Sodium channels and their genes: dynamic expression in the normal nervous system, dysregulation in disease states

Stephen G. Waxman\textsuperscript{a,b,*}, Sulayman Dib-Hajj\textsuperscript{a,b}, Theodore R. Cummins\textsuperscript{a,b}, Joel A. Black\textsuperscript{a,b}

\textsuperscript{a}Department of Neurology and PVA/EPVA Neuroscience Research Center, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA
\textsuperscript{b}Rehabilitation Research Center, VA Connecticut, West Haven, CT 06516, USA

Accepted 3 August 2000

Abstract

Although classical neurophysiological doctrine rested on the concept of the sodium channel, it is now clear that there are nearly a dozen sodium channel genes, each encoding a molecularly distinct channel. Different repertoires of channels endow different types of neurons with distinct transduction and encoding properties. Sodium channel expression is highly dynamic, exhibiting plasticity at both the transcriptional and post-transcriptional levels. In some types of neurons within the normal nervous system, e.g. hypothalamic magnocellular neurosecretory neurons, changes in sodium channel gene expression occur in association with the transition from a quiescent to a bursting state; these changes are accompanied by the insertion of a different set of sodium channel subtypes in the cell membrane, a form of molecular plasticity which results in altered electrogenic properties. Dysregulation of sodium channel genes has been observed in a number of disease states. For example, transection of the peripheral axons of spinal sensory neurons triggers down-regulation of some sodium channel genes, and up-regulation of other sodium channel genes; the resultant changes in sodium channel expression contribute to hyperexcitability that can lead to chronic pain. There is also evidence, in experimental models of demyelination and in post-mortem tissue from patients with multiple sclerosis, for dysregulation of sodium channel gene expression in the cell bodies of some neurons whose axons have been demyelinated, suggesting that an acquired channelopathy may contribute to the pathophysiology of demyelinating diseases such as multiple sclerosis. The dynamic nature of sodium channel gene expression makes it a complex topic for investigation, but it also introduces therapeutic opportunities, since subtype-specific sodium channel modulating drugs may soon be available. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

One of the features that makes neurons special is their ability to produce regenerative electrical signals, a characteristic that rests, in large part, on the presence of voltage-gated sodium channels within neurons. The seminal studies of Hodgkin and Huxley \cite{27} revealed that, in most mammalian neurons, ion flux through sodium channels underlies the regenerative upstroke of the action potential. Although Hodgkin and Huxley were not able to visualize sodium channels or directly discern their molecular structure, they were able to infer some important features of these channels and to predict, e.g., that they possess 'gates' which opened as the channels were activated by depolarization, allowing sodium ions to pass through. Nearly forty years later, Numa and his colleagues took another large step forward with the cloning of the first sodium channel \cite{34}.

The early electrophysiological and pharmacological experiments provided hints that there might be more than one sodium channel. However, the idea that there are multiple, molecularly distinct sodium channels had its formal birth when Numa and his colleagues demonstrated that, within the mammalian brain, there were (at least) three sodium channel genes, each encoding a different but...
related molecule [35] Other genes encoding additional sodium channels sharing a common overall motif, but with subtly different amino acid sequences, were discovered soon thereafter. Some neurons co-express several different sodium channel genes, indicating that their membranes contain several types of sodium channels. The expression of different repertoires of sodium channels in different types of neurons endows them with different transducing and encoding properties [49]. We now know that there are, in fact, nearly a dozen genes, each encoding a distinct sodium channel. Physiological signatures have been established for only some of these sodium channels. Nonetheless, the available evidence makes it clear that, from a functional point of view, sodium channels are not all the same: different channels can have different physiological characteristics, and can play different roles in the physiology of excitable cells.

Recent research has also made it apparent that the expression of sodium channels within neurons is not a static or fixed process. While neuronal plasticity has been most extensively studied with respect to synaptic potentiation and depression, sprouting and pruning of neurites, and the recruitment of pre-existing or new neurons into functional circuits, it is now clear that plasticity in expression of the genes encoding voltage-gated sodium channels can result in plasticity in the deployment of sodium channels which, in turn, can produce significant changes in the electroresponsive properties of neurons. These changes may be especially important in disease states characterized by hypo- or hyper-excitability, or where altered impulse trafficking contributes to pathophysiology.

