Rat brain Na$^+$ channel mRNAs in non-excitable Schwann cells

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Abstract

The expression of rat brain voltage-sensitive Na$^+$ channel mRNAs in Schwann cells was examined using in situ hybridization cytochemistry and RT-PCR. The mRNAs of rat brain Na$^+$ channel subtype II and III, but not subtype I, were detected in cultured Schwann cells from sciatic nerve and in intact sciatic nerve, which contains Schwann cells but not neuronal cell bodies. These results indicate that rat brain Na$^+$ channel mRNAs, which have been considered as mainly neuronal-type messages, are also expressed in glial cells in vitro and in vivo.

Key words: Ion channel; Sciatic nerve; Glial cell

1. Introduction

Voltage-sensitive Na$^+$ channels mediate changes in Na$^+$ permeability during the rising phase of action potentials in electrically excitable cells [1–3]. Na$^+$ channels purified from rat brain are composed of α (260 kDa), $\beta 1$ (36 kDa), and $\beta 2$ (33 kDa) subunits [4]. The primary structure of the rat brain Na$^+$ channel α subunit has been deduced from cDNA clones to show the existence of three distinct subtypes (I, II, and III) [1–3]. These subtypes are highly homologous to each other and have about 87% identity at the amino acid level but contain unique 5′ and 3′ untranslated regions. Functional expression studies in *Xenopus* oocytes and mammalian cells have shown that the α subunit alone can encode functionally active Na$^+$ channels and the $\beta 1$ subunit can influence the kinetic properties of expressed Na$^+$ channels [2].

Expression of rat brain Na$^+$ channel mRNAs has traditionally been considered to be restricted to excitable cells, but recent studies suggest that they can be also expressed in astrocytes [5,6], which are non-excitable glial cells in CNS. In the present study, using in situ hybridization cytochemistry and RT-PCR, we have further examined the expression of rat brain Na$^+$ channels in another type of non-excitable glial cell: the Schwann cell, which is the major glial cell in PNS. Schwann cells have been shown to play important roles in the development, function, and regeneration of peripheral nerves [7,8]. Our results demonstrate that Schwann cells in vivo and in vitro can express subtype II and III rat brain Na$^+$ channel mRNAs; in addition, the mRNA for a putative glial-specific Na$^+$ channel a subunit [9] was amplified from both sciatic and optic nerves. Our data suggest that glial cells express multiple forms of Na$^+$ channel mRNAs in vivo.

2. Materials and methods

2.1. Cell cultures

Schwann cells were cultured from postnatal day 0 Sprague–Dawley rat sciatic nerves. Tissue was minced and incubated in a solution containing 30 units papain/ml, Earle's salts, 0.5 mM EDTA, and 1.65 mM L-cysteine at 37°C followed by trituration in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum and penicillin/streptomycin (500 U/ml each) supplemented with trypsin inhibitor and bovine serum albumin (1.5 mg/ml each). Resuspended cells were plated onto 12 mm circular glass coverslips treated with polyornithine/laminin, and maintained at 37°C for 4 days in a 5% CO$_2$/95% air atmosphere. Schwann cells were readily identified by their characteristic bipolar spindle-shape morphology in culture [7,10].

2.2. In situ hybridization

In situ hybridization was performed as previously reported [5]. Briefly, a portion of the 3′ untranslated regions of each subtype of rat brain Na$^+$ channel a subunit was amplified via RT-PCR and cloned into pBluescript SK(−) vector. From these cloned vectors, digoxigenin-labeled sense or antisense single-strand riboprobes were generated using DIG RNA labeling kit (Boehringer Mannheim). Specific staining of cultured Schwann cells was observed with antisense but not with sense riboprobes. These probes have been recently used to localize rat brain Na$^+$ channel α subunit mRNAs in rat CNS neurons [11] and cultured astrocytes [5]. On Northern blot analysis, these probes were hybridized to expected sizes of rat brain Na$^+$ channel α subunit mRNAs ranging from 7.5 to 9.5 kb in rat brain [11] and cultured astrocytes [5], consistent with previous reports [2].

In control experiments, including (i) hybridization of cells in the absence of labeled probes and (ii) pretreatment of the cells with RNase A, no specific labeling was observed.

