Published in final edited form as:

J Neurophysiol. 1994 December ; 72(6): 2796–2815.

Slow Sodium Conductances of Dorsal Root Ganglion Neurons: Intraneuronal Homogeneity and Interneuronal Heterogeneity

MARCO A. RIZZO, JEFFERY D. KOCSIS, and STEPHEN G. WAXMAN

Department of Neurology, Yale University School of Medicine, New Haven 06510; and Paralyzed Veterans of America/Eastern Paralyzed Veterans Association Neuroscience Research Center, Veterans Affairs Medical Center, West Haven, Connecticut 06516

SUMMARY AND CONCLUSIONS

1. Voltage-dependent Na^+ conductances were studied in small (18–25 μm diam) adult rat dorsal root ganglion (DRG) neurons with the use of the whole cell patch-clamp technique. Na^+ currents were also recorded from larger (44–50 μm diam) neurons and compared with those of the small neurons.
2. The predominant Na^+ conductance in the small neurons was selective over tetramethylammonium by at least 10-fold and was resistant to 1 μM external tetrodotoxin (TTX). Na^+ conductances in many larger DRG neurons were kinetically faster and, in contrast, were blocked by 1 μM TTX.
3. The Na^+ conductance in the small neurons was kinetically slow. Activation half-times were voltage dependent and ranged from 2 ms at -20 mV to 0.7 ms at $+50$ mV. Approximately 50% of the activation half-time was comprised of an initial delay. Inactivation half-times were voltage dependent and ranged from 11 ms at -20 mV to 2 ms at $+50$ mV.
4. Peak slow Na^+ conductances were near maximal with conditioning potentials negative to -120 mV and were significantly reduced or eliminated with conditioning potentials positive to -40 mV. The slow Na^+ conductance increased gradually with test potentials extending from -40 to $+40$ mV. In some cells the conductance could be saturated at $+10$ mV. Peak conductance/voltage relationships, although stable in a given neuron, revealed marked variability among neurons, spanning >20 - and 50-mV domains for steady-state activation and inactivation (current availability), respectively.
5. Kinetics remained stable within a given neuron over the course of an experiment. However, considerable kinetic variation was exhibited from neuron to neuron, such that the half-times of activation and of inactivation spanned an order of magnitude. In all small neurons studied there appeared to be a singular kinetic component of the current, based on sensitivity to the conditioning potential, voltage dependence of activation, and inactivation half-time.
6. Unique closing properties were exhibited by Na^+ channels of the small neurons. Hyperpolarization following a depolarization-induced fully inactivated state resulted in tail currents that appeared to be the consequence of reactivation of the slow Na^+ conductance. Tail currents recorded at various times during a fixed level of depolarization revealed that the underlying channels accumulated into a volatile inactivated state over the course of the preceding depolarization.
7. Larger neurons had a different repertoire of Na^+ conductances, with either only a TTX-sensitive, kinetically fast type, or a combination of fast TTX-sensitive and slow currents.

In larger neurons the kinetically separable fast current had a greater sensitivity to the conditioning potential, i.e., a left-shifted steady-state inactivation curve.

8. The different properties of the slow Na⁺ conductance in different neurons is likely to reflect heterogeneity of the structure of the underlying channel molecule. Although consistent with what others have found in equivalent preparations, this heterogeneity is far broader in scope than what has so far been described. We suggest that biosynthetic constraints within a given small neuron maintain ion channel uniformity.

INTRODUCTION

Heterogeneity of mammalian Na⁺ channels was initially recognized by the differential toxin sensitivity of various tissues. Whereas axons of vertebrates and invertebrates are sensitive to nanomolar concentrations of tetrodotoxin (TTX), cardiac muscle requires several micromolar to produce the equivalent blockade (Brown et al. 1981), and higher concentrations are required for blockade in denervated skeletal muscle (Harris and Thesleff 1971; Pappone 1980), embryonic muscle (Frelin et al. 1986; Weiss and Horn 1986), and certain neural tissues (Neumcke 1990; Spitzer 1979; reviewed by Trimmer and Agnew 1989). Kinetic comparisons of Na⁺ channels among different tissues are hampered by the multiplicity of preparations, the variability of space-clamp configuration, differences in ambient solutions and temperatures, and other technical differences among laboratories.