Gene expression is controlled at the transcriptional (DNA→mRNA), translational (mRNA→protein), and post-translational (protein→modified protein) levels. While regulation at all of these levels is important, transcriptional regulation plays a particularly important role in controlling the molecular architecture of cells. This article will first provide examples of some of the diverse physiological functions that are subserved by the distinct sodium channels that are encoded by different genes. It will then discuss dynamic aspects of sodium channel gene expression in normal neurons, and dysregulated expression of sodium channels in injured neurons.

2. Sodium channels can modulate resting potential and can amplify subthreshold inputs

Given that all sodium channels share a common overall structural motif [10], why should the expression of one type of channel, rather than another, have functional significance? Although the concept of the ‘fast’, rapidly-activating and inactivating sodium channel as a mediator of the rising phase of the action potential is now regarded as classical, it has become clear from patch clamp and other physiological studies that some sodium channels have other properties and other functions. The molecular identities of some of these ‘non-conventional’ sodium channels have recently been established.

One example is provided by sodium channel NaN, which was cloned and sequenced by Dib-Hajj et al. [17]. NaN is preferentially expressed in small DRG neurons. The presence of a serine at position 355 in the amino acid sequence of NaN suggests that NaN is TTX-resistant [17,44]. The gene for NaN is localized, together with the genes encoding two other TTX-resistant sodium channels, SNS and SkM2, within a conserved linkage group at 3p21–3p24 within human chromosome 3, suggesting that the three TTX-resistant sodium channels arose by duplication from a common ancestral gene [19].

NaN and SNS are the only TTX-resistant sodium channels that are present within DRG neurons. Thus, when DRG neurons are exposed to TTX and studied by patch clamp, the NaN and SNS currents can be recorded in isolation. SNS-knockout mutant mice provide a model system in which the SNS current is absent [2]. Using this model, Cummins et al. [13] identified a TTX-resistant (K_i=39 μM) persistent sodium current (Fig. 1A) attributable to NaN. A similar TTX-resistant persistent current, which appears to be produced by NaN, has been observed in wild-type mouse and rat DRG neurons [13] and human DRG neurons [20]. The NaN current is unique in exhibiting a hyperpolarized dependence of activation (threshold ≈ −70 mV; midpoint of activation = −41 mV in mouse DRG neurons) and steady-state inactivation (midpoint = −44 mV), with a substantial overlap between

![Fig. 1. Persistent TTX-resistant sodium currents are produced by NaN channels in small DRG neurons.](image-url)

(A) Representative TTX-resistant sodium currents recorded from a DRG neuron from a SNS-null mouse with 100 ms test pulses. (B) Activation (unfilled squares) and steady-state inactivation (filled squares) curves for the NaN current show significant overlap. Steady-state inactivation was measured with 500 ms prepulses. (C) NaN currents from a SNS-null neuron elicited with 2 s step depolarizations to the voltages indicated. Recordings were made with 250 nM TTX, 100 μM cadmium (to block calcium currents) and V_hold = −120 mV in an SNS-null neuron. Modified from Cummins et al. [13].
activation and steady-state inactivation curves (Fig. 1B) which extends from −70 to −30 mV [13]. Because the resting potential of small DRG neurons is close to −55 mV [9], the properties of NaN suggest that it should generate a ‘window’ current close to resting membrane potential. Moreover, the low threshold for activation of NaN channels suggests that they should open in response to small subthreshold depolarizations close to membrane potential, thus contributing to subthreshold electrogenesis. Computer simulations, in fact, suggest that NaN channels contribute a 10–15 mV depolarizing influence to resting potential, and amplify small depolarizing inputs by more than 50%, in small DRG neurons (Herzog, Cummins and Waxman, unpublished results). NaN thus appears to regulate the excitability of DRG neurons.