2.3. RT-PCR

The procedure for RT-PCR was described previously in detail [6,12]. Briefly, total RNA was extracted from postnatal day 7–14 Sprague–Dawley rat sciatic and optic nerves, or from adult rat brain, liver, and kidney, or cultured B104 neuronal cells with a single-step guanidinium thiocyanate acid-phenol-chloroform method. B104 cells were used as a control since they express only rat brain Na$^+$ channel subtype III [13]. cDNA was synthesized from the prepared RNA using AMV reverse transcriptase, and PCR was performed in 1× PCR buffer (10 mM

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Abbreviations: RT-PCR, reverse transcription-polymerase chain reaction; CNS, central nervous system; PNS, peripheral nervous system.

Tris-HCl, pH 9.0, 50 mM KCl, 2.5 mM MgCl₂, 0.1% Triton X-100). Each PCR cycle consisted of a denaturation step (94°C, 1 min), an annealing step (58°C, 2 min), and an elongation step (72°C, 3 min). DNA was amplified for 30–35 cycles in a programmable thermal controller (PTC-100, MJ Research Inc.). For controls, RNA samples were pretreated with RNase A for 30 min at 37°C and subjected to RT-PCR; in these controls, no specific amplification was observed.

2.4. Primers

All three subtypes of rat brain Na⁺ channel α subunits were simultaneously amplified using two different primer sets (A and B), which were designed to fall into regions that are highly conserved between subtypes. The amplified regions contain the first intracellular domains which are known to contain introns [14]. The 5′ primer of *primer set A* corresponds to nucleotides 1,557–1,586 (numbered according to GenBank) of subtype I; 1,522–1,551 of subtype II; and 1,722–1,751 of subtype III. The 3′ primer of *set A* corresponds to 2,601–2,629 of subtype I; 2,533–2,561 of subtype II; and has one mismatch with subtype III (nucleotides 2,592–2,620). The 5′ primer of *primer set B* corresponds to nucleotides 1,217–1,238 of rat brain Na⁺ channel subtype III with one nucleotide substitution at 1,219 (thymine substitution by cytosine); *primer set B* used the same 3′ primer as *primer set A*. The *primer set B* was previously used to amplify all three subtypes of rat brain Na⁺ channel α subunits from rat brain and astrocytes [6].

Besides noting their expected sizes, restriction mapping analysis was performed to validate the amplified PCR products [6]. PCR products used in enzyme digestion reactions were purified from the gel using Gene-Clean II (Bio 101), and the digested PCR products were run on 2–3% agarose gel. Table 1 shows the predicted sizes of restriction enzyme-digested fragments.

A putative glial Na⁺ channel α subunit (NaG) [9] was amplified using a 5′ primer corresponding to nucleotides 76–95, and a 3′ primer corresponding to nucleotides 1,289–1,308; six nucleotides (5′-CCAAGC-3′) were additionally attached to the 5′ end of the 3′ primer. From the same RNA preparations used to amplify Na⁺ channels, we have also amplified rat β-actin. The use of β-actin primers (Clontech) will give predictably different sizes of PCR products depending upon whether the DNA is amplified from genomic DNA (1,440 bp) or mRNA (764 bp) because of the presence of introns in the amplified regions. From all RNA samples used in the present study, β-actin was amplified to give one size (764 bp) of PCR products, suggesting that there is only a negligible amount of genomic DNA contamination in our RNA samples.

3. Results and discussion

Cultured Schwann cells were hybridized with digoxigenin-labeled subtype-specific riboprobes against rat brain Na⁺channel α subunits to show the specific expression of subtype II and III mRNAs, but not subtype I (Fig. 1). The hybridization signal, revealed by using anti-digoxigenin antibody conjugated with alkaline phosphatase reaction, was detectable in the cell body and bipolar processes of Schwann cells, indicating the pres-
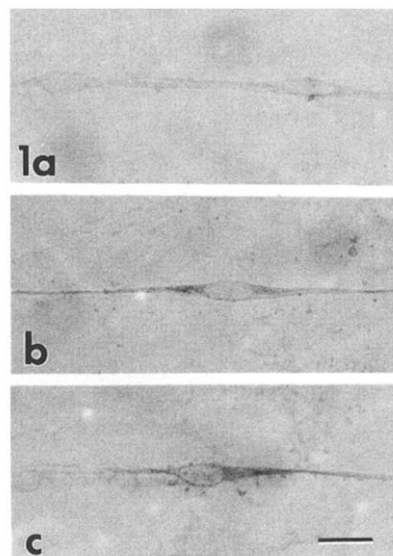

Fig. 1. Na⁺ channel mRNAs in cultured sciatic nerve Schwann cells. Schwann cells were hybridized with riboprobes specific for subtype I (a), II (b), or III (c), respectively. Note the staining in the cell body and bipolar processes of Schwann cells when using subtype II- and III-specific probes. Bar = 20 μm.

ence of mRNA and perhaps protein-synthesizing machinery in these regions. Similar observations have been reported in neurons in which mRNA is localized in the cell body and dendritic processes, but not in axons [15,16].

