J. Membrane Biol. 125, 193–205 (1992)

The Journal of
Membrane Biology

Topical Review

Tissue-Specific Expression of the Voltage-Sensitive Sodium Channel

Gail Mandel
Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794

Introduction

The voltage-sensitive ion channels are a class of trans-membrane proteins that lie at the heart of cellular excitability, the ability to transmit information via electrical signals. In response to changes in membrane potential, these molecules mediate rapid ion flux through a highly selective pore in the membrane. If the channel density is high enough a large, regenerative depolarization, called the action potential, results.

The voltage-sensitive sodium channel is the ion channel most often responsible for generating the action potential. Although sodium-based action potentials in different excitable tissues look very similar (Hille, 1984) recent electrophysiological studies indicate that sodium channels in different cells differ with respect to their functional properties. In accordance with this observation, many sodium channels with distinct primary structures have now been identified. This review describes the impact of molecular approaches on questions of sodium channel diversity and regulation. Specifically, recent experiments revealing both tissue-specific and temporal regulation of the sodium channel alpha subunits are summarized.

Sodium Channels Exhibit Diversity in Properties

Although action potentials are "all-or-none" signalling events, a single excitable cell can transduce chemical or electrical information by regulating the duration and frequency of its action potentials. As the basis of the action potential in many different cells, sodium channels with different functional properties may be important in this type of signalling. For example, a sodium channel that turns on and off rapidly can drive the membrane potential up and down rapidly, permitting high firing frequencies. By contrast, a more slowly-inactivating sodium channel can contribute to a broadening of the action potential and, in conjunction with potassium channels, can limit the maximum firing frequency of a neuron. An example of this type of channel diversity is seen in sensory neurons of the dorsal root ganglion (DRG). These neurons, which transmit a variety of sensory stimuli to the spinal nerves, must be capable of following stimulation frequencies varying between two and several hundred hertz (Harper & Lawson, 1985). This ability has been achieved, at least in part, by the use of two types of neurons. One group (A-alpha and A-beta) produce action potentials that can follow, because of their short durations, stimulation frequencies of several hundred hertz. The other group (c-type neurons) can only follow very low frequencies (under five hertz) and exhibit broader action potentials of increased durations. Along with potassium channels, fast and slow-inactivating sodium channels have been implicated in mediating the "short" and "long" duration DRG action potentials, respectively. Interestingly, these different channels are associated with morphologically distinct cell types in the DRG (Campbell, 1989).

Functionally distinct sodium channels have been described in a variety of neuronal cell types (Llinas & Sugimori, 1980; Kostyuk, 1981; Bossu & Feltz, 1984; Gilly & Armstrong, 1984; French & Gage, 1985; Ikeda, Schofield & Weight, 1986; Jones, 1987; Alonso & Llinas, 1989; Gilly & Brismar, 1989) and in skeletal muscle (Gonoi, Sherman & Catterall, 1985; Weiss & Horn, 1986). The kinetics of sodium currents in glia and neurons can also be distinguished (Barres, Chun & Corey, 1989). In contrast to nerve and skeletal muscle,

Key Words sodium channels · regulation · development · excitability · ion channels · nervous system

functional diversity of sodium channels in cardiac muscle is not well documented.

The functional distinctions among sodium channels, although not always dramatic, suggest the presence of channel proteins with different structures. This suggestion is reinforced by pharmacological and immunochemical studies of sodium channels. For example, antibodies raised against the sodium channels in brain and skeletal muscle often do not cross-react (Wollner & Catterall, 1985; Casadei & Barchi, 1987). Similarly, several neurotoxins have been identified which block sodium channels differentially in different tissues (for reviews *see* Catterall, 1986; Trimmer & Agnew, 1989). The extent of block depends upon the affinity of the toxin for different sodium channels. For example, with the puffer fish toxin tetrodotoxin (TTX), three pharmacologically distinct sodium channels in mammals have been identified. One class of sodium channels, present in brain and adult skeletal muscle, is blocked by application of TTX in the nanomolar range (TTX-sensitive). Sodium channels in developing or denervated skeletal muscle, and in cardiac muscle, on the other hand, are only blocked by higher TTX concentrations, in the 1–10 μM range (TTX-insensitive; Harris & Thesleff, 1971; Pappone, 1980; Brown, Lee & Powell, 1981). The TTX-insensitive sodium channels in the DRG neurons described above are blocked by TTX concentrations in the 100 μM range, representing yet a third pharmacological class of sodium channels (Jones, 1987; Campbell, 1991). In neurons and skeletal muscle, the TTX-sensitive and insensitive sodium channels exhibit different gating behavior and kinetics (Bossu & Feltz, 1984; Jones, 1987; Ikeda et al., 1986; Gonoi et al., 1985; Weiss & Horn, 1986; Campbell, 1991). Because of the different recording and cell culture conditions it is difficult to say whether the more slowly-inactivating sodium channel in nerve is equivalent to the functionally similar sodium channel in skeletal muscle. However, on the basis of similarities in functional properties, Moczydlowski et al. (1986) predicted that the TTX-insensitive sodium channels in denervated skeletal muscle and in cardiac muscle would be encoded by the same gene.

This leads to the following problem: Although clearly establishing the presence of multiple sodium channel types, none of the above experiments indicate whether differences in the primary structures, differences in post-translational modifications of the alpha subunit, or contributions of other subunits were responsible for the observed differences in channel properties. Molecular cloning and expression of individual complementary DNAs (cDNAs) provided the first direct evidence that at least some of the diversity in sodium channels was due to the expression of distinct alpha subunit genes.

The Sodium Channel Is Encoded by a Multi-Gene Family

Voltage-sensitive sodium channels have been purified from many tissues and exist either as a single protein or a complex of proteins (reviewed in Trimmer & Agnew, 1989; Catterall, 1986; Barchi, 1988). Irrespective of the source, all of the channels contain a large, highly glycosylated alpha subunit of approximately 260 kDa. The eel electroplax and chick cardiac nervous system sodium channels consist of only an alpha subunit (Trimmer & Agnew, 1989; Lombert & Lazdunski, 1984). In contrast, mammalian brain and skeletal muscle contain, in addition to the alpha subunit, one or two smaller beta subunits of 37 and 39 kDa (Catterall, 1986; Barchi, 1988). The alpha subunits expressed alone in heterologous cells are voltage-dependent and sodium-selective ion channels. However, the kinetics of the expressed sodium channels are often different than that of native channels (Krafte et al., 1988; Trimmer et al., 1989); normal kinetics are restored by the addition of small molecular size mRNA, suggesting the involvement of accessory proteins (Auld et al., 1988). The physiological importance of the beta subunits, and their primary structures, have not yet been elucidated.

Molecular studies of the eel *Electricus electrophorus* sodium channel revealed the first primary structure of a voltage-sensitive ion channel (Noda et al., 1984). Using an antibody raised against the purified eel sodium channel, partial cDNA clones were isolated by immunoscreening a cDNA expression library made from electroplax, an enriched source of sodium channel mRNA. The putative immunopositive cDNA clones were authenticated by hybridization to oligonucleotides based on partial amino acid sequence of the purified channel proteins. The full-length cDNA encoded a protein of 1820 amino acids containing four internal regions of homology (approx. 50%), each postulated to act as a subunit in pore formation (Fig. 1).