Another example of a channel that has functions other than as a mediator of the action potential upstroke is provided by the PN1 sodium channel. Like NaN, PN1 is selectively expressed within dorsal root ganglion (DRG) neurons [47]. To establish the physiological role of PN1, Cummins et al. [12] used patch-clamp techniques to study its human ortholog PN1/hNE expressed in a model expression system (HEK293 cells), in a ‘bottom-up’ analysis. For comparison, Fig. 2A shows patch-clamp recordings from muscle (SkM1) sodium channels, expressed in transfected HEK293 cells. Like most traditional sodium channels, the SkM1 channels require sudden, relatively large depolarizations for activation. These channels do not open in response to slow depolarizations close to resting potential. When stimulated, for example, by slow, small depolarizations (e.g. a ramp at 0.23 mV/ms), the SkM1 channels do not generate a response (Fig. 2A). PN1/hNE channels display slow closed-state inactivation, up to five-fold slower than SkM1 [12]. As a result of this, PN1/hNE channels activate and generate a sodium current in response to small and gradual depolarizations close to resting potential (Fig. 2B). PN1/hNE channels also display a unique pharmacological profile, including block by tetradotoxin (Fig. 2C) and enhancement by cadmium (Fig. 2D).

Having established the functional signature for PN1/hNE channels using this bottom-up analysis in a heterologous expression system, the next step was to use this information to determine, in a top-down approach, whether these channels display similar properties in their native milieu within DRG neurons [12]. Because small, ramp-like depolarizations constitute an effective stimulus for the PN1/hNE sodium channel in HEK293 cells, these stimuli were applied to intact DRG neurons, and were found to evoke depolarizing responses within these cells (Fig. 2E), similar to those seen in the HEK expression system (Fig. 2B). The current evoked by these ramp stimuli within intact DRG neurons displayed responses to TTX and cadmium (Fig. 2F) that are identical to those of PN1/hNE channels within the heterologous expression system. The convergence of the bottom-up and top-down analyses suggests that, in intact DRG neurons, PN1/hNE channels respond to small, slow depolarizations close to resting potential, activating so as to produce inward (depolarizing) currents and thereby amplifying inputs such as generator

![Fig. 2](https://example.com/figure2.png)

‘Bottom-up’ and ‘top-down’ analyses reveal similar properties of the PN1/hNE sodium channel in HEK 293 cells and in DRG neurons. (A) SkM1 sodium channels, transfected into HEK293 cells, do not activate in response to slow ramp-like (0.23 mV/ms) depolarizations. (B) In contrast, these slow ramp stimuli activate PN1/hNE channels transfected into HEK293 cells, generating distinct inward currents which are evoked close to resting potential. (C) PN1/hNE currents are blocked by TTX. (D) PN1/hNE currents are enhanced by Cd²⁺. (E) In a ‘top-down’ analysis, nearly identical stimuli yield a similar inward current in DRG neurons. (F) Ramp currents in DRG neurons display a similar pharmacologic profile, being blocked by TTX and enhanced by Cd²⁺. (G) The threshold for activation of ramp currents within intact DRG neurons is similar to that for isolated PN1/hNE currents. (H) PN1/hNE currents in HEK293 cells, and ramp currents in intact DRG neurons, plotted together. Modified from Cummins et al. [12].
potentials. Consistent with this function, PN1/hNE channels are localized at the distal ends of neurites of spinal sensory neurons [47]. Given that NaN and PN1 both activate at voltages close to resting potential, it is possible that they cross-activate each other; cross-activation between these two types of channels, however, remains speculative.

3. Sodium channel gene expression: a dynamic process in normal neurons

It is almost axiomatic that, as neurons pass from a quiescent state to a high-frequency firing state, they use their (pre-existing) sodium channels differently, i.e., they activate these channels repetitively. But do neurons deploy a different set of sodium channels when they make these state transitions? An example of such a change is provided by the magnocellular neurosecretory cells within the supraoptic nucleus [43]. These specialized neurons send their axons to the neurohypophysis where they release vasopressin, a response that is triggered by increases in plasma osmolality. In their basal state these cells are relatively quiescent, firing irregularly at low frequencies (<3 impulses/s) but, in response to changes in the osmotic milieu, these cells respond by generating high-frequency bursts of action potentials which elicit vasopressin release [31]. To test the hypothesis that the transition from the quiescent to the bursting state involves a change in the expression of sodium channel genes, Tanaka et al. [43] studied neurons under normal conditions and following salt-loading, which triggers a transition to a bursting state. As seen in Fig. 3, in situ hybridization reveals that salt-loading triggers an up-regulation of mRNAs for two sodium channels, α-II and Na6, in these cells.

