears to be expressed in both neurons and glia, more predominantly in neurons.

DISCUSSION

SCN12A is a novel human voltage-gated sodium channel α subunit gene. Comparison of the deduced amino acid sequence demonstrated that the molecular features of sodium channels are well conserved in *SCN12A* as described above. Interestingly, SCN12A contains a serine residue at a position in the SS2 segment of domain I that is occupied by an aromatic residue in the TTX-S channels (Fig. 1B). Rat *Scn10a* (rPN3/SNS) and rat *Scn11a* (NaN/SNS2) are TTX-R channels that contain a serine residue at the position (22, 25). Recently, expression studies have demonstrated that rats Scn10a and Scn11a produce TTX-R sodium currents in *Xenopus* oocytes (22) and HEK293 cells (30), respectively. These results suggest that SCN12A is a TTX-R sodium channel.

Among the previously reported mammalian sodium channels, rat Scn11a showed the highest similarity to SCN12A (73%) (Table 1). These two channels have less than 50% similarity to the other channels. The interdomain loops L1 and L2 of SCN12A showed especially low similarities to those of the other channels, confirming that cytoplasmic L1 and L2 loops may characterize

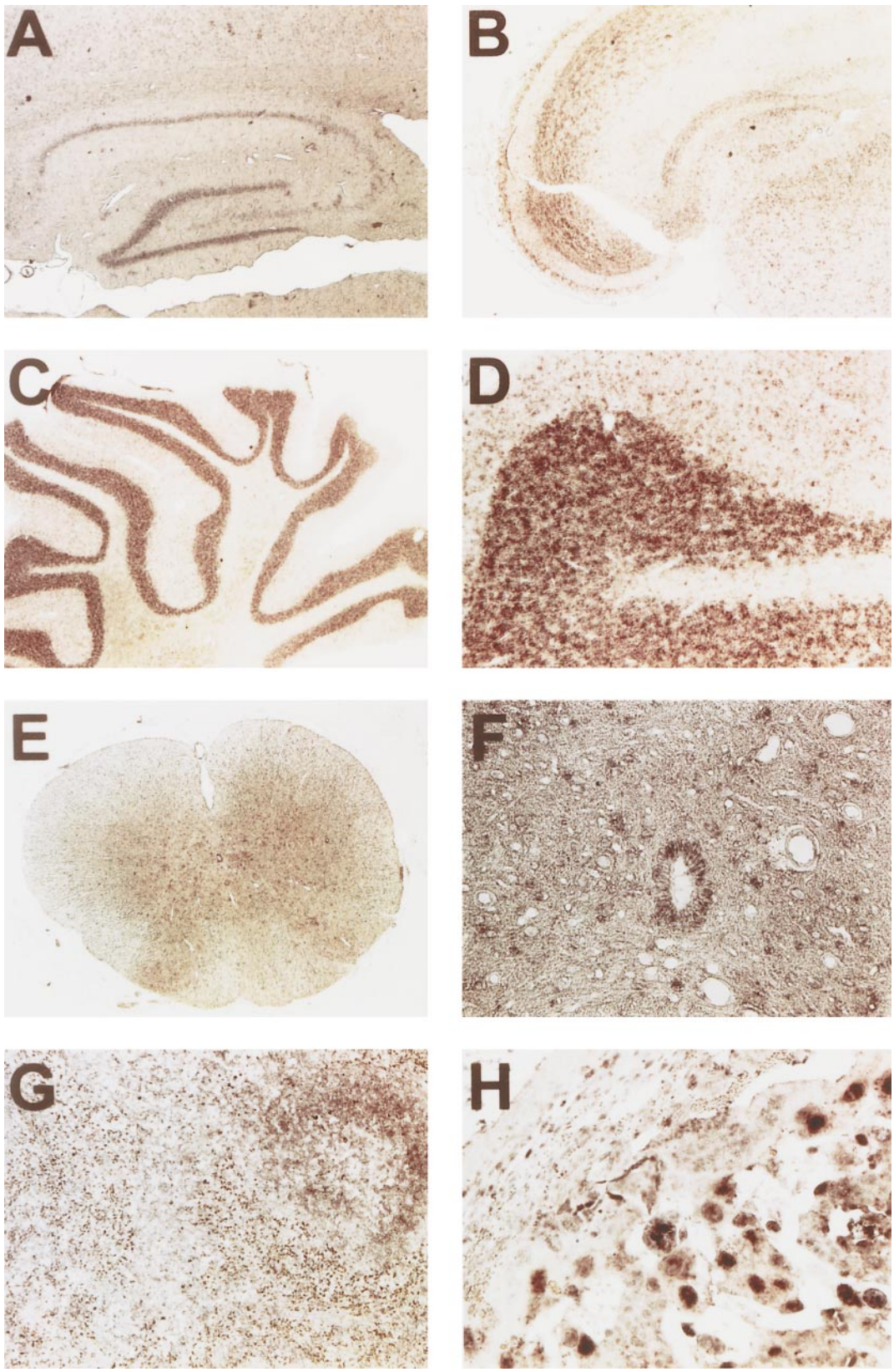

FIG. 4. *In situ* hybridization of *SCN12A* in rat tissues. *In situ* hybridization to adult rat sections using DIG-labeled RNA probes specific to *SCN12A*. Sections show hippocampus (A), olfactory bulb (B), lower magnification of cerebellum (C), higher magnification of cerebellum (D), lower magnification of spinal cord (E), higher magnification of spinal cord (F), spleen (G), and placenta (H) hybridization with antisense probe.

a unique feature of an individual sodium channel. These loops contain multiple putative sites of cAMP-dependent phosphorylation that give distinct effects to the kinetics of sodium channels (31).