The overall functional similarities among sodium channels in different animal species suggested that a cDNA encoding the eel alpha subunit would be a suitable probe for identifying the homologous subunit in rat. Using this approach, Noda and his colleagues cloned cDNAs encoding not one, but two distinct alpha subunits, termed types I and II, in rat brain (Noda et al., 1986*a*). The sequence of a full-length cDNA coding for a related channel, type III, was published more recently (Kayano et al., 1988).

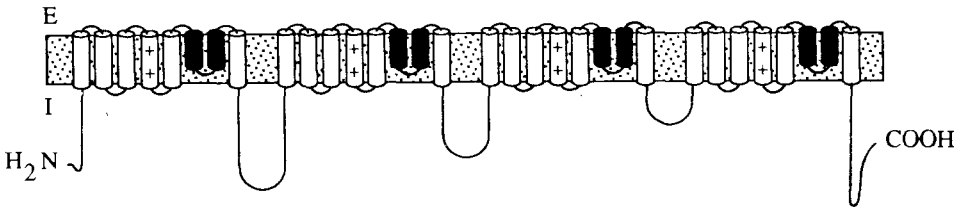

Fig. 1. Proposed secondary structure and membrane topology of the sodium channel alpha subunit (after Noda et al., 1986*a*; Catterall, 1988; Guy, 1988; Hartmann et al., 1991). Each repeated domain consists of at least six trans-membrane peptides. The positive charges indicate proposed voltage-sensing regions, and the filled cylinders indicate pore-lining regions. The amino (H_2N) and carboxy (COOH) termini are assumed to be on the inside (*I*) of the plasma membrane.

The primary structures of all three mammalian channels are approximately 87% identical at the amino acid level and roughly 60% identical to the eel alpha subunit. Auld et al. (1988) identified a novel sodium channel mRNA from a brain cDNA library, termed type IIA, which differs from type II mRNA by an exon containing six different amino acids, and by unique 5′ and 3′ untranslated regions (Sarao et al., 1991). Proof that the mammalian cDNAs, and by analogy the eel cDNA, encoded functional sodium channels was obtained by expression in *Xenopus* oocytes. The brain type II, IIA and type III cDNAs direct the synthesis of functional, TTX-sensitive sodium channels in *Xenopus* oocytes when expressed from synthetic RNAs (Noda et al., 1986*b*; Suzuki et al., 1988; Moorman et al., 1990); the type I channel was expressed too poorly for detailed characterization. Thus, the isolated cDNAs represent distinct primary structures of the rat brain voltage-dependent sodium channel. While smaller subunits may influence the kinetic properties of sodium channels, they are apparently not essential for basic functioning of the channel.

Because the nucleotide differences in the cDNAs of the mammalian type I, II, and III sodium channels occur all along their length, and because distinct gene promoters have been mapped for the type I (G. Mandel, *unpublished*) and type II sodium channels (Maue et al., 1990), the three "brain" cDNAs likely represent the transcripts of different genes. The existence of a multi-gene family coding for the sodium channel is not confined to vertebrates. Two distinct sodium channel genes have been identified and characterized in *Drosophila melanogaster* using different approaches. One approach used a probe encoding a portion of the rat brain type cDNA to screen a *Drosophila* genomic library at low stringency (Salkoff et al., 1987; Ramaswami & Tanouye, 1989). The deduced amino acid sequence of the cloned fly cDNAs revealed extensive homology with the vertebrate alpha subunits. An alternative approach was to clone DNA from the *para* (for paralyzed) locus of *Drosophila*, a locus known to be associated with an alteration of sodium channel function (Loughney, Kreber & Ganetzky, 1989). There are as yet no expression studies or genetic "rescue" experiments proving that the fly genes encode functional sodium channels. However, analysis of the cloned *para* cDNAs again predicted a structure similar (60% identical) to the vertebrate sodium channels. Interestingly, the two fly sodium channel proteins are no more closely related to each other than they are to the vertebrate channels, suggesting that the gene duplication event leading to the two channels predates the evolutionary origin of *Drosophila* (Loughney et al., 1989).

Sodium Channel Genes Are Expressed in a Tissue-Specific Fashion

Cloning of the "brain" type sodium channel cDNAs raised the question of whether excitability in other tissues was due to expression of these same genes. It was shown several years ago, using Northern blot assays, that brain type II sodium channel complementary RNA (cRNA) cross-hybridized to an approximately 9.5 kilobase (kb) mRNA in skeletal muscle (Cooperman et al., 1987). However, skeletal muscle failed RNase protection tests using probes specific for the brain type I and type II sodium channels (Cooperman et al., 1987). This latter result suggested the presence of a related but distinct sodium channel mRNA in skeletal muscle. Similarly, the major sodium channel mRNA species detected in cardiac muscle with a brain type I cRNA probe appeared to be somewhat smaller (approximately 7.5 kb) than the predominant sodium channel mRNA in brain and skeletal muscle, suggesting the possibility of cardiac-specific sodium channels (Sills et al., 1989).

Immunochemical studies using alpha subunit-specific antisera also suggested tissue-specific expression of sodium channel genes. Anti-peptide antibodies raised against unique regions of the predicted brain type I and type II alpha subunits reacted with

brain and spinal cord tissue, but did not cross-react with sodium channel epitopes in skeletal or cardiac muscle, or in peripheral neurons (Gordon et al., 1987). These data, in conjunction with the above hybridization studies, provided an impetus for the isolation and characterization of the three new muscle sodium channel cDNAs described below.

In rapid succession, two distinct full-length cDNAs, termed Mu1 (or SkM1) and SkM2, were isolated from rat skeletal muscle (Rogart et al., 1989; Trimmer et al., 1989; Kallen et al., 1990). SkM1 was identified by low-stringency screening of a rat denervated skeletal muscle cDNA library with probes specifying portions of the rat brain type I and type II channels (Trimmer et al., 1989). SkM1 is approximately 76% identical to the brain sodium channels at the amino acid level and encodes a functional, TTX-sensitive alpha subunit when expressed in *Xenopus* oocytes. The full-length SkM2 predicted protein is equally related (approximately 75% amino acid identity) to SkM1 and the rat brain channels (Rogart et al., 1989; Kallen et al., 1990). Despite the similarities in predicted primary structures, the mRNAs coding for SkM1 and SkM2 are regulated quite differently (*see below*).

SkM1 does not constitute a significant component of the sodium channel mRNAs expressed in either brain or cardiac muscle (Trimmer et al., 1989, 1990). By contrast, a full-length alpha subunit cDNA isolated from a rat cardiac cDNA library, called rat heart 1 (Rogart et al., 1989), is apparently identical in the coding and noncoding sequences to SkM2. This result indicates that rat heart 1 and SkM2 likely represent products of the same gene. Because SkM2 is present in cardiac muscle, in denervated skeletal muscle, and in the L6 skeletal muscle cell line (Kallen et al., 1990), three cell types which contain predominantly TTX-insensitive sodium channels, it was tempting to speculate that SkM2 encoded a TTX-insensitive sodium channel. In fact, *Xenopus* oocytes microinjected with synthetic mRNA derived from cloned SkM2 (and rat heart 1) resulted in expression of a TTX-insensitive sodium channel (Cribbs et al., 1990; White et al., 1991). These studies raise the interesting, but still unanswered, question of whether TTX insensitivity in different tissues is always due to expression of the same gene.