Structural determinations of the primary amino acid sequences of several mammalian ionic channels have revealed structural heterogeneity of the γ -aminobutyric acid-A (GABA_A) receptor (White 1992), the non-*N*-methyl-D-aspartate (non-NMDA) type glutamate-activated channels (Sommer and Seeburg 1992; Verdoorn et al. 1991), and the transient K⁺ channel of *Drosophila* (Jan and Jan 1990) in addition to the Na⁺ channel: three in rat brain (Kayano et al. 1988; Noda et al. 1986a), one in rat heart (Rogart et al. 1989), and one in rat skeletal muscle (Trimmer et al. 1989). Injection of *Xenopus* oocytes with mRNA specific for two of the brain Na⁺ channels produces functional expression on the oocyte membrane (Noda et al. 1986b; Suzuki et al. 1988). Moreover, manipulation of specific regions of the primary amino acid sequence alters specific channel properties (Heinemann et al. 1992; Noda et al. 1989; Stühmer et al. 1989). It is interesting, in this regard, that recent studies of the voltage-dependent Na⁺ channel of rat skeletal muscle (Schaller et al. 1992) and rat brain (Gustafson et al. 1993; Schaller et al. 1992) reveal that the differential splicing of RNA transcripts is a potential mechanism for conferring structural heterogeneity. Molecular biological investigations have provided important results in revealing Na⁺ channel structural heterogeneity, yet may be limited with respect to a full appreciation of heterogeneity resulting from variable quaternary structure, posttranslational modification, or interactions with local, native membrane components or soluble cytoplasmic factors. On the other hand, electrophysiological studies have the advantage of revealing behavioral diversity of Na⁺ currents in their native neuronal membrane.

Mammalian dorsal root ganglion (DRG) neurons have been shown to support TTX-resistant Na⁺ spikes of relatively long duration (Fukuda and Kameyama 1980; Ransom and Holz 1977; Yoshida et al. 1978). The underlying Na⁺-selective channel has emerged as a kinetically and pharmacologically distinct subclass (Caffrey et al. 1992; Elliot and Elliot 1993; Kostyuk et al. 1981; McLean et al. 1988; Ogata and Tatebayashi 1992a; Roy and Narahashi 1992; Schwartz et al. 1990). This channel is found in the soma of small DRG neurons that give rise primarily, although not exclusively, to C-type primary afferent fibers (Rose et al. 1986; Yoshida and Matsuda 1979). Its function remains obscure. Recent evidence has inferred the expression of this form of channel on the myelinated axons of primary cutaneous afferents (Honmou et al. 1994); however, these fibers conduct TTX-sensitive action potentials, and the axonal density of the TTX-resistant channel is not sufficient to support regenerative activity (Honmou et al.

1994; Yoshida and Matsuda 1979). One hypothesis (Bossu and Feltz 1984) is that the TTX-resistant channel may serve to prolong the interspike interval by prolonging the inactivated state of TTX-sensitive channels during sustained depolarization in rat nodose neurons. An alternative idea is that it may confer slow-adaptive properties to the neuron, because of the channel's slow component of inactivation (Ogata and Tatebayashi 1992b) and rapid repriming capability following complete inactivation (Elliot and Elliot 1993).

