Structure and Function of a Novel Voltage-gated, Tetrodotoxin-resistant Sodium Channel Specific to Sensory Neurons*

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Small neurons of the dorsal root ganglion (DRG) are known to play an important role in nociceptive mechanisms. These neurons express two types of sodium currents, which differ in their inactivation kinetics and sensitivity to tetrodotoxin. Here, we report the cloning of the α-subunit of a novel, voltage-gated sodium channel (PN3) from rat DRG. Functional expression in Xenopus oocytes showed that PN3 is a voltage-gated sodium channel with a depolarized activation potential, slow inactivation kinetics, and resistance to high concentrations of tetrodotoxin. In situ hybridization to rat DRG indicated that PN3 is expressed primarily in small sensory neurons of the peripheral nervous system.

Voltage-gated sodium channels play a fundamental role in the regulation of neuronal excitability. In addition to differences in primary structure and kinetic properties (1), these channels can be distinguished pharmacologically on the basis of their relative sensitivity to the neurotoxin, tetrodotoxin (TTX) (2). Two types of sodium currents are expressed by sensory neurons within the dorsal root ganglion (DRG), a fast inactivating TTX-sensitive current and a slow inactivating, TTX-resistant current that appears to be expressed by a high proportion of the small afferent neurons (3–8). Of the large and small neurons of the DRG, the latter is of primary importance in the processing of nociceptive information within the somatosensory system (9, 10). In order to define the molecular basis of sodium channel conductance in sensory neurons, we have attempted to identify and clone novel sodium channel α-subunits. As described here, this work has led to the isolation and functional expression of PN3, a novel voltage-gated, TTX-resistant sodium channel expressed predominantly by small sensory neurons within the peripheral nervous system.

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1 The abbreviations used are: TTX, tetrodotoxin; DRG, dorsal root ganglion; PCR, polymerase chain reaction; RT, reverse transcription; SCN, sodium channel β-subunit; h, human; r, rat.

EXPERIMENTAL PROCEDURES

cDNA Cloning—cDNA was prepared from normal adult male Sprague-Dawley rat DRG poly(A)⁺ RNA using the SuperScript Choice System (Life Technologies, Inc.). cDNA (10 μg) was selected by sucrose gradient fractionation (11) ligated into the Zap Express vector (Stratagene) and packaged with the Gigapack II XL lambda packaging extract (Stratagene). Phage (3.5 × 10⁶) were screened by filter hybridization with a 32P-labeled probe (bases 4637–5868 of rb11a) (12). Filters were hybridized in 50% formamide, 5 × saline/sodium phosphate/EDTA, 5 × Denhardt’s solution, 0.5% SDS, 250 μg/ml salmon sperm DNA, and 50 ng salmon sperm DNA at 42 °C and washed in 0.5 × SSC, 0.1% SDS at 50 °C. Positive clones were excised into vivo into pBk-CMV using the ExAssist/XLORL system (Stratagene). Southern blots of cDNA-digested plasmids were hybridized with a 32P-labeled DNA probe representing a novel domain IV segment amplified from DRG RNA by PCR with degenerate oligonucleotides (9, 10). In order to define the molecular basis of sodium channel conductance in sensory neurons, we have attempted to identify and clone novel sodium channel α-subunits. As described here, this work has led to the isolation and functional expression of PN3, a novel voltage-gated, TTX-resistant sodium channel expressed predominantly by small sensory neurons within the peripheral nervous system.

In situ Hybridization—Histology—Oligonucleotide probe sequences were synthesized from the unique 3′-untranslated region of PN3 and defined a 410-base pair amplicon. Thermal cycler parameters were: 30 s at 94°C, 30 s at 57°C, 1 min at 72°C (24 cycles); 30 s at 94°C, 30 s at 57°C, 5 min at 72°C (1 cycle). A positive control (1 ng of pBk-CMV/PN3) and a no-template control were also included. cDNA from each tissue was also PCR amplified using primers specific for glyceraldehyde-3-phosphate dehydrogenase (13) to demonstrate template viability. PN3 PCR amplicons from nodose ganglia and sciatic nerve were confirmed by nucleotide sequence analysis.

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There was a slow "rundown" of the current with time, and a correction was made for the resulting sloping baseline. Varying concentrations of TTX (Sigma) in bathsolution were perfused over the oocyte, and the current amplitude was allowed to attain steadystate before the effect was recorded.

**RESULTS AND DISCUSSION**

Sequence Analysis of PN3—To identify novel sodium channel α-subunits from the peripheral nervous system, we used degenerate oligonucleotide-primed RT-PCR analysis of RNA from rat DRG and homology cloning from a rat DRG cDNA library. PCR fragments from domain I and interdomain I–II were cloned and sequenced. The sequences matched those of clones 7.3 and 17.2 that were isolated from the rat DRG cDNA library by homology cloning. Clone 7.3 (PN3, peripheral nerve sodium channel 3) was sequenced entirely. Several other full-length and partial clones for PN3 were isolated.

Nucleotide sequence analysis of the PN3 cDNA identified a 5868-base open reading frame, coding for a 1956-amino acid protein. In common with other sodium channels, there is an ATG 5 base pairs upstream of the genuine ATG. The deduced amino acid sequence of PN3 (Fig. 1) exhibited the primary structural features of an α-subunit of a voltage-gated sodium channel. PN3 contains four homologous domains (I–IV), each consisting of six putative α-helical transmembrane segments (S1–S6). The positively charged residues in the voltage sensor (S4 segments) and the inactivation gate between III S6 and IV S1 are highly conserved in PN3; sites for cAMP-dependent phosphorylation and N-linked glycosylation shown to exist in other sodium channels (1) are also present in PN3. However, there are two unique consensus sites for cAMP-dependent phosphorylation sites, one in domain II between S3 and S4 and another in the interdomain II–III (Fig. 1). Modulation of rBIIa channel function by cAMP-dependent protein kinase A has been demonstrated (1, 17, 18). The significance of the unique cAMP-dependent protein kinase A consensus sites in PN3 in vitro and in vivo remains to be demonstrated. In addition, there is an insertion of an additional Gln between Pro583 and Ala584 in several partial clones. The significance of the glutamine insertion has not been determined.