In addition to SkM2, a partial cDNA clone encoding 130 amino acids of a novel sodium channel-like protein has been isolated from a cardiac library using a rat brain type II cDNA probe (Sills et al., 1989). The transcript detected by the cardiac cDNA probe is approximately 7.5 kb in size, and is present in cardiac muscle but not brain, innervated or denervated skeletal muscle. Although the cloned cardiac cDNA does not encode a full-length protein, the high amino acid identities to other functional sodium channels suggests that it is also a member of the family. However, authentication of this cDNA clone awaits further molecular and functional characterization.

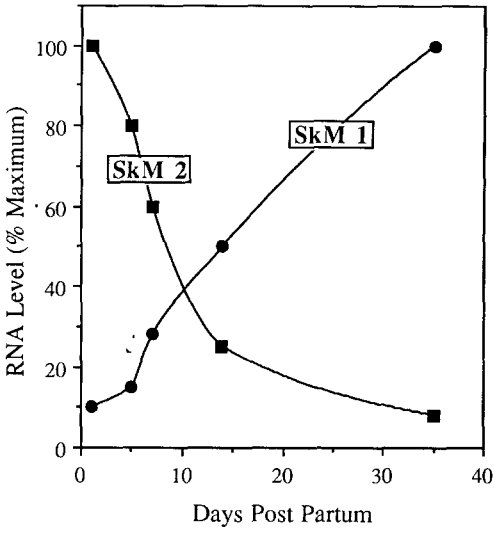

Fig. 2. Differential temporal regulation of sodium channel mRNAs in skeletal muscle (after Kallen et al., 1990).

Sodium Channel Genes Are Differentially Regulated during Development in Skeletal Muscle and Nerve

Regulation in Skeletal Muscle

During skeletal muscle development there is a switch in the TTX sensitivity of its sodium channels (Harris & Marshall, 1973; Sherman & Catterall, 1982; Frelin, Vigne & Lazdunski, 1983). Embryonic muscle is almost exclusively TTX insensitive, due to a preponderance of TTX-insensitive sodium channels at this stage. During development, there is a switch in the proportion of TTX-sensitive and insensitive sodium channels such that adult muscle is predominantly TTX sensitive. Denervation causes a return to the embryonic TTX phenotype (Redfern & Thesleff, 1971; Pappone, 1980; Rogart & Regan, 1985). If SkM1 and SkM2 encoded the TTX-sensitive and insensitive sodium channels, respectively, and if the genes were regulated at the transcriptional level, SkM1 and SkM2 mRNAs should exhibit reciprocal patterns of appearance during development. This prediction has been borne out (Fig. 2). Steady-state levels of SkM1 mRNA are barely detectable in

the early embryonic stages, increase steadily after birth, and plateau in the adult. Conversely, expression of SkM2 is restricted to the embryonic stages of development. Although the hybridization data are supportive, at least two other types of experiments would have to be done before concluding that the cloned cDNAs represent the native functional sodium channels. First, immunochemical measurements of the relative amounts of SkM1 and SkM2 alpha subunit protein should be correlated with the mRNA levels. Second, and exhibiting a higher level of difficulty, SkM1 or SkM2 transcripts or proteins should be selectively eliminated in vivo. Recordings from muscle should then reveal an absence of the characteristic TTX-sensitive or insensitive type currents. The "knock out" experiments could theoretically be done using either antisense RNA or antibodies. This type of experiment has not yet been accomplished for any of the cloned sodium channel cDNAs. However, skeletal muscle offers the advantages that the two sodium channels are well characterized and functionally distinct, that microinjection techniques in muscle have been used successfully for other genes (Tanabe et al., 1988), and that the developmental stages when SkM1 and SkM2 are predominant are well separated in time.

The correlation of SkM1 and SkM2 mRNA levels and sodium channel function is not as clear in denervated muscle as it is in developing skeletal muscle. Following denervation, levels of SkM1 and mRNA increase 10-fold (Trimmer et al., 1989, 1990) while levels of SkM2 mRNA increase approximately two orders of magnitude (Rogart et al., 1989; Kallen et al., 1990). The magnitude of increase in expression of the two transcripts is a bit surprising because only approximately 25% of the TTX-sensitive channels are replaced by TTX-insensitive channels in denervated muscle (Pappone, 1980). In the absence of knowledge of the turnover rates of alpha subunit protein following denervation, it is difficult to interpret the biological significance of this regulation of SkM1 and SkM2 transcripts.

Whether or not SkM1 and SkM2 turn out to be the only sodium channel transcripts in skeletal muscle, the results of the developmental and denervation experiments suggest regulation of sodium channel expression by nerve. This situation is very reminiscent of the regulation of another muscle membrane channel, the pentameric acetylcholine receptor (AchR). The AchR exhibits different functional properties during development (Brehm & Henderson, 1988) and some of the subunit mRNAs are dramatically up regulated by denervation. Much of the regulation of the AChR subunits by nerve is controlled at the transcriptional level (Tsay & Schmidt, 1989). It is tempting to speculate that a similar molecular mechanism also underlies certain aspects of regulation of the sodium channel alpha subunits. Experiments determining whether the increases in sodium channel mRNA levels after denervation are transcriptional or post-transcriptional will be a critical first step toward addressing the issue of coregulation of different channel types.

Regulation in the Nervous System

RNA blot hybridization and immunoprecipitation studies both indicate that sodium channel genes are regulated differentially during brain and spinal cord development (Grubman et al., 1988; Beckh et al., 1989; Scheinman et al., 1989). In the studies described here, RNA blot hybridization studies were performed using probes constructed from the divergent 3′ untranslated regions of the brain sodium channel mRNAs (Beckh et al., 1989); there is no cross-reactivity between the type I, II, or III mRNAs with these probes. The immunoprecipitation studies were performed with affinity-purified anti-peptide antibodies generated against divergent amino acids in the type I and type II alpha subunits. Only three amino acids out of 18 were in common between the two immunogenic peptides. The anti-peptide antibodies reacted almost as well with native sodium channels as they did with the peptides, and they were specific for the two alpha subunit types (Gordon et al., 1987).

In rat brain, the mRNAs encoding the three types of sodium channels were shown to be temporally regulated, showing distinct time courses during development (Fig. 3). Levels of type II and type III mRNAs increased significantly between embryonic day 10 and birth coincident with the time of rat neurogenesis. Type II mRNA levels continued to stay high in the adult, whereas type III mRNA levels decreased gradually over the next two weeks to a low level in the adult. Although present, type I mRNA levels were quite low until birth, gradually rising to adult levels over the next two weeks or so. Immunoprecipitation studies revealed the same time course of expression of type I and type II alpha subunit proteins as the corresponding mRNAs (Gordon et al., 1987). The increase in type I and type II sodium channel gene expression during development could underly the increases in TTX binding and current seen during mouse brain development or in developing neuronal cultures (Couraud et al., 1986; Huguenard, Hamill & Prince, 1988).