The work presented here uses the patch-clamp technique to study the slow, voltage-dependent Na^+ conductance in small (18–24 μm diam, 20–30 pF, corresponding to C-type primary afferents) DRG neurons of adult rat. The initial focus of the study dealt with gating aspects of the slow Na^+ conductance to establish that the underlying channel is similar to the slow Na^+ conductance of sensory neurons that others have studied. The gating was found to be unique in several respects. Its relatively weak voltage dependence and unique closing properties set this channel apart from other known TTX-resistant or -sensitive Na^+ selective channels. The second focus of the study addressed issues relating to physiological variations that we observed among different neurons. Systematic studies comparing Na^+ currents from different DRG neurons in the same size range have not previously been reported. The Na^+ current recorded from each small neuron had a distinct and stable kinetic pattern, suggesting the presence of a single type of Na^+ channel in a given neuron. However, when compared across the population of neurons studied, voltage and time dependencies varied such that they exhibited a virtual continuum of steady-state and kinetic properties over domains spanning an order of magnitude, implying neuron-to-neuron variability in the type of channel present in each neuron's membrane. Larger neurons (44–50 μm diam) tended to have kinetically faster Na^+ currents that, although more uniform among cells, would occasionally reveal multiple currents in a single cell, suggesting that multiple channel subtypes are present in the membrane. The differences in Na^+ channels among small neurons and the apparent ability to maintain channel uniformity within a given neuron suggest an electrophysiological strategy whereupon each sensory neuron is uniquely yet uniformly tuned. Some results have been previously reported in abstract form (Rizzo et al. 1993).

METHODS

Cell culture

Adult male Wistar rats were exsanguinated under pentobarbital anesthesia. The lumbar ganglia (L_4 , L_5) were excised after freeing the nerve trunks and connective tissue sheath. Pooled ganglia were incubated with gentle agitation for 25 min in *solution A*, which consisted of complete saline solution (CSS), 1 mg/ml collagenase (Boehringer Mannheim Biochemicals, Indianapolis, IN), 0.2 mg/ml cysteine, 1.5 mM CaCl_2 , and 0.5 mM EDTA (ethylenedinitrilotetraacetic acid, disodium salt). The CSS contained (in mM) 137 NaCl, 5.3 KCl, 1 MgCl_2 , 25 sorbitol, 3 CaCl_2 , 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) titrated to pH 7.2 with NaOH. Ganglia were then incubated in 30 U/ml papain (Worthington Biochemicals, Freehold, NJ), followed by a rinse and second incubation with *solution A* for 25 min, then transferred to culture media containing 1:1 Dulbecco's modified Eagles's medium (DMEM), and Ham's F12 medium containing 10% fetal calf serum, 1.5 mg/ml trypsin inhibitor, 1.5 mg/ml bovine serum albumin, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (GIBCO, Grand Island, NY). The ganglia were then gently triturated with the use of a siliconized Pasteur pipette and plated with a density of 500–1,000 cells/ mm^2 on a polyornithine/laminin coated glass coverslip. They were maintained at 37°C in a 5% CO_2 incubator for 18–48 h before recording. Cells were studied during the first 48 h in culture, because at later times most cells had an extensive neuritic outgrowth that caused voltage-clamp recordings to be quantitatively unreliable. Cells chosen for recording were round, 18–25 μm ("small") in diameter, had no neuritic processes, and therefore were most ideal for space

clamping. In some experiments, Na⁺ currents were recorded from 44- to 50- μ m-diam neurons and compared with those recorded from small neurons.

Solutions for voltage-clamp experiments

Before electrical recording, the coverslips containing cultured DRG were rinsed, then placed in a protein-free saline (*E1*, see Table 1). In some experiments, 30 mM external Na⁺ solution was used (*solutions E2 and E3*) with 110 mM tetramethylammonium⁺ (TMA⁺), the primary nonpermeant monovalent cation used to replace external Na⁺ to reduce current amplitude and therefore errors caused by series resistance artifact. Cells placed in a recording chamber maintained their morphological and electrical properties at 18–20°C for at least 2 h. Recording pipettes were filled with one of the internal solutions listed in Table 1.