We also performed in situ hybridization with the Na⁺ channel subtype-specific riboprobes in intact sciatic nerves, but intriguingly specific labeling has not been observed (not shown). Thus, we suspect that rat brain Na⁺ channel mRNAs in Schwann cells in vivo are normally down-regulated via inhibitory mechanisms that are lost when the cells are isolated from the tissue and cultured. As noted below, our RT-PCR experiments, which have a lower threshold for detection of mRNA [17,18], demonstrate the presence of rat brain Na⁺ channel mRNAs in intact sciatic nerves, most likely derived from Schwann cells, although their level of expression is too low to be detected by in situ hybridization.

The RT-PCR amplification of rat brain Na⁺ channels from sciatic nerves and restriction digestion of the ampli-

Table 1
Predicted sizes of restriction enzyme-digested fragments for primer sets A and B to amplify rat brain sodium channels (in base pairs)

	Primer Set A			Primer Set B		
	AvaII	SmaI	HindIII	EcoRI	SmaI	KpnI
I*	661, 332, 80	NC	NC	823, 752	NC	NC
II	NC**	586, 454	NC	NC	1,091, 454	NC
III	NC	NC	602, 152, 145	NC	NC	1,074, 330

*Rat brain sodium channel subtype.
**NC indicates that restriction enzyme would not be expected to cut the PCR products. The uncut sizes of subtype I, II, and III using primer set A are 1,073, 1,040, and 899, respectively, and using primer set B are 1,575, 1,545, and 1,404, respectively.

fied Na$^+$ channels using primer sets A (Fig. 2) and B (Fig. 3) reveal the presence of subtype II and III mRNAs in sciatic nerve, consistent with the in situ hybridization results. As expected, only subtype III mRNA from B104 cells, and all three subtypes (I, II, and III) from adult rat brain were amplified (Fig. 2). Neither subtype I, II, or III was amplified from adult rat liver or kidney (not shown).

Several lines of evidence suggest that the amplified Na$^+$ channel mRNAs from sciatic nerves are likely derived from Schwann cells. First, electrophysiological measurements including whole-cell patch clamping and pharmacological binding assays have demonstrated the presence of voltage- and tetrodotoxin-sensitive Na$^+$ channels in Schwann cells in vitro and in vivo [10,19,20]. Second, fibroblasts, the other major cell type in sciatic nerve, do not express voltage-sensitive Na$^+$ channels [12,21]. Third, sciatic nerves are composed of axon bundles and Schwann cells, and most axons do not contain neuronal mRNAs [15,16,22].

The present study demonstrates several interesting aspects of voltage-sensitive Na$^+$ channel expression in the nervous system. Contrary to the generally accepted notion of the restricted expression of voltage-sensitive Na$^+$ channels in neurons, our results show that non-excitable Schwann cells, as well as in astrocytes [5,6], can also express these Na$^+$ channel mRNAs. These results imply a broad expression of rat brain Na$^+$ channels in both CNS and PNS glial cells as well as in neurons, and raise an interesting question: what is the physiological role of rat brain Na$^+$ channels in glial cells? Astrocytes and Schwann cells can not generate action potentials in vitro or in situ [20,23]. At present, there are two major hypoth-