In rat spinal cord, the time course of detection during development of the individual sodium channel mRNAs was similar to that observed in brain (Fig. 3). Type II mRNA levels deviated slightly,

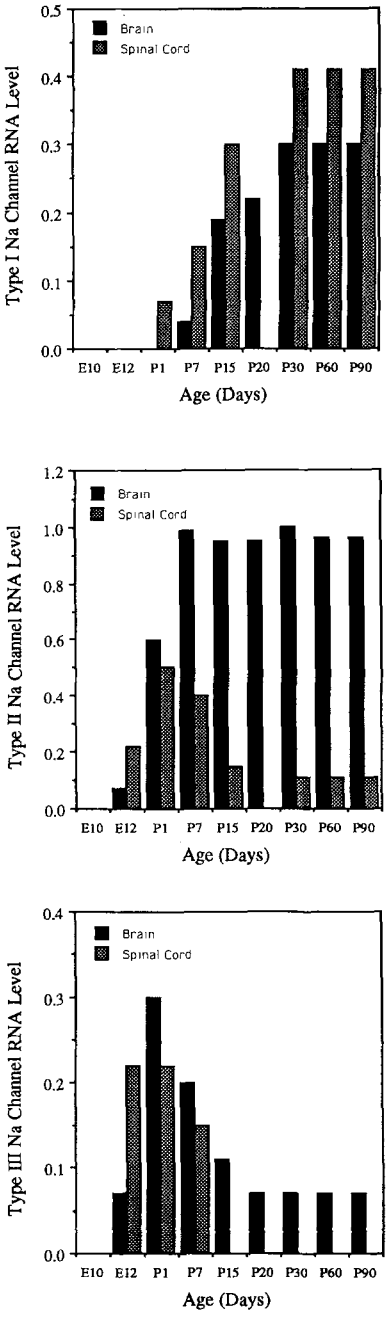

Fig. 3. Differential temporal regulation of sodium channel mRNAs in brain and spinal cord (after Beckh et al., 1989).

peaking around the same age (birth) in brain and spinal cord, but then declining in spinal cord to the relatively low levels characteristic of the adult animal. As above, the developmental profile for the type I and type II alpha subunit proteins detected immunochemically was similar to that observed for the corresponding mRNAs (Gordon et al., 1987).

The observed changes in sodium channel expression lead naturally to speculation about functional correlates in individual neurons. For example, it has been suggested (Beckh et al., 1989; Westenbroek, Merrick & Catterall, 1989) that the type I sodium channel, because of its anatomical location and developmental time course, could be responsible for the persistant sodium current noted in maturing pyramidal and nonpyramidal cells (Huguenard et al., 1988). As yet, there is no direct evidence for this association. Although the types I, II, IIA, and III sodium channels have been expressed in heterologous cells, only the type III, not the type I channel, displays kinetic properties resembling a persistant sodium current (Moorman et al., 1990). Additionally, the immunological data do not reveal whether the channel detected on the cell surface is functional. It is conceivable that during development an alpha subunit appears on the cell surface prior to a post-translational event that confers functionality (*see* for example, Schmidt, Rossie & Catterall, 1985). Thus, in cells expressing both type I and type II sodium channel alpha subunits, perhaps only one of them actually contributes to the sodium currents at the time of detection. These uncertainties notwithstanding, it is likely that the changes in mRNA and subunit levels provide a molecular correlate to at least some of the changes recorded electrophysiologically.

Sodium Channels Are Differentially Localized within the Nervous System

The same cDNA and antibody probes used in the above studies have been used to examine differential sodium channel expression in the central and peripheral nervous systems. Using these reagents, two questions have been asked: (1) are certain sodium channels preferentially located in distinct anatomical regions within the nervous system, and (2) is there spatial segregation of sodium channels in an individual neuron?

A Comparison of Peripheral and Central Nervous Systems

The results from both RNA hybridization and immunochemical studies indicated differential expression among the "brain" sodium channel genes. A logical next step was to ask whether the sodium channels expressed in the central nervous system (CNS) were the same as those expressed in the peripheral nervous system (PNS). The answer to this question appears to be both "yes" and "no." The type II and type III genes, expressed widely in the CNS, are expressed at very low levels in some cells in the

PNS (Beckh, 1990). The type II and III mRNAs were barely detectable, by Northern blot analysis, in dorsal root ganglion (DRG), cranial nerves and sciatic nerves. On the other hand, type I mRNA was present in moderately high amounts in DRG and cranial nerve, but in low levels in sciatic nerve. A comparison of the amount of all three brain mRNAs, relative to total sodium channel mRNA detected with a conserved cDNA probe, suggested the presence of additional, as yet unidentified, sodium channel types in DRG neurons. Consistent with the mRNA studies, immunochemical studies showed that neither type I nor type II sodium channel alpha subunits made up a significant component of the total sodium channels in the superior cervical ganglion or sciatic nerve (Gordon et al., 1987). This result again suggests the presence of novel sodium channel types. No immunochemical data is available for DRG for comparison with the mRNA studies and there is no data yet on the presence of type III alpha subunit protein in the PNS.

As described earlier, a population of neurons in vertebrate DRG has been identified electrophysiologically that contains, in addition to the more conventional channels, a distinct sodium channel type; this DRG channel has a k_D for TTX approximately tenfold higher than the k_D of sodium channels in either skeletal muscle or heart (Jones, 1987; Campbell, 1991). The DRG TTX-insensitive sodium channel may represent one of the distinct gene products suggested by the hybridization and immunochemical data. All of these data taken together suggest that sodium channel excitability in the PNS is due, at least in part, to the expression of genes which are not expressed in the CNS. It will be of interest to determine, using *in situ* hybridization techniques, what the relative levels of different mRNAs are in more purified subpopulations of neurons.

A Comparison of Brain and Spinal Cord

As described earlier, Northern blot analysis indicated that mRNA types I and II accounted for most of the sodium channel mRNA in adult spinal cord and brain, respectively. When different regions in the CNS were analyzed for expression of these genes, interesting variations were revealed (Table 1; data from Beckh et al., 1989). Type II mRNA was the most abundant of the three mRNAs in the rostral regions of the brain (cortex, hippocampus, striatum, midbrain). There were subtle differences in type II mRNA levels among these different anatomical regions. In the more caudal regions of the brain (colliculus, medulla pons) and in spinal cord, there was very little type II mRNA and type I mRNA was the most abundant species. Type III mRNA was the least abundant mRNA in every CNS region examined but, like type II mRNA, was present in higher amounts in rostral regions of the brain and barely detectable in adult spinal cord.

Table 1. Distribution of sodium channel types in the adult rat central nervous system

Region	Protein[a] Ratio of type I: type II	mRNA[b]	
		Type I	Type II
Total brain	0.18	+ +	+ + +
Hippocampus	0.07	+/−	+ + +
Cerebellum	0.09	+/−	+ + +
Cerebral cortex	0.17	+	+ + +
Medulla oblongata	0.98	+ + +	+/−
Spinal cord	2.20	+ + +	+/−

[a] From Gordon et al., 1987.
[b] Adapted from Beckh et al., 1989; the + and − symbols in each column indicate the relative amounts of mRNA for each sodium channel type, estimated from autoradiograms of Northern blots.

Immunoprecipitation studies using anti-peptide antibodies specific for the type I and type II channels revealed the same pattern of expression as the hybridization approach (Table 1; data from Westenbroek et al., 1989). There were only minor variations in type II channel reactivity among several rostral brain regions. In all of these regions, and in total brain, the type II protein was much more abundant than type I. In hippocampus, cerebellum, and cerebral cortex, the ratios of the type I to type II alpha subunit were 0.07, 0.09, and 0.17, respectively (but *see below*). On the other hand, the ratio of type I to type II protein was nearly unitary (0.98) in the medulla oblongata, and higher (2.20) in spinal cord. Thus, the different amounts of type I and type II alpha subunits in brain and spinal cord are accounted for by the differences in proportions of the two mRNAs. The lack of a specific antibody for the type III alpha subunit precluded the ability to evaluate whether this mRNA was translated in the regions studied. The data from the developmental and anatomical studies indicate that all three sodium channels are differentially controlled during development and according to anatomical region.