Every effort was made to reduce contamination of the Na⁺ currents by other voltage-activated currents. Cs⁺ salts were used to minimize current through voltage-dependent K⁺ channels. In some experiments involving 44- to 50- μ m-diam neurons (Fig. 12), a small amount of sustained outward current remained at the end of a strong depolarizing pulse, indicating that not all K⁺ had been completely dialyzed from the internal milieu, or a significant anionic conductance was present. Up to 20 mM external tetraethylammonium (TEA, Sigma) was ineffective in blocking the non-inactivating outward K⁺ current in these neurons. Larger concentrations of TEA were not used out of concern that it might have a nonspecific effect. 4-Aminopyridine (1 mM) was used in many experiments to eliminate contamination of the records by transient outward K⁺ current. This was particularly important during experiments in 44- to 50- μ m-diam neurons. No kinetic studies were performed on the large neurons because of a relatively limited space-clamp configuration and the often-encountered persistent outward current during sustained depolarization. In spite of all measures taken to minimize contamination of the Na⁺ currents by other currents, some persistent inward or outward current remained at the end of strong depolarizing pulses. Of 40 neurons studied, 13 had either a persistent, noninactivating component of inward current, or a slight outward current after the P/4 procedure was applied (below), in excess of ~5% of the maximal Na⁺ conductance; the remaining 27 were used for kinetic comparisons.

Constant external and internal divalent cation concentrations were maintained with the following exceptions. All experiments were done with external 0.1 mM cadmium, without which cells exhibited additional components of slowly inactivating and non-inactivating inward current, presumably carried by Ca²⁺ ion. To eliminate MgF₂ precipitation, Mg²⁺ ion was not used in internal solutions in experiments where internal CsF was the main salt. To successfully perform such experiments, a small quantity of non-magnesium-containing internal CsCl solution was drawn into a pipette tip already filled with CsF solution. Slight positive pressure was then maintained during micromanipulation of the pipette onto the cell, extruding the CsCl into the (Mg²⁺-containing) external saline, yet avoiding MgF₂ precipitation.

Voltage-clamp recording technique

The whole cell patch-clamp method (Hamill et al. 1981) was used to record voltage-dependent Na⁺ currents from the cultured DRG neurons. Patch electrodes (Corning No. 7052 capillary glass) of 1.6–3.3 M Ω were constructed and fire-polished with the use of a Narashige PP-83 vertical puller and an MF-83 fire-polisher. The electrodes were mounted on the headstage of a Medical Systems Corp. APC-8 patch-clamp amplifier with the use of a 500-M Ω feedback resistor. The electrode was micromanipulated (Narashige MO 103R) onto the cell that lay in a saline bath on the stage of an inverted phase-contrast microscope (Nikon Diaphot). The bath temperature in all experiments was maintained at 18–20°C.

Customized Ag-AgCl pellets (E. W. Wright, Guilford, CT) were used for headstage and bath electrodes. These had contact exclusively with an electrode standard solution containing 150 mM NaCl, and 10 mM HEPES-NaOH buffered to pH 7.4. Before obtaining the whole cell clamp configuration, junction potentials between the electrode solutions and those in the pipette and recording chamber were compensated. If voltage-offset after an experiment was ≥ 2 mV, the experiment was excluded from analysis.

The shunt capacitance between the pipette and bath was reduced by maintaining the bath level ~ 50 μ m above the cell being clamped, and by analog capacity compensation. Series resistance was generally 1.2–1.7 times the pipette resistance before obtaining the gigaseal, and usually measured 2–4 M Ω . Uncompensated, this conferred time constant limitations in the small neurons of maximally 120 μ s, and errors in voltage measurements of 2–8 mV (with 30 mM external Na⁺ ion). These parameters were improved upon by 20–40% series resistance compensation. Further compensation tended to generate oscillations because of the shunting of the series resistance by the pipette/bath capacitive coupling. Capacity current was further reduced, along with linear leak currents, via P/4 pulse protocols (Bezanilla and Armstrong 1977). Each test stimulation was followed by four control sweeps in which the command potential was one-fourth amplitude and opposite in sign to the test potential. The control sweeps were added to the test sweep before analysis. Test pulses were applied in 1-s intervals. Between test pulses, cells were held at -60 mV to -100 mV.

The current was sampled and converted into (16-bit) digital form with the use of an acquisition system (ITC-16, Instrutech Corp.) that interfaced the patch-clamp amplifier with an Apple Macintosh computer. The current was sampled at 50 kHz, low-pass filtered (4-pole Bessel) at 10 kHz unless otherwise stated, and stored on magneto-optical media (DGR Technologies). Generally, four sweeps were averaged at each test pulse. Before analysis, stored current records were inspected to exclude sweeps with recognizable artifacts.