Twelve voltage-gated sodium channel α subunits have been identified in mammals forming a large gene family. *SCN5A* and *SCN10A* have been mapped to human chromosome 3p where *SCN12A* is localized (Fig. 2). Mouse *Scn11a* has been mapped to mouse chromosome 9 that is syntenic to that region (26). These four channels are TTX-resistant and form a cluster on human chromosome 3p, suggesting that the TTX-R sodium channels may be closely related in evolution (6). All other channels are TTX-sensitive. Among them *SCN2A, SCN6A, SCN9A* and the human orthologs of *Scn1a, Scn3a* and *Scn7a* are located on human chromosome 2q in a cluster (6). *SCN4A* and *SCN8A* are located on human chromosome 17q and 12q, respectively (6). All of the sodium channel α subunit genes map to within four paralogous chromosome segments including the HOX gene clusters (32). It has been inferred that the expansion of the sodium channel α subunit gene family was initially associated with the large-scale genomic duplications of the ancestral chromosome and intrachromosomal gene duplications occurred after the divergence of the four chromosome segments (6). The results of mapping and comparative analysis of *SCN12A* support the inference of divergent evolution of sodium channel α subunit genes.

Alternative splicing has been reported in *Scn1a, Scn2a, Scn3a, Scn8a/SCN8A* and *Scn9a* (6). The truncated forms of Scn1a and Scn8a have been identified in astrocytes and neuroblastoma cells (33, 34). These contain the stop codon in domain III. There are at least two alternative-splicing variants of *SCN12A*. One of them, SCN12A-s, is a truncated form that lacks domain IV and C-terminal region (Fig. 1). The stop codon is located in the transmembrane segment S3 of domain IV. The exon boundary is the same as human *SCN5A* (35) and mouse *Scn10a* (36). RT-PCR analysis demonstrated that both transcripts of *SCN12A* are expressed similarly (data not shown). Several EST sequences (Accession Nos. AA885211 and AA91388, etc) identical to the end of the ORF and 3′-UTR region of the SCN12A-s have been registered in the GenBank database, demonstrating the existence of truncated splicing variant, SCN12A-s. The function of the truncated sodium channel α subunit has not yet been elucidated.