Sodium Channel Alpha Subunits Are Spatially Segregated in Some Neurons

It has been appreciated for some time that sodium channels are not uniformly distributed in a single cell. For example, electrophysiological studies indicate that, in some neurons, the threshold for action

Table 2. Segregation of sodium channel alpha subunits in neurons

Region	Presence of alpha subunit[a]	
	Type I	Type II
Hippocampus		
Pyramidal cell bodies	+	−
Dentate granule cell bodies	+	−
Unmyelinated axons (mossy fiber pathway)	−	+
Cerebellum		
Purkinje cell bodies	+	−
Unmyelinated axons	−	+
Spinal cord		
Motor neuron cell bodies	+	−
Motor neuron axons	−	−

[a] Adapted from Westenbroek et al., 1989; the + and − symbols indicate the presence or absence of moderate to high immunoreactivity to antibodies for each type of sodium channel.

potentials is lower in cell bodies compared to axons (Coombs, Curtis & Eccles, 1957; Nishi & Koketsu, 1960; Geduldig & Gruener, 1970; Barrett & Crill, 1980). This observation, or similar examples of subcellular functional diversity, could be explained if the sodium channel type located in the axon differed in its voltage sensitivity from the sodium channel type in the cell body (*see* e.g., Gilly & Armstrong, 1984). The increased density of the axonal type of sodium channel could be achieved by preferential expression of its gene and subsequent association with localized cytoskeletal elements. The latter explanation has recently been proposed (Westenbroek et al., 1989) and there is some evidence for its validity. Biochemical measurements have shown that sodium channels are indeed expressed at high densities in the axon hillock (Catterall, 1981; Wollner & Catterall, 1986) and Node of Ranvier (Ellisman & Levinson, 1982; Boudier et al., 1985; Waxman & Ritchie, 1985; Angelides et al., 1988), regions which serve as foci for electrical activity. Additionally, neuronal sodium channels have been shown to be associated with cytoskeletal elements (Angelides et al., 1988; Edelstein, Catterall & Moore 1988; Srinivasan et al., 1988), providing a potential anchoring mechanism for different membrane regions. One key question is whether different alpha subunits are spatially segregated in neurons. Recent immunocytochemical studies, described below, show that the brain type I and type II sodium channel alpha subunits are preferentially located in neuronal cell bodies and axons, respectively (Westenbroek et al., 1989).

The rat hippocampus and cerebellum contain cells showing segregation of type I and type II sodium channels (Table 2). As mentioned above, in hippocampus, the staining pattern is dominated by the type II alpha subunit (Table 1). More sensitive immunocytochemistry showed that the type II sodium channels were localized mainly to the axons of unmyelinated fibers in the mossy fiber pathway or to other fiber layers, giving rise to the dense staining in cross sections of this region. By contrast, type I sodium channels were restricted to cell bodies of the pyramidal and dentate granule neurons. In the cerebellum, type II sodium channels were restricted primarily to unmyelinated fibers in the molecular layer, resulting in the pattern of intense reactivity in cross-sections of this region. Type I sodium channels were localized mainly in Purkinje cell bodies, in the adjacent cell layer, although the intensity of staining in individual cells was not dramatic. In spinal cord, where the type I sodium channel comprised much more of the immunoreactivity than type II, the type I channel was again localized preferentially to cell bodies of spinal motor neurons. The amount of reactivity in an individual cell body was not dense and, in addition, the axons appeared to contain a different type of sodium channel altogether.

Thus, the distinct staining patterns of CNS sodium channel reactivity may be accounted for, at least in part, by a different distribution of the type I and type II alpha subunits in the membranes of individual neurons (summarized in Table 2). If the type I and type II sodium channels were functionally distinct, something that is not yet known, the preferential location of these channels in different parts of the cell could subserve a functional role.

Cell-Specific Expression of Sodium Channel Genes Can Be Ascribed to Activity of Specific Genetic Elements

The localization of different sodium channels to specific regions in the nervous system supports the possibility that cell-specific regulation of this gene family is at the transcriptional level. By analogy with other eukaryotic genes, distinct DNA elements should be present which mediate cell-specific and temporal regulation of individual sodium channel genes. Although these DNA regulatory elements can be anywhere in the gene, they are often located within the 5′ flanking region, upstream of the start site of the mRNA. Using the tools of molecular genetics, unique DNA elements specifying neural-specific expression have recently been identified in the type II sodium channel gene. Some of these experiments are summarized below.

Studies of sodium channel gene regulation have been facilitated by the use of well-characterized cell lines. In particular, rat pheochromocytoma (PC12)

cells, a popular cell model for neuronal differentiation (Green & Tischler, 1976) have been very useful. These cells acquire phenotypic characteristics similar to a sympathetic neuron when treated in culture with nerve growth factor (NGF; reviewed in Halegoua, Armstrong & Kremer, 1991). In addition to extending neurites and initiating synthesis of certain neurotransmitters, NGF-treated PC12 cells acquire the ability to generate sodium-based action potentials (Dichter, Tischler & Greene, 1977). This ability is conferred by an increase in the density of functional sodium channels in the membranes of the NGF-treated cells (Rudy et al., 1987; Mandel et al., 1988; O'Lague & Huttner, 1980). Northern blot analysis revealed that undifferentiated PC12 cells contained a basal level of sodium channel mRNA which increased coincident with the increase in channel activity observed after treatment with NGF (Mandel et al., 1988).

RNase protection analysis of PC12 mRNAs demonstrated that the effect of NGF on sodium channel gene expression was selective. A probe containing 5′ untranslated sequences common to the type II and IIA mRNAs, but divergent from all others, indicated that this mRNA was present at low basal levels in the undifferentiated PC12 cells. In parallel with the increase in density of functional sodium channels after NGF treatment, was an increase in the level of type II/IIA mRNAs; type I mRNA was not detected in either the undifferentiated or differentiated PC12 cells (Mandel et al., 1988). No probes specific for the type III gene were available at the time of this study. The PC12 data indicated that in a single population of cells, sodium channel mRNAs could be regulated differentially in response to a specific growth factor. Unfortunately, no published work bears on the question of whether type II sodium channel alpha subunits are present on the surface of NGF-treated cells. Therefore, the physiological importance of changes in type II/IIA mRNA levels awaits further characterization.