Analytic methods

The significant conclusions of this paper, the heterogeneity of Na⁺ channels among the different neurons studied, were arrived at without making a priori assumptions of the homogeneity of the Na⁺ channel population in a given neuron. Kinetic comparisons among different neurons were limited to the study of half-times, which was sufficient to demonstrate kinetic heterogeneity. Curve fitting the inactivation phase of the currents to simple exponentials was abandoned for two reasons. 1) The slow inactivation rate revealed by many of the Na⁺ conductances was the consequence of a slow activation rate. The inactivation phase of the current therefore often did not conform to simple exponential decay. 2) Uniformity of the underlying population of channels could not be assumed, although this question is addressed by the studies to follow.

Peak conductances were calculated as $g = I(E - E_{\text{Na}})$ where I is the peak current, E is the voltage-clamp potential, and E_{Na} is the experimental reversal potential for Na⁺ ion. As a matter of convention in the text, the “current availability” curve will refer to “steady-state” inactivation, indicating the fraction of channels available for opening upon depolarization as a function of the conditioning potential. “Inactivation” will refer to the kinetic process of channel closure during sustained depolarization.

Comparisons of the peak conductance/voltage relation across neurons studied was limited to the comparison of the voltage of half-maximal activation or inactivation; we chose this conservative approach for two reasons. 1) In 14/40 of the neurons studied, the peak conductance could not be saturated, and attempts to fit the form of the Boltzmann equation that describes the fraction of open channels versus voltage failed because of the arbitrary choice of slope factors and voltages of half-maximal activation. 2) Although one conclusion of this paper

argues strongly in favor of the uniformity of underlying channels within a given neuron, the application of the Boltzmann equation as a standard for comparison among neurons may have been erroneous because of the striking capability for heterogeneity of this channel. The voltages of half-maximal conductance over a fixed domain were adequate to demonstrate Na⁺ channel heterogeneity.

A form of fluctuation analysis, the variance of an ensemble mean, was used to examine intraneuronal channel homogeneity versus heterogeneity, and to approximate the single-channel conductance. The variance of an ensemble mean of n current records at high bandwidths, as advanced by Sigworth (1980)) and given by $\sigma^2 = \Sigma[(I - I^*)^2/(n - 1)]$, reveals a valley in the vicinity of the peak of the mean current I^* if two conditions are met: 1) the fraction of open channels is >0.5 and 2) the population of channels is uniform (Conti et al. 1984). Thus only in cells exhibiting such a minimum in the variance could the underlying single-channel conductance be approximated by the ratio of the variance versus the mean at low levels of the current (reviewed by Hille 1992).

RESULTS

Cells

This study focuses on the transient, voltage-dependent Na⁺ conductances that are particular to the small neurons shown in Fig. 1. Figure 1 is a video photomicrograph showing several 18- to 25- μ m-diam DRG neurons (arrows) 24 h after plating. At this stage they are nearly spherical, have few or no processes, and have capacitances of 20–30 pF. Larger DRG neurons on the plate (*) have passive and active properties distinguishable from the small neurons (Caffrey et al. 1992). Neurons 44–50 μ m diam, comprising multiple populations of cell bodies of both deep (muscle) and cutaneous afferent fibers, each have unique Na⁺ channel populations (Honmou et al. 1994). Many of these neurons support a rapid, TTX-sensitive Na⁺ current that underlies an action potential whose duration is one-third to one-half of that that the small variety of neuron can generate. Properties of the fast Na⁺ current in these larger neurons were measured 1) for comparison with the Na⁺ current in small neurons and 2) to demonstrate that time constant limitations of the whole cell clamp configuration permitted the recording of faster Na⁺ currents under less favorable space-clamp conditions in neurons with up to three times the capacitance.