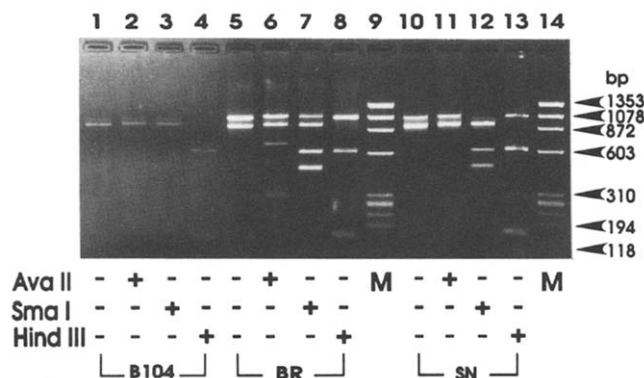Fig. 3. RT-PCR amplification of the rat brain Na$^-$ channel α subunit using primer set B. Restriction mapping analysis (run on 2% agarose gel) in sciatic nerve (SN; lanes 2–5) and B104 cells (B104; lanes 6–9). Lanes 1 and 10 contain ϕX174/HaeIII digests, and lane 11 contains lambda DNA/HindIII fragments. Lanes 2 and 6 are the amplified PCR products without restriction enzyme treatment. PCR products were treated with subtype specific enzymes in the amplified regions: EcoR I (subtype I-specific, lanes 3,7); SmaI (subtype II-specific, lanes 4,8); or KpnI (subtype III-specific, lanes 5,9). The asterisks indicate the largest enzyme-digested fragment for the respective subtypes. The results demonstrate the presence of subtype II and III mRNAs in sciatic nerve and only subtype III mRNA in B104 cells.

eses about the possible role of glial Na$^+$ channels. First, glial Na$^+$ channels may be involved in extracellular ionic homeostasis by providing a 'return pathway' for Na$^+$ ions that is required for Na$^+$/K$^+$-ATPase operation [24]. Second, it has been speculated that glial cells constitute an extra-source of axolemmal Na$^+$ channels, which reduces the biosynthetic load on the neuron [19]. In this regard, the present study and a previous study [6,12] demonstrate the expression of both α and β1 subunit mRNAs of rat brain Na$^+$ channels in Schwann cells and astrocytes, thus providing a substrate for nearly complete rat brain Na$^+$ channel biosynthesis in these cells. If Na$^+$ channels are transferred from glial cells to axons, the most likely candidate for transfer is subtype II because immunocytochemical studies have shown that subtype II is preferentially localized in axons [25,26].

Another interesting aspect from the present study is the in situ hybridization detection of subtype II and III Na$^+$ channel mRNAs in cultured Schwann cells but not in intact sciatic nerves. Similar findings were reported in studies on astrocytic Na$^+$ channels in which subtype II and III Na$^+$ channel mRNAs were localized in cultured spinal cord astrocytes but not in intact spinal cord white matter [5,11]. Similarly, rat brain Na$^+$ channel β1 subunit mRNAs have been detected in cultured glial cells but not in intact tissue [12,27]. It might be argued from these apparently contradictory results that the expression of rat brain Na$^+$ channel mRNAs in cultured glial cells reflects a culture-induced artifact, and thus glial cells in