Ion channel expression within the cell membrane is controlled at both the transcriptional and translational levels. Thus increased mRNA levels are not necessarily accompanied by increased synthesis of channel protein. To determine whether increased levels of sodium channel protein were produced as mRNA levels rose, sodium channel protein expression was measured in magnocellular neurons, using immunocytochemical and immunoblotting methods with an antibody directed against a conserved region of sodium channels. These studies demonstrated a distinct increase in the level of sodium channel protein within the magnocellular neurons of salt-loaded rats [43]. Sodium channel protein molecules become functional in an electrogentic sense when they are inserted (together with accessory subunits) in the cell membrane, but they can also be sequestered within intracellular pools. Thus, the next question was whether the changes in sodium channel gene transcription are accompanied by the insertion of functional channels in the membranes of magnocellular neurons. The presence of two different sodium channels (α-II and Na6) in the magnocellular neurons suggested that

---

Fig. 3. α-II and Na6 sodium channel mRNA are up-regulated, together with sodium channel β1 and β2 mRNA, in supraoptic magnocellular neurons following salt-loading. The micrographs, from control (left column) and salt-loaded (right column) rats, were digitally enhanced to show in situ hybridization with subtype-specific riboprobes for Na channel subunits α-I, α-II, α-III and Na6. α-I and α-III mRNA are not detectable, and low levels of α-II and Na6 mRNA are present in the control supraoptic nucleus (no asterisks). Expression of the α-II and Na6 transcripts is up-regulated following salt-loading (asterisks). Optical densities from unenhanced micrographs (graph) provide a quantitative measure of mRNA levels and show a significant increase in α-II and Na6 mRNA following salt-loading. *P<0.01. Bar=100 μm. Modified from Tanaka et al. [43].
these cells should produce two sodium currents. Studies on other neuronal cell types, such as Purkinje cells, indicated that the Na\textsubscript{6} sodium channel can produce a slow or persistent TTX-sensitive sodium current [37,48]. The \(\alpha\)-II channel, in contrast, has been shown to produce a fast transient current [3,35]. Using patch clamp methods, Tanaka et al. [43] showed that, consistent with the expression of these two types of sodium channels, two distinct sodium currents are present in control magnocellular neurons: (i) a fast transient sodium current which would be expected to contribute to the rapid depolarizing upstroke of the action potential; and (ii) a persistent 'threshold' sodium current which activates closer to resting potential. Both currents were increased in magnocellular neurons from salt-loaded rats, but to different degrees. There was an increase of 20% in the density of the fast transient sodium current, while the density of the threshold current was approximately 60% larger in salt-loaded rats (Fig. 4). The disproportional increases in the two currents encoded by these two channels would be expected to lower the threshold for action potential generation. Thus, molecular changes at the sodium channel gene transcription level produce functional changes in electrogenesis in these neurons as they move from a quiescent to a bursting state.

4. Sodium channel gene expression: down-regulation in axotomized neurons

The dynamic nature of sodium channel gene expression in normal neurons raises the question: is sodium channel gene expression altered in injured neurons? A hint that sodium channel expression might change in injured neurons was provided by early microelectrode observations of altered somato-dendritic excitability in axotomized motor neurons [22,30]. Early pharmacological and ion-substitution experiments suggested that this involves a sodium conductance [41,44], but these studies did not examine the roles of sodium channels in this process, or the expression of sodium channel genes.

More recent studies on this question have used molecular biological methods to study sodium channel gene expression together with patch clamp methods to study the currents produced by these channels. Dorsal root ganglion (DRG) neurons have been especially well-studied in this respect. These studies have shown that, in these cells, expression of some sodium channel genes is up-regulated, and expression of others is down-regulated, following axonal transection (Fig. 5).