Importantly, the hybridization data (Mandel et al., 1988) indicated that undifferentiated PC12 cells, which express basal levels of type II/IIA mRNA, would be a good system for examining the molecular basis of cell-specific expression of the type II gene. It was hypothesized, by analogy with other eukaryotic tissue-specific genes, that elements required for neural-specificity of the type II sodium channel gene would be present in the 5′ flanking region of the gene. To test this hypothesis, 1154 base pairs (bp) of 5′ flanking sequence, including the promoter, were cloned and characterized for biological activity. Chimeric genes consisting of the type II 5′ flanking sequence fused to the bacterial reporter gene, chloramphenicol acetyltransferase (CAT), were introduced into several different mammalian cell lines: PC12 cells, a neuronal-like cell line, L6 cells, a skeletal muscle cell line, and 3T3 cells, a fibroblast cell line. Expression of the endogenous type II gene, as determined by RNase protection analysis, would predict that the cloned type II 5′ flanking region should direct CAT expression in PC12 cells, but not in the L6 or 3T3 cells. This prediction was borne out in the transient expression assays (Fig. 4). Reporter activity, indicative of transcription directed by sodium channel regulatory elements, was high only in PC12 cells. Very low levels of CAT activity were detected in the nonexcitable 3T3 fibroblast cell line. A viral promoter, which is not expressed in a cell-specific fashion, directs CAT expression in both the neuronal and nonneuronal cell lines (Fig. 4).

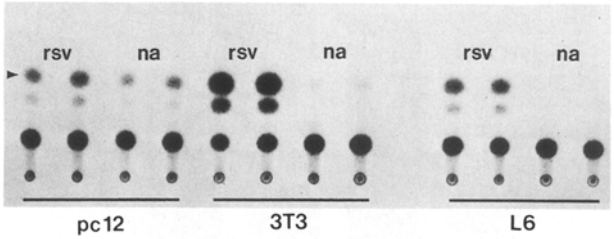

Fig. 4. Cell-specific expression of a type II sodium channel-CAT fusion gene. Autoradiogram of a CAT assay showing thin layer chromatography fractionation of acetylated forms of chloramphenicol (arrow head). Lysates for the assay were from separate dishes of cells transiently transfected with a rous sarcoma virus - CAT fusion gene (*rsv*) or a sodium channel-CAT (*na*) fusion gene.

The results from transient expression assays of the rat L6 skeletal muscle cell line were particularly interesting. L6 cells expressed very low levels of sodium channel CAT activity despite their high density of functional sodium channels, in keeping with the observation that muscle cells express the SkM2 sodium channel gene, but not the brain type II gene (Trimmer et al., 1989, 1990; Kallen et al., 1990). The transfection data suggest two levels of regulation of sodium channel expression. The first level is a genetic on-off switch, which is flipped on in excitable cells (PC12, L6) or off in inexcitable cells (3T3). In the "off" position, none of the sodium channel genes are expressed. In the "on" position, a second level of regulation comes into play, controlling which member of the sodium channel gene family should be expressed in a specific excitable cell type.

To begin to locate the genetic elements mediating such regulation, a deletional analysis was done of the type II 5′ flanking region in PC12 and L6 cells (Maue et al., 1990). Sequential removal of sequences from the 5′ end of the flanking sequence resulted in very little change in the CAT expression in PC12

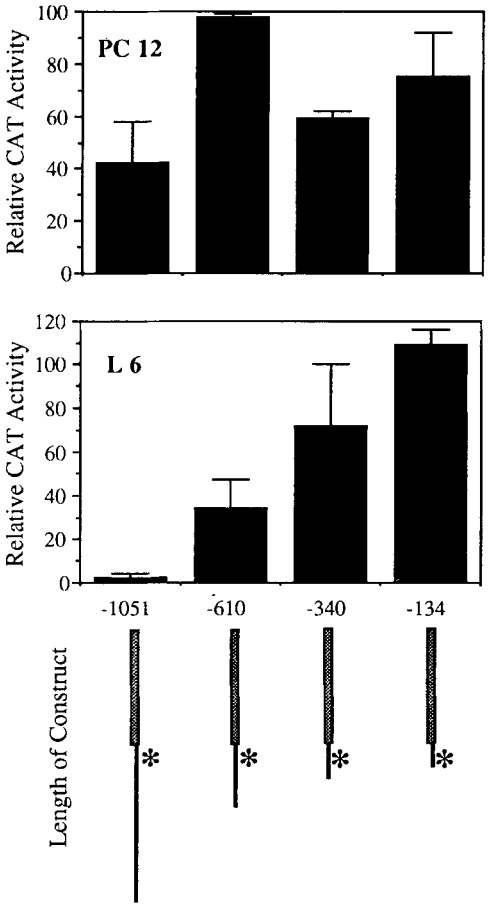

Fig. 5. Deletional analysis of the type II 5′ flanking region showing regulation by negatively-acting elements (after Maue et al., 1990). Asterisks indicate transcriptional start sites in the type II gene. The length of the constructs (−1051, −610, −340, −134) are relative to the start sites.

cells. Postively-acting elements responsible for high-level fusion gene expression in PC12 cells were present in the first 250 bp upstream from the start sites of transcription (Fig. 5; data from Maue et al., 1990). Surprisingly, the same deletion mutations resulted in extremely high levels of CAT activity, driven by the type II promoter, in the L6 skeletal muscle cells. This data is compatible with the idea that there are negatively-acting elements in the 5′ flanking region that normally repress expression of the type II gene in skeletal muscle cells and, by analogy, in other cells not normally expressing this gene. Because the negatively-acting sequences were approximately 50-fold more effective in repressing promoter activity in L6 than in PC12 cells, it suggests that the repression is also "cell-specific." Deletional analysis of the type II gene suggests the presence of at least two negatively-acting regions, and one positively-acting region, upstream of the type II promoter (Fig. 5).

By analogy with other eukaryotic genes, it is likely that the DNA elements identified in the type II gene serve as binding sites for specific transcription factors. Specific binding of nuclear proteins from L6 cells to the negative elements in the type II 5′ flanking region has recently been described (Kraner et al., 1991). However, scrutiny of the nucleotide sequence of the type II 5′ flanking region has failed to reveal significant identities with negatively-acting elements identified in other eukaryotic genes, suggesting that novel transcription factors are involved in neural-specific expression of the type II gene. Thus far, repressor proteins which bind to the negatively-acting elements in the type II gene have been identified only in extracts prepared from L6 cells and skeletal muscle (Kraner et al., 1991). Because the type II gene is expressed poorly in the peripheral nervous system, it is reasonable to hypothesize the existence of similar repressors in subpopulations of PNS neurons. This hypothesis is easily tested by preparing nuclear extracts from PNS neurons and assaying them for binding activity to the type II 5′ flanking sequence, as described above for the L6 extracts.

The type II gene is so far the only sodium channel gene which has been examined in detail for the presence of DNA regulatory elements conferring cell specificity. It obviously will be of interest to compare the regulatory regions of other members of this gene family for both common and unique sequences. There are no obvious identities in the first 1200 bp of the type I and type II 5′ flanking regions (G. Mandel, *unpublished*). This observation, in addition to the observation that the 5′ untranslated regions of the brain type I and type II sodium channel mRNAs are also divergent, suggests that the regions upstream of the coding sequence have evolved to provide a means of differentially regulating the mRNAs in different neuronal types. As more sodium channel gene regulatory regions are cloned and characterized, the importance of the regulatory regions in aspects of sodium channel regulation, in addition to cell-specific expression, should emerge.

Future Directions

One of the most puzzling, but most interesting, aspects of the sodium channel gene family is the extent of its diversity. Unlike the case for functionally divergent voltage-sensitive potassium and calcium channels, identification of the large number of different sodium channel mRNAs was initially unexpected. This is in part historical, because the func-

tion of the sodium channel was associated exclusively with the generation of action potentials. From this association came the idea that there would be enormous pressure to rigidly conserve the structure of alpha subunits in the different tissues which use action potentials as their signalling mechanism. Despite these ideas, it is clear that members of the sodium channel gene family are many, and that they are regulated tightly in terms of cell-specific expression and development. Ironically, because of its diversity, the sodium channel gene family has turned out to be an excellent model for examining the molecular basis of regulation of neuronal multi-gene families.