Fig. 2. RT-PCR amplification of the rat brain Na$^+$ channel α subunit using primer set A. Restriction mapping analysis (run on 2% agarose gel) in B104 cells (B104; lanes 1–4), rat brain (BR; lanes 5–8), and sciatic nerve (SN; lanes 10–13). Lanes 9 and 14 contain ϕX174/HaeIII digests. Lanes 1, 5, and 10 are the amplified PCR products without restriction enzyme treatment. PCR products were treated with subtype specific enzymes in the amplified regions: AvaI (subtype I-specific, lanes 2,6,11); SmaI (subtype II-specific, lanes 3,7,12); or HindIII (subtype III-specific, lanes 4,8,13). The results demonstrate the presence of all three subtypes of rat brain Na$^+$ channel α subunits in rat brain, subtype II and III in sciatic nerve, and only subtype III in B104 cells.
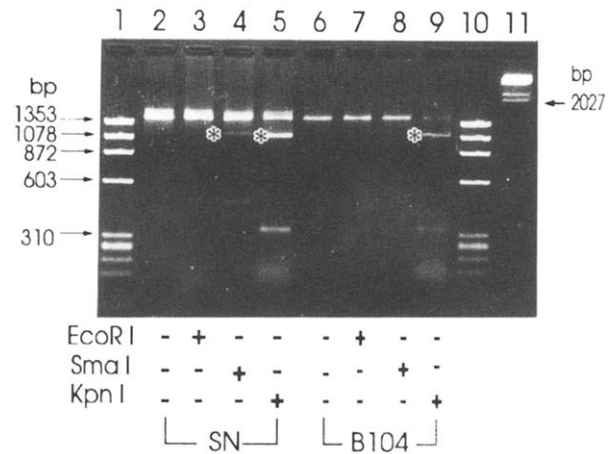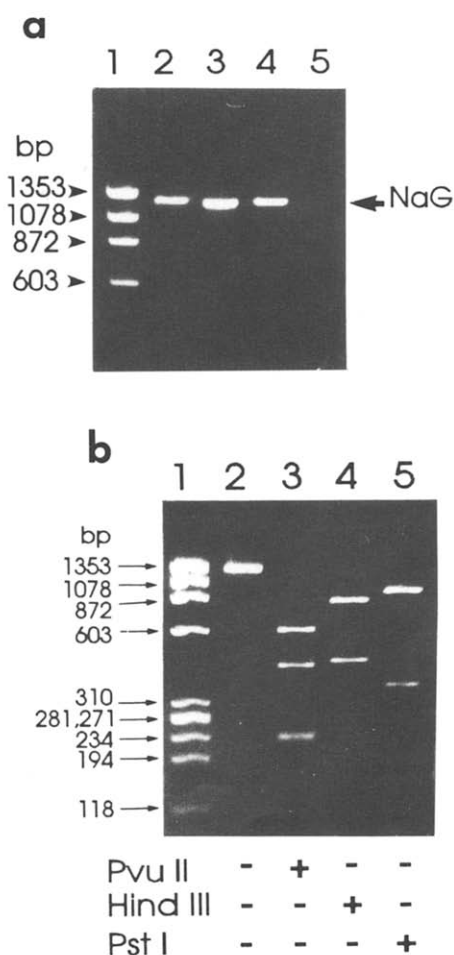
Fig. 4. (a) RT-PCR amplification of a putative Na⁺ channel α subunit (NaG) in optic nerve (lane 2), sciatic nerve (lane 3), rat brain (lane 4), and rat liver (lane 5). Lane 1 contains φX174/HaeIII digests. (b). Restriction mapping analysis (run on 3% agarose gel) of the amplified PCR product from sciatic nerve. Lane 1 contains φX174/HaeIII digests. Lane 2 is the amplified PCR products without restriction enzyme treatment. Digestion of the PCR products with PvuII, HindIII, or PstI will predictably generate DNA fragments of (589 + 425 + 225, in bp), (796 + 443), or (891 + 348), respectively. The results demonstrate the presence of multiple forms of Na⁺ channel messages, including rat brain Na⁺ channels and NaG, in optic and sciatic nerves in vivo.

vivo do not express rat brain Na⁺ channels. However, the Na⁺ channel mRNA amplification from intact sciatic nerves in the present study and from optic nerve in a previous study [6] argue against this possibility. Moreover, Na⁺ currents have been detected in some astrocytes within hippocampal slice preparations [23], and recently astrocytes within spinal cord white matter have been immunolabeled with Na⁺ channel antibody [28]. Thus, the inability to detect Na⁺ channel α and β1 subunit mRNAs in glial cells in intact tissues using the present in situ hybridization technique may represent a relative lack of sensitivity of this method compared to RT-PCR. This view is consistent with the recent report of Jensen and Chiu [29], who observed the expression of glutamate receptor 1 (GluR1) and GluR3 subunits in optic nerves

by RT-PCR, but only GluR1 by in situ hybridization. It is also possible that neuronal factors may down-regulate glial Na⁺ channel expression in vivo [30].

Recently, Gautron et al. [9] reported the isolation and characterization of a partial cDNA encoding a putative Na⁺ channel α subunit (NaG), which is highly expressed in cultured glial cells. Fig. 4A shows the RT-PCR amplification of NaG from rat optic and sciatic nerves as well as from adult rat brain but not from liver. Optic nerve, which is similar to sciatic nerve in that it contains axons and glia but not neuronal cell bodies, provides a counterpart of sciatic nerve in the CNS. The amplified PCR products were verified by restriction mapping analysis as shown in Fig. 4B. The expression of NaG in glial cells further demonstrates the complexity of glial Na⁺ channel mRNA expression. In summary, Schwann cells in PNS and astrocytes in CNS may express multiple forms of Na⁺ channel mRNAs including the messages for rat brain Na⁺ channels. The functional role of these glial Na⁺ channels, and their regulation, represent important areas for future study.

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