![Fig. 1. Deduced amino acid sequence of peripheral nerve sodium channel type 3 (PN3) showing putative transmembrane domains.](image)

![Fig. 2. A, amino acid homology comparison of PN3 with selected sodium channels.](image)

![Fig. 2. B, tissue distribution profile of PN3 by RT-PCR analysis after 35 cycles of amplification. SCG, superior cervical ganglia.](image)
have detected other sodium channels including rBI (23), rBIII (24), rH1 (25), peripheral nerve sodium channel type 1 (PN1) (26), SCP6 (27), and other novel sodium channel \( \alpha \)-subunits (data not shown).

In situ hybridization using PN3-specific oligonucleotide probes showed that PN3 mRNA was expressed predominantly by small neurons in rat DRG (Fig. 3). Approximately 76% of the small cell population (400–1000 \( \mu \)m\(^2\)) and 33% of the large cell population (1400–2000 \( \mu \)m\(^2\)) were hybridized with probes for PN3 (Fig. 4). Recently, Akopian and Wood (28) isolated a partial cDNA clone, G7, from a rat DRG cDNA library with homology to known sodium channels and specific expression in subsets of sensory neurons. Whether PN3 and G7 are the same gene is unknown at present.

Functional Analysis of PN3—Two electrode voltage clamp recordings from Xenopus oocytes injected with PN3 cRNA indicated that expression of PN3 produced an inward current with slow inactivation kinetics (Fig. 5A). This current was voltage-dependent (Fig. 5B) and is carried by sodium ions; reduction of extracellular sodium ion concentration (by substituting N-methyl-D-glucamine) from 91 to 50 and 21 mM resulted in hyperpolarizing shifts in the reversal potential from +43 mV to +12 mV and –22 mV, respectively. Examination of the current-voltage relationship for PN3 (Fig. 5B) reveals a strikingly depolarized activation potential. In this expression system, PN3 exhibits little or no activation at –10 mV, whereas most cloned sodium channels begin to activate between –60 and –30 mV (29–31).

The currents produced by injection of PN3 cRNA had slow inactivation kinetics (Fig. 5A). rBIIa, rBIII, and rSkM1 sodium channels also produce currents with slow inactivation kinetics when injected into Xenopus oocytes; coexpression of the \( \beta \)1-subunit greatly accelerates the inactivation kinetics of these channels (22, 32–35). However, coinjection of 1.3 ng of human sodium channel \( \beta \)1-subunit (hSCN\( \beta \)1) (36) cRNA, which is homologous to the rat brain and DRG SCN\( \beta \)1, with PN3 cRNA did not accelerate the inactivation kinetics (data not shown). In contrast, coexpression of this quantity of hSCN\( \beta \)1 cRNA with rSkM1 cRNA was sufficient to accelerate the inactivation kinetics of rSkM1 maximally. Therefore, PN3 may possess inherently slow kinetics.

When expressed in Xenopus oocytes, the PN3 sodium current is highly resistant to TTX (IC\(_{50} \approx 100 \mu M\) ) (Fig. 5C). The TTX-sensitive brain and skeletal muscle sodium channels are blocked by nanomolar TTX concentrations, whereas the TTX-insensitive cardiac sodium channels are blocked by micromolar TTX concentrations (2). In rat heart sodium channel 1 (rH1), Cys\(^{374}\) is a critical determinant of TTX insensitivity (37–39); in the TTX-sensitive rBI, rBII, rBIII, and rSkM1, the corresponding residue is an aromatic amino acid, either Phe or Tyr, the aromatic ring of which facilitates the binding of TTX to the protein. In PN3, this position is occupied by a Ser residue (Ser\(^{356}\)), which may explain the unique response to TTX. Site-directed mutagenesis of this residue to Phe/Tyr or Cys will determine whether this amino acid residue is solely responsible for TTX resistance.

TTX-resistant sodium currents have been implicated in peripheral and central neuronal sensitization mediated by the
the effect was allowed to attain steadystate. Each concentration and proceeding to the highest. For each concentration, the full range of TTX concentrations shown, beginning with the lowest

current-voltage relationship of the data in

produced by step depolarizations of an oocyte injected with 18 ng of PN3 sodium current. Each oocyte was exposed to

mean

6

2.10−10. Woolf, C. J., and Doubell, T. P. (1994)

FIG. 5. Expression of PN3 in Xenopus oocytes. A, currents produced by step depolarizations of an oocyte injected with 18 ng of PN3 cRNA from a holding potential of −100 to −30 mV through +50 mV in 10-mV increments. The Gened 

currents in other sensory ganglia of the peripheral nervous system such as nodose ganglia (40).

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C-fibers of small neurons following peripheral tissue damage and nerve injury. The biophysical and pharmacological properties of PN3 suggest that it contributes to the TTX-resistant sodium currents in small neurons of DRG. PN3 may, therefore, play a role in the sensory function and dysfunction that is characteristic of pathophysiological pain processing. In addition, we suggest that PN3 may conduct TTX-resistant sodium currents in other sensory ganglia of the peripheral nervous system such as nodose ganglia (40).

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