Whole cell Na⁺ currents: voltage dependence

Figure 2 shows whole cell currents recorded from a small neuron under conditions that favor recording isolated Na⁺ current. In the experiment shown in Fig. 2 and all that follow, Cs⁺ ion replaced K⁺ ion in the pipette, reducing or eliminating outward current carried by K⁺ ion. To separate transient and sustained components of inward current, external 100 μ M Cd²⁺ was applied. Although others have reported that 5 mM Cd²⁺ (Kostyuk 1981; Roy and Narahashi 1992) or 50 μ M Cd²⁺ (Bossu and Feltz 1984) significantly reduced or blocked the slow, TTX-resistant Na⁺ current in DRG neurons and nodose neurons, respectively, we found that 100 μ M Cd²⁺ effectively blocked Ca²⁺ current although retaining the peak conductance and other properties of the slow Na⁺ current.

Figure 2A shows a family of currents recorded by varying the depolarizing test pulse, stepping from a fixed prepulse. Figure 2B shows a family of currents recorded from the same neuron with a variable conditioning prepulse and fixed test pulse, the “availability” protocol. To activate the current, conditioning potentials less than –40 mV were required. From conditioning potentials more negative than –70 mV, depolarizations to –40 or –30 mV activated the current. Peak conductances are plotted against conditioning and test pulses in Fig. 2C. To activate maximal conductances, the prepulses had to be more negative than –100 mV, and the test pulses had to be more positive than 0 mV. The peak conductance appeared to increase when

the conditioning potential was increased from -140 to -120 mV. This may have been due to partial removal of a slow form of inactivation (Ogata and Tatebayashi 1992b), which generally had a time course on the order of minutes, but whose removal could be accelerated with such hyperpolarized voltages. In the neuron whose Na^+ currents are shown in Fig. 2, *A* and *B*, a conditioning potential more positive than -40 mV completely eliminated the current. The peak conductance saturated at test potentials >0 mV and decreased at test potentials greater than $+20$ mV. In the section of this paper that discusses variability in gating characteristics, it will be seen that the peak conductance/voltage relationship varied considerably from neuron to neuron, and peak Na^+ conductances in some cells could not be saturated.

Na^+ selectivity and TTX sensitivity

Figure 3 shows the results of exposure to TTX of two varieties of current. The kinetically faster variety (Fig. 3*A*), generally found in neurons >40 μm diam, was sensitive to 1 μM TTX (3 cells tested). In contrast, Na^+ currents recorded from small neurons (Fig. 3*B*) were resistant (8/8 cells tested) to this concentration of TTX. This is consistent with previous reports (Elliot and Elliot 1993; Kostyuk et al. 1981; Roy and Narahashi 1992) that demonstrated TTX-resistant, kinetically slow Na^+ channels in the somatic membrane of small DRG neurons. However, these results contrast with the TTX sensitivity of frog DRG neurons (Campbell 1992). In neurons whose capacitance was <25 pF, the fraction of Na^+ current remaining after 1 μM TTX application spanned from 0.0 to 0.7 in a continuous fashion.

Figure 4 compares the TMA^+ versus Na^+ selectivity of the channel. In this neuron, current was recorded before and after replacement of external 140 mM Na^+ ion by 110 mM TMA^+ and 30 mM Na^+ . The currents reversed close to the theoretical reversal potentials ($+86$ mV, $+46$ mV respectively) for Na^+ . The reversal potential shifted by -30 mV, short of the expected shift by 10 mV, indicating a TMA^+/Na^+ permeability ratio of 0.086. This is consistent with findings of Ikeda and Schofield (1987) on the Na^+ -selective TTX-resistant channel of rat nodose neurons, in which the TMA^+/Na^+ permeability ratio was <0.1 . In this and most experiments to follow, external 30 mM Na^+ and 110 mM TMA^+ were used to reduce the magnitude of the currents, and therefore errors caused by series resistance artifact. In >30 experiments the currents reversed near $+45$ mV, indicating selectivity for Na^+ ion.