As illustrated in Fig. 5 (middle and bottom rows),

---

![Fig. 4. Two distinct sodium currents in supraoptic neurons show differential increases following salt-loading.](image)

(A) Recordings from representative supraoptic neurons acutely isolated from control (left panel) or salt-loaded (right panel) rats, showing the fast transient sodium current. The currents were elicited by 40 ms test pulses to various potentials from \(-60\) to \(30\) mV. Cells were held at \(-100\) mV. (B) Normalized activation (circles) and steady-state inactivation (squares) curves show only small differences between control (filled symbols) and salt-loaded (open symbols) neurons. Error bars indicate s.e. (C) Ramp currents are elicited by slow ramp-like depolarizations (extending from \(-100\) to \(+40\) mV over 600 ms) in supraoptic neurons. The left panel shows that TTX (250 nM) blocks the ramp current in salt-loaded supraoptic neurons, demonstrating that this current is produced by sodium channels. The right panel shows the TTX-sensitive ramp currents in representative control and salt-loaded supraoptic neurons. Note the larger amplitude in salt-loaded neurons. (D) Fast transient and ramp current densities (estimated by dividing the maximum currents by the cell capacitance) are both increased following salt-loading; however, the increase is proportionately greater for the ramp currents. Error bars indicate s.e., *indicates \(P<0.005\). From Tanaka et al. [43].
Fig. 5. Sodium channel expression can change strikingly in neurons following injury. mRNA for sodium channels α-III (top) is up-regulated, and mRNA for SNS (middle) and NaN (bottom) are down-regulated, in DRG neurons following transection of their axons within the sciatic nerve. The in situ hybridizations (right side) show α-III, SNS, and NaN mRNA in control DRG, and at 5–7 days post-axotomy. RT-PCR (left side) shows products of co-amplification of α-III and SNS together with β-actin transcripts in control (C) and axotomized (A) DRG (days post-axotomy indicated above gels), with computer-enhanced images of amplification products shown below gels. Co-amplification of NaN (392 bp) and GAPDH (6076 bp) shows decreased expression of NaN mRNA at 7 days post-axotomy (lanes 2, 4, 6) compared to controls (lanes 1, 3, 5). Top, middle panels modified from Dib-Hajj et al [15]. Bottom modified from Dib-Hajj et al [17].

Following transection of the peripheral axons of DRG neurons (by ligation within the sciatic nerve), expression of the SNS [15] and NaN [17] sodium channels is down-regulated at the transcriptional level. The reductions in SNS and NaN mRNA are accompanied by reduced levels of SNS and NaN sodium channel protein [42]. This change in sodium channel gene expression is not a response to axotomy per se. In contrast to transection of the peripheral axons of DRG neurons, which triggers a down-regulation of SNS and NaN, transection of the centrally-directed branches of DRG neurons (within dorsal roots) does not alter the expression of these channels [42]. One explanation of this might be that down-regulation of SNS and NaN is due to loss of access to a peripheral pool of trophic factors. Consistent with this hypothesis, nerve growth factor (NGF) up-regulates SNS expression [4], while glial derived neurotrophic factor (GDNF) up-regulates NaN and SNS expression [26] in DRG neurons in vitro. Similarly, in vivo delivery of NGF [18] and GDNF (Cummins, Black, Dib-Hajj and Waxman, unpublished results) to axotomized DRG neurons can rescue the expression of SNS and NaN in these cells.

In parallel with the down-regulation of SNS and NaN mRNA and protein in DRG neurons following peripheral axotomy, the slowly-inactivating and persistent TTX-resistant sodium currents produced by these channels are reduced [11,42] (Fig. 6A, B). These electrophysiological changes persist for at least 60 days post-axotomy [11]. As noted above, the persistent current produced by NaN appears to contribute a depolarizing influence to resting potential (Herzog, Cummins and Waxman, unpublished results). Cummins and Waxman [11] suggested that down-regulation of NaN channels shifts membrane resting potential in axotomized DRG neurons in a hyperpolarizing direction, reducing resting inactivation of fast sodium channels, and thereby producing hyperexcitability which can contribute to pain and paraesthesia [11]. Consistent with a contribution of these changes to neuropathic pain, Dib-Hajj et al. [21] observed down-regulation in SNS and NaN channels and their currents in DRG neurons in the chronic constriction injury model of neuropathic pain.