One of the most pressing questions arising from the molecular studies is the reason for the extensive multiplicity of genes and alternatively-spliced mRNAs. At least two ideas, which are not mutually exclusive, seem plausible: (1) that the amino acid substitutions are responsible for targeting distinct sodium channels to different subcellular membranes (segregation of sodium channels) and (2) that the different primary structures result in distinct functional properties. Although both ideas are testable, the first is much more amenable to experimentation than the second. For example, genetic engineering could be used to mix and match variant alpha subunits. The chimeric molecules could be expressed in heterologous cells and then measured immunologically for subcellular localization. These experiments could be done in both skeletal muscle and in neuronal cells where segregation of sodium channels has been hypothesized to occur. Ideally, to assess the functional role of different alpha subunits, it would be preferable to substitute one type of sodium channel for another, in a single cell, and thereby determine the contribution of each sodium channel type to the changes in electrophysiological properties. It is not immediately apparent how this experiment could be done effectively, but the technical advances in microinjection techniques and use of antisense oligonucleotides suggest that reconstitution experminets will begin to provide insights over the next few years.

In the process of sorting out the biological significance of the multiplicity of sodium channels, the sodium channel will provide an excellent system for understanding cell-specific and developmental regulation of gene families expressed in the nervous system. For example, a major regulatory mechanism for sodium channel gene expression appears to be negative, involving the activities of cell-specific repressors (for general review *see* Levine & Manley, 1989). This may turn out to be a more general regulatory mechanism in the nervous system. To fully understand the extent and impact of this type of regulation, the transcriptional factors will have to be identified from the relevant cells, and their interactions with specific sodium channel promoters characterized. Antibodies raised against these transcriptional factors can be used to generate topological maps of these proteins. Such maps will be useful for determining the extent of negative gene regulation for a specific sodium channel type.

It is likely that positively-acting genetic elements, in conjunction with the negative elements, confer cell-specific expression of sodium channels. Studies identifying such elements for the sodium channel genes have been hampered somewhat by the limited types of cell lines available for transfection studies and by the limited biological repetoire of these lines. The activities of 5′ flanking regions of different sodium channel genes should be examined *in vivo* for their abilities to confer cell-specific and developmental regulation. Transgenic mouse studies, for example, will be useful for identifying all of the important genetic elements dictating cell-specific and temporal expression in individual cell types as well as confirming results from tissue-culture studies. The transcriptional factors binding to positive genetic elements can then be compared with respect to their activity during development and their potential contributions to neuronal plasticity in the adult animal.

The past decade has witnessed a steep increase in our knowledge of regulation of the sodium channel gene family. Molecular reagents and techniques for identifying and characterizing the multiplicity of sodium channels have become almost routine; determining the genomic structures, the extent of the multiplicity, and the biological importance of differentially spliced mRNAs lies ahead. A unique advantage of studies of the voltage-sensitive sodium channel is the strength provided by the combination of molecular, biochemical and electrophysiological approaches, all of which can be brought to bear on questions of regulation.

The author is indebted to Laurinda Jaffe, Paul Brehm, and, especially, to Simon Halegoua for many insightful suggestions and helpful discussions. The work was supported by grants from the NIH and Muscular Dystrophy Association.

References

Alonso, A., Llinas, R.R. 1989. *Nature* **342:**175–177

Angelides, K., Elmer, L.W., Loftus, D., Elson, E. 1988. *J. Cell Biol.* **106:**1911–1925