The voltage-gated sodium channels are essential for excitability of neurons and muscle cells. Individual sodium channel subtypes play unique physiological roles and show differences in tissue specificity (6). Briefly, seven sodium channels are primarily expressed in neurons; *Scn1a, SCN2A, Scn3a, Scn8a/SCN8A, Scn9a/SCN9A, Scn10a/SCN10A* and *Scn11a*. *Scn4a/SCN4A* is expressed primarily in skeletal muscle and *Scn5a/SCN5A* in cardiac muscle (11) and the CNS (37). *SCN6A* is expressed in heart and uterus (12). *Scn7a* is expressed in various tissues including lung and smooth muscles (38). In the CNS, glial cells express sodium channels as well as neurons. *Scn2a/SCN2A, Scn3a, Scn7a, Scn8a/SCN8A* and *Scn9a/SCN9A* are expressed in glia. Although SCN12A and Scn11a share similar structural features and are located on syntenic regions of their respective chromosomes, their tissue distributions are strikingly different. Rat Scn11a is expressed specifically in sensory neurons of DRG and trigeminal ganglia (25). Our new gene, *SCN12A*, is expressed in the CNS and non-neural tissues as well as DRG (Figs. 3 and 4).

TTX-R sodium current was observed in both neurons (39) and glial cells (40) in the central nervous system. The previously reported TTX-R sodium channels are expressed primarily in DRG (15, 22, 25), cardiac muscle (11) and the CNS (37). Along with SCN5A, SCN12A may be responsible for TTX-R sodium current observed in the CNS. Further studies including the functional expression of *SCN12A* will help to elucidate its physiological roles in the CNS and other tissues.

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REFERENCES

1. Catterall, W. A. (1992) *Physiol. Rev.* **72,** S15–S48.
2. Noda, M. (1993) *Ann. NY Acad. Sci.* **707,** 20–37.
3. Catterall, W. A. (1995) *Annu. Rev. Biochem.* **64,** 493–531.
4. Isom, L. L., De Jongh, K. S., and Catterall, W. A. (1994) *Neuron* **12,** 1183–94.
5. Isom, L. L., Scheuer, T., Brownstein, A. B., Ragsdale, D. S., Murphy, B. J., and Catterall, W. A. (1995) *J. Biol. Chem.* **270,** 3306–3312.
6. Plummer, N. W., and Meisler, M. H. (1999) *Genomics* **57,** 323–331.
7. Lu, C. M., Han, J., Rado, T. A., and Brown, G. B. (1992) *FEBS Lett.* **303,** 53–58.
8. Ahmed, C. M., Ware, D. H., Lee, S. C., Patten, C. D., Ferrer-Montiel, A. V., Schinder, A. F., McPherson, J. D., Wagner-McPherson, C. B., Wasmuth, J. J., Evans, G. A., and Montal, M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8220–8224.
9. Lu, C. M., and Brown, G. B. (1998) *J. Mol. Neurosci.* **10,** 67–70.
10. George, A. L., Jr., Komisarof, J., Kallen, R. G., and Barchi, R. L. (1992) *Ann. Neurol.* **31,** 131–137.
11. Gellens, M. E., George, A. L. Jr., Chen, L. Q., Chahine, M., Horn, R., Barchi, R. L., and Kallen, R. G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 554–558.
12. George, A. L. Jr., Knittle, T. J., and Tamkun, M. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4893–4897.
13. Plummer, N. W., Galt, J., Jones, J. M., Burgess, D. L., Sprunger,