5. Sodium channel gene expression: up-regulation in axotomized neurons

As described above, early electrophysiological studies [22,30] demonstrated that, following axonal transection,
there are changes in excitability of motor neurons. Subsequent studies established that this abnormal excitability is sodium-dependent [41,44]. Abnormal accumulations of sodium channels were visualized in early immunohistochemical studies at injured axonal tips within experimental neuromas, but these studies used generic antibodies that do not discriminate between different types of sodium channels [14,24,25]. Delineation of the mechanism underlying aberrant sodium channel expression, and determination of whether abnormal types of sodium channels are produced in injured neurons, however, required the study of sodium channel gene expression. The first studies were carried out by Waxman et al. [50] and Dib-Hajj et al. [15] who used in situ hybridization and RT-PCR to demonstrate that there is a an up-regulation of the previously silent α-III sodium channel gene in adult DRG neurons following transection of their axons within the sciatic nerve (Fig. 5, top). Iwahashi et al. [28] observed a similar up-regulation of the α-III sodium channel gene following axotomy of adult facial motor neurons. These changes are not due to a global increase in channel protein synthesis because, as described above, other sodium channel genes are downregulated in these axotomized neurons. Black et al. [5] have demonstrated that increased synthesis of type III sodium channel protein accompanies the up-regulation of type III mRNA in axotomized DRG neurons.

In tandem with activation of the type III sodium channel gene in axotomized DRG neurons, there is a switch in the sodium currents that are expressed by these cells (Fig. 7A, B). Patch clamp studies have demonstrated the emergence, in axotomized DRG neurons, of a TTX-sensitive sodium current that recovers (reprimes) rapidly from inactivation [11]. Recovery from inactivation is accelerated by as much as four-fold in the axotomized neurons. Recordings from many neurons indicate that the change to a rapidly-repriming current is a discrete, step-like transition, and not a gradual shift as might be expected if a population of pre-existing channels had been modulated. Thus axotomy appears to trigger a transition from expression of a slowly repriming TTX-sensitive sodium channel, to expression of a rapidly-repriming sodium channel. It has been suggested that this contributes to hyperexcitability of these injured neurons [11].

It has been proposed that type III channels produce the rapidly-repriming TTX-sensitive current in axotomized neurons [11]. Several observations support this conclusion: First, rapidly-repriming TTX-sensitive sodium current and expression of type III sodium channels show parallel patterns of up-regulation following the transection of peripherally-directed (sciatic nerve) axons of DRG neurons but not following transection of the centrally-directed (dorsal root) axons of these cells [5]. Second, type III sodium channels display rapid repriming when expressed in mammalian expression systems (HEK293 cells; Cummins, Dib-Hajj, and Waxman, unpublished observations). Third, abnormal accumulations of type III sodium channel proteins can be detected by immunocytochemistry with subtype-specific antibodies, close to the tips of injured axons within experimental neuromas [5], a site where abnormal hyperexcitability has been demonstrated [8,33,39]. Axotomized DRG neurons provide an example of up-regulation of a sodium channel gene following axonal injury, and this change in gene expression appears to be maladaptive, contributing to the development of hyperexcitability.

6. Sodium channel gene expression: up-regulation in demyelinated neurons

While multiple sclerosis has classically been viewed as a ‘demyelinating’ disease in which myelin is the primary target, there is recent evidence for abnormal expression of sodium channel genes in several experimental models of demyelinating diseases, and in multiple sclerosis. Studies in our laboratory have demonstrated abnormal up-regulation of Sensory Neuron Specific sodium channel SNS within cerebellar Purkinje cells in these disorders [6,7]. SNS is normally expressed in a highly specific manner within primary sensory neurons within DRG and trigeminal ganglion, and is not normally expressed within the brain [1,38]. This sensory neuron specific TTX-resistant sodium channel exhibits a depolarized voltage-dependence and slow activation and inactivation kinetics [1,38], together with a more rapid recovery from inactivation [16,23] than traditional ‘fast’, TTX-sensitive sodium chan-
nels. Because of the distinct electrophysiological characteristics of SNS-type channels, the presence of these channels can alter the firing properties of neurons [2,40].