Auld, V.J., Goldin, A.L., Krafte, D.S., Marshall, J., Dunn, J.M., Catterall, W.A., Lester, H.A., Davidson, N., Dunn, R.J. 1988. *Neuron* **1**:449–461
Barchi, R.L. 1988. *Annu. Rev Neurosci.* **11**:455–495
Barres, B.A., Chun, L.L.Y., Corey, D.P. 1989. *Neuron* **2**:1375–1388
Barrett, J.N., Crill, W.E. 1980. *J. Physiol.* **304**:231–249
Beckh, S. 1990. *FEBS Lett.* **262**:317–322
Beckh, S., Noda, M., Lubbert, H., Numa S. 1989. *EMBO J.* **8**:3611–3616
Bossu, J.L., Feltz, A. 1984. *Neurosci. Lett.* **51**:241–246
Boudier, J.A., Berwald-Netter, Y., Dellmann, H.D., Boudier, J.L., Couraud, F., Koulakoff, A., Cau, P. 1985. *Dev. Brain Res.* **20**:137–142
Brehm, P., Henderson, L. 1988. *Dev. Biol.* **129**:1–11
Brown, A.M., Lee, K.S., Powell, T. 1981. *J. Physiol.* **318**:479–500
Campbell, D. 1989. *Biophys. J.* **55**:176*a*
Casadei, J.M.; Barchi R.L. 1987. *J. Neurochem.* **48**:773–778
Catterall, W.A. 1981. *J. Neurosci.* **1**:777–783
Catterall, W.A. 1986. *Annu. Rev. Biochem.* **55**:953–985
Catterall, W.A. 1988. *Science* **242**:50–60
Coombs, J.S., Curtis, D.R., Eccles, J.C. 1957. *J. Physiol.* **139**:232–249
Cooperman, S.S., Grubman, S.A., Barchi, R.L., Goodman, R.H., Mandel, G. 1987. *Proc. Natl. Acad. Sci. USA* **84**:8721–8725
Couraud, R., Martin-Moutot, N., Koulakoff, A., Berwald-Netter, Y. 1986. *J. Neurosci.* **6**:192–198
Cribbs, L.L., Satin, J., Fozzard, H.A., Rogart, R.B. 1990. *FEBS Lett.* **275**:195–200
Dichter, M.A., Tischler, A.S., Greene L.A. 1977. *Nature* **268**:501–504
Edelstein, N.G., Catterall, W.A., Moore, R.J. 1988. *Biochemistry* **27**:1818–1822
Ellisman, M.H., Levinson, S.R. 1982. *Proc. Natl. Acad. Sci. USA* **79**:6707–6711
Frelin, C., Vigne, P., Lazdunski, M. 1983. *J. Biol. Chem.* **258**:7256–7259
French, C.R., Gage, P.W. 1985. *Neurosci. Lett.* **56**:289–294
Geduldig, D., Gruener, R. 1970. *J. Physiol.* **211**:217–244
Gilly, W.F., Armstrong, C.M. 1984. *Nature* **309**:448–450
Gilly, W.F., Brismar, R. 1989. *J. Neurosci.* **9**:1362–1374
Gonoi, T., Sherman, S.J., Catterall, W.A. 1985. *J. Neurosci.* **5**:2559–2564.
Gordon, D., Merrick, D., Auld, V., Dunn, R., Goldin, A.L., Davidson, N., Catternall, W.A. 1987. *Proc. Natl. Acad. Sci. USA* **84**:8682–8686
Green, L.A., Tischler, A.S. 1976. *Proc. Natl. Acad. Sci. USA* **73**:2424–2428
Grubman, S.A., Cooperman, S.S., Weintraub, J., Begley, M., Goodman, R.H., Mandel, G. 1988. *In:* Molecular Biology of Ion Channels. W. Agnew, editor. Academic, New York
Guy, H.R. 1988. *Curr. Top. Membr. Transp.* **33**:277–288
Halegoua, S., Armstrong, R.C., Kremer, N.E. 1991. *Curr. Top. Microbiol. Immunol.* **165**:119–170
Harris, J.B., Marshall, M.W. 1973. *Nature New Biol.* **243**:191–192
Harris, J.B., Thesleff, S. 1971. *Acta Physiol. Scand.* **83**:382–388
Hartmann, H.A., Kirsch, G.E., Drewe, J.A., Taglialatela, M., Joho, R.H., Brown, A.M. 1991. *Science* **251**:942–944
Hille, B. 1984. *In:* Ionic Channels of Excitable Membranes. pp. 70–71. B. Hille, editor. Sinauer, Sunderland (MA)
Huguenard, J.R., Hamill, O.P., Prince, D.A. 1988. *J. Neurophysiol.* **59**:778–795
Ikeda, S.R., Schofield, G.G., Weight, F.F. 1986. *J. Neurophysiol.* **55**:527–539
Jones, S. 1987. *J. Physiol.* **389**:605–627
Kallen, R.G., Sheng, Z.-H., Yang, J., Chen, L., Rogart, R.B., Barchi, R.L. 1990. *Neuron* **4**:233–242
Kayano, T., Noda, M., Flockerzi, V., Takahashi, H., Numa, S. 1988. *FEBS Lett.* **228**:187–194
Kostyuk, P.G., Veselovsky, N.S., Tsyndrenko, A.Y. 1981. *Neuroscience* **6**:2423–2430
Krafte, D.S., Snutch, T.P., Leonard, J.P., Davidson, N., Lester, H.A. 1988. *J. Neurosci.* **8**:2859–2868
Kraner, S.D., Dains, K.M., Chong, J.A., Mandel, G. 1991. *Soc. Neurosci. Abstr.* **17**:1286
Levine, M., Manley, J.L. 1989. *Cell* **59**:405–408
Llinas, R., Sugimori, M. 1980. *J. Physiol.* **305**:197–213
Lombet, A., Lazdunski, M. 1984. *Eur. J. Biochem.* **141**:651–660
Loughney, K., Kreber, R., Ganetzky, B. 1989. *Cell* **58**:1143–1154
Mandel, G., Cooperman, S.S., Maue, R.A., Goodman, R.H., Brehm, P. 1988. *Proc. Natl. Acad. Sci. USA* **85**:924–928
Maue, R.A., Kraner, S.D., Goodman, R.H., Mandel, G. 1990. *Neuron* **4**:223–231
Moczydlowski, E., Uehara, A., Guo, X., Heiny, J. 1986. *Ann. N.Y. Acad. Sci.* **479**:269–292
Moorman, J.R., Kirsch, G.E., VanDongen, A.M.J., Joho, R.H., Brown, A.M. 1990. *Neuron* **4**:243–252
Nishi, S., Koketsu, K. 1960. *J. Cell. Comp. Physiol.* **55**:15–30
Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H., Numa, S. 1986*a*. *Nature* **320**:188–192
Noda, M., Ikeda, T., Suzuki, H., Takeshima, H., Takahashi, T., Kuno, M., Numa, S. 1986*b*. *Nature* **322**:826–828
Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H. Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsua, H., Raftery, M., Hirose, T., Inayama, S., Hayashida, H., Miyata, T., Numa, S. 1984. *Nature* **312**:121–127
O'Lague, P.H., Huttner, S.L. 1980. *Proc. Natl. Acad. Sci. USA* **77**:1701–1705
Pappone, P. 1980. *J. Physiol.* **306**:377–410
Ramaswami, M., Tanouye, M.A. 1989. *Proc. Natl. Acad. Sci. USA* **86**:2079–2082
Redfern, P., Thesleff, S. 1971. *Acta Physiol. Scand.* **82**:70–78
Rogart, R.B., Cribbs, L.L., Muglia, L.K., Kephart, D.D., Kaiser, M.W. 1989. *Proc. Natl. Acad. Sci. USA* **86**:8170–8174
Rogart, R.B., Regan, L.J. 1985. *Brain Res.* **329**:314–318
Rudy, B., Kirschenbaum, B., Ruckenstein, A., Greene, L.A. 1987. *J. Neurosci.* **7**:1613–1625
Salkoff, L., Butler, A., Wei, A., Scavarda, N., Giffen, K., Ifune, C., Goodman, R.H., Mandel, G. 1987. *Science* **237**:744–749
Sarao, R., Gupta, S.K., Auld, V.J., Dunn, R.J. 1991. *Neuron (in press)*
Schaller, K., Krzemien, D.M., McKenna, N.M., Caldwell, J. 1991. *Neuron (in press)*
Scheinman, R.I., Auld, V.J., Goldin, A.L., Davidson, N., Dunn, R.J., Catterall, W.A. 1989. *J. Biol. Chem.* **264**:10660–10666
Schmidt, J., Rossie, S., Catterall, W.A. 1985. *Proc. Natl. Acad. Sci. USA* **82**:4847–4851
Sherman, S.J., Catterall, W.A. 1982. *J. Gen. Physiol.* **80**:753–768
Sills, M.N., Xu, Y.C., Baracchini, E., Goodman, R.H., Cooperman, S.S., Mandel, G., Chien, K.R. 1989. *J. Clin. Invest.* **84**:331–336

Srinivasan, Y., Elmer, L., Davis, J., Bennett, V., Angelides, K. 1988. *Nature* **333:**177–180

Suzuki, H., Beckh, S., Kubo, H., Yahagi, N., Ishida, H., Kayano, T., Noda, M., Numa, S. 1988. *FEBS Lett.* **228:**195–200

Tanabe, T., Beam, K.G., Powell, J.A., Numa, S. 1988. *Nature* **336:**134–139

Trimmer, J.S., Agnew, W.S. 1989. *Annu. Rev. Physiol.* **51:**401–418

Trimmer, J.S., Cooperman, S.S., Agnew, W.S., Mandel, G. 1990. *Dev. Biol.* **142:**360–367

Trimmer, J.S., Cooperman, S.S., Tomiko, S.A., Zhou, J., Crean, S.M., Boyle, M.B., Kallen, R.G., Sheng, Z., Barchi, R.L., Sigworth, F.J., Goodman, R.H., Agnew, W.S., Mandel, G. 1989. *Neuron* **3:**33–49

Tsay, H.-J., Schmidt, J. 1989. *J. Cell Biol.* **108:**1523–1526

Waxman, S.G., Ritchie, J.M. 1985. *Science* **228:**1502–1507

Weiss, R.E., Horn, R. 1986. *Science* **233:**361–364

Westenbroek, R.E., Merrick, D.K., Catterall, W.A. 1989. *Neuron* **3:**695–704

White, M.M., Chen, L., Kleinfeld, R., Kallen, R.G., Barchi, R.L. 1991. *Mol. Pharmacol.* **29:**604–608

Wollner, D.A., Catterall, W.A. 1985. *Brain Res.* **331:**145–149

Wollner, D.A., Catterall, W.A. 1986. *Proc. Natl. Acad. Sci. USA* **83:**8424–8428

Received 16 August 1991