L. K., Kohrman, D. C., and Meisler, M. H. (1998) *Genomics* **54,** 287–296.

14. Klugbauer, N., Lacinova, L., Flockerzi, V., and Hofmann, F. (1995) *EMBO J.* **14,** 1084–1090.
15. Rabert, D. K., Koch, B. D., Ilnicka, M., Obernolte, R. A., Naylor, S. L., Herman, R. C., Eglen, R. M., Hunter, J. C., and Sangameswaran, L. (1998) *Pain* **78,** 107–114.
16. Guy, H. R., and Conti, F. (1990) *Trends Neurosci.* **13,** 201–206.
17. Yang, N., and Horn, R. (1995) *Neuron* **15,** 213–218.
18. Stuhmer, W., Conti, F., Suzuki, H., Wang, X. D., Noda, M., Yahagi, N., Kubo, H., and Numa, S. (1989) *Nature* **339,** 597–603.
19. Guy, H. R., and Seetharamulu, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 508–12.
20. Murphy, B. J., Rossie, S., De Jongh, K. S., and Catterall, W. A. (1993) *J. Biol. Chem.* **268,** 27355–27362.
21. Catterall, W. A. (1980) *Annu. Rev. Pharmacol. Toxicol.* **20,** 15–43.
22. Akopian, A. N., Sivilotti, L., and Wood, J. N. (1996) *Nature* **379,** 257–262.
23. Satin, J., Kyle, J. W., Chen, M., Bell, P., Cribbs, L. L., Fozzard, H. A., and Rogart, R. B. (1992) *Science* **256,** 1202–1205.
24. Wang, Q., Shen, J., Splawski, I., Atkinson, D., Li, Z., Robinson, J. L., Moss, A. J., Towbin, J. A., and Keating, M. T. (1995) *Cell* **80,** 805–811.
25. Dib-Hajj, S. D., Tyrrell, L., Black, J. A., and Waxman, S. G. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 8963–8968.
26. Dib-Hajj, S. D., Tyrrell, L., Escayg, A., Wood, P. M., Meisler, M. H., and Waxman, S. G. (1999) *Genomics* **59,** 309–318.
27. Hirai, M., Kusuda, J., and Hashimoto, K. (1996) *Genomics 34,* 263–265.
28. West, J. W., Patton, D. E., Scheuer, T., Wang, Y., Goldin, A. L., and Catterall, W. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10910–10914.
29. Hashida, H., Goto, J., Zhao, N., Takahashi, N., Hirai, M., Kanazawa, I., and Sakaki, Y. (1998) *Genomics* **54,** 50–58.
30. Tate, S., Benn, S., Hick, C., Trezise, D., John, V., Mannion, R. J., Costigan, M., Plumpton, C., Grose, D., Gladwell, Z., Kendall, G., Dale, K., Bountra, C., and Woolf, C. J. (1998) *Nat. Neurosci.* **8,** 653–655.
31. Smith, R. D., and Goldin, A. L. (1996) *J. Neurosci.* **16,** 1965–74.
32. Bailey, W. J., Kim, J., Wagner, G. P., and Ruddle, F. H. (1997) *Mol. Biol. Evol.* **14,** 843–853.
33. Oh, Y., and Waxman, S. G. (1998) *NeuroReport* **9,** 1267–1272.
34. Plummer, N. W., McBurney, M. W., and Meisler, M. H. (1997) *J. Biol. Chem.* **272,** 24008–24015.
35. Wang, Q., Li, Z., Shen, J., and Keating, M. T. (1996) *Genomics* **34,** 9–16.
36. Souslova, V. A., Fox, M., Wood, J. N., and Akopian, A. N. (1997) *Genomics* **41,** 201–209.
37. Hartmann, H. A., Colom, L. V., Sutherland, M. L., and Noebels, J. L. (1999) *Nat. Neurosci.* **2,** 593–595.
38. Akopian, A. N., Souslova, V., Sivilotti, L., and Wood, J. N. (1997) *FEBS Lett.* **400,** 183–187.
39. Mercuri, N. B., Stratta, F., Calabresi, P., and Bernardi, G. (1993) *Neurosci. Lett.* **153,** 192–196.
40. White, J. A., Alonso, A., and Kay, A. R. (1993) *Neuron* **11,** 1037–1047.