We first studied the taiep rat, a mutant model in which myelin is initially formed normally, but then lost as a result of an oligodendrocyte abnormality, using in situ hybridization and immunocytochemistry with subtype-specific sodium channel antibodies. These studies demonstrated the abnormal expression of SNS sodium channel mRNA and protein in Purkinje cells after they have lost myelin (but not prior to loss of myelin) in this model of demyelination [7]. More recently, we have demonstrated that SNS mRNA and protein, which are not present within the cerebellum of control mice, are expressed within cerebellar Purkinje cells in a mouse model of multiple sclerosis, chronic relapsing experimental allergic encephalomyelitis [6]. We have also demonstrated the expression of SNS mRNA (Fig. 8a) and protein (Fig. 8d) within cerebellar Purkinje cells from tissue obtained post-mortem from MS patients, but not in controls with no neurological disease (Fig. 8e) [6]. These new results provide evidence for dysregulation of sodium channel gene expression within Purkinje neurons in at least three ‘demyelinating’ disorders including two experimental models and one human disease, multiple sclerosis.

As illustrated by the studies on DRG neurons summarized above, altered sodium channel gene expression can result in significant changes in neuronal physiological properties. Normal cerebellar functioning depends on the precise timing of impulses. Non-pathological Purkinje cells produce multiple sodium currents which interact to determine the firing properties of these cells [32,36]. Mutations which alter the voltage-dependence of the sodium channels that are expressed in Purkinje cells produce abnormal patterns of impulse activity in these cells, and these physiological changes are associated with cerebellar ataxia [29,37]. We have hypothesized that, in a similar manner, the mis-expression of SNS sodium channels within Purkinje cells in demyelinating diseases may alter the firing patterns of these neurons, thereby contributing to clinical abnormalities such as ataxia in these disorders [6]. The hypothesis that multiple sclerosis and related diseases include an ‘acquired channelopathy’ has important functional and clinical implications.

7. Dynamic aspects of sodium channel expression: conclusions and prospects

As outlined above, it is now clear that sodium channels represent a diverse family of related proteins, encoded by different genes, and with different physiological properties and functions. It has also become clear that the activation of sodium channel genes is not a fixed, static process. On the contrary, the activation of sodium channel genes is highly dynamic, changing in tandem with state-transitions in normal neurons, and subject to dysregulation in a variety of disease states.

At first glance, the multiplicity of sodium channel genes and the changing nature of their activation may appear to make sodium channel expression a difficult subject for research. On the other hand, however, the complexity of sodium channel expression may present experimental and therapeutic opportunities. As selective sodium channel modulating drugs are developed, it may become possible to alter the activities of specific types of sodium channels, without changing the activities of others. This will undoubtedly help us to understand the specific roles of various channel subtypes in shaping neuronal behavior. More importantly, it may open up new avenues for the treatment of neurological disorders in which there is hypo- or hyperexcitability, or altered impulse trafficking.

Fig. 8. Sensory Neuron Specific sodium channel SNS is not present within the normal cerebellum, but is expressed in cerebellar Purkinje cells within brains obtained at post-mortem from MS patients [6]. Panels on left (a–c) show in situ hybridization with SNS-specific risoprobes, and illustrate the absence of SNS mRNA in control cerebellum (b) and its presence in Purkinje cells in post mortem tissue from patients with MS (a). No signal present is following hybridization with sense riboprobe (c). Panels on right show immunostaining with antibody directed against SNS, and illustrate absence of SNS protein in control cerebellum (c; arrowhead indicates Purkinje cell) and its presence in MS (d). a: ×120, inset ×280; b, c: ×165; d, e: ×175. Modified from Black et al. [6].
Acknowledgements

Research described in this article has been supported, in part, by grants from the National Multiple Sclerosis Society, and from the Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs. We also thank the Eastern Paralyzed Veterans Association and the Paralyzed Veterans of America for support.

References

[36] J.G. Waxman, Sensory neuron specific sodium channel SNS is abnormal-...


[48] S.G. Waxman, J.D. Kocsis, J.A. Black, Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is re-expressed following axotomy, J. Neurophysiol. 72 (1994) 466–471.