Whole cell Na^+ currents: kinetics

Figure 5*A* shows the rising phases of the currents of Fig. 2*A* scaled, superimposed, and plotted on a different time scale. The delay before activation and time to half-activation are reduced with stronger depolarizations as shown in Fig. 5*B*. In this cell the half-times to peak ranged from 1.8 to 0.6 ms, with the delay comprising $\sim 50\%$ of this interval. The corresponding peaks occur at from >5 ms down to 1.4 ms. Figure 5*C* shows the mean (—) of a large ensemble of currents recorded to study fluctuations during the activation process. The current rises after a delay, reaches a peak, and then spontaneously decreases. The corresponding ensemble variance (+++) was scaled to superimpose on the mean at low levels of the current. The variance clearly reveals a minimum in the vicinity of the peak of the current (Fig. 5*D*, shorter time base). This would be expected for a uniform population of channels whose peak open probability was >0.5 (Conti et al. 1984; Sigworth 1980). Data from four other experiments revealed similar contours in the variance/mean temporal relationship; however, three experiments did not show a minimum variance near the peak of the mean current; an example is shown in Fig. 5, *E* and *F*.

Although these results may seem equivocal, there are limited possible interpretations. Either some cells did not have a uniform population of channels, or the underlying channels had a different sensitivity to the stimulating pulse, such that an open probability of 0.5 was not achieved. The latter interpretation is in fact consistent with the calculated conductance/voltage

relation in some neurons (Fig. 8, A–C). The question of whether or not an individual neuron can have more than one population of channels in its somatic membrane is addressed below. The ratio of the variance to mean current calculated at points of low open probability, either very early or late in the trace, yielded a lower limit for the single-channel conductance on the order of 13 pS.

Inactivation kinetics are shown in Fig. 6. The data of Fig. 2, A and B, are scaled and superimposed in Fig. 6, A and B, respectively. In this cell the Na⁺ current had a half-time of decay of > 10 ms at –20 mV, decreased to 2.4 ms at +50 mV (Fig. 6C), and exhibited an *e*-fold decrease versus 14 mV. In Fig. 6B, which shows currents evoked by variable conditioning prepulses and a fixed test pulse, the current traces overwrite each other signifying that 1) the inactivation rate was not dependent on the conditioning potential and 2) if there was more than one population of Na⁺-selective channel in this neuron, they could not be distinguished by their inactivation kinetics and their voltage dependence of inactivation. If we assume that more than one channel subpopulation does underly these currents, for these scaled currents to overwrite, maximally only one of the following three parameters may vary among the channel types: the availability function, activation kinetics, or inactivation kinetics.

Tail currents recorded late in the depolarizing pulse (Fig. 7A) at a fixed potential reveal that 1) if the depolarizing test pulse was relatively weak such that a sustained inward current remained at the end of the test pulse, a finite tail current could be recorded, 2) the tail current amplitude increased with increasing prior depolarization, and 3) even after strong depolarizations, which had minimal or no measurable current at the end of the pulse, a significant tail current could be measured. This form of current could be identified in 27/40 small neurons in which a kinetically slow variety of Na⁺ current was present, and that unambiguously fully inactivated with strong depolarizations. Such tail currents could not be identified in 14/14 large (44–50 μm diam) neurons that had a single, kinetically fast variety of channel (an example is shown in Fig. 12, B and D). Attempts to identify the ion responsible for the tail currents by extrapolating the peak current-voltage (*I*-*V*) failed because of rectification of the peak *I*-*V* at membrane potentials negative to approximately –80 mV, as if these were not true “instantaneous” currents. Several possible sources of these tail currents were considered. One strong possibility was relaxation of a Ca²⁺-selective channel. Ca²⁺ tail currents recorded in rat DRG neurons (Carbone and Lux 1987) decreased in amplitude at the end of the depolarization and were blocked by external 100 μM Cd²⁺. A 20-fold increase in external [Ca²⁺] (20 mM) was required to match our Na⁺ tail current amplitudes. Others who have studied Ca²⁺ currents in DRG neurons have not demonstrated such tail currents (Leybaert and de Hemptinne 1993); however, external divalent cations including Cd²⁺ have been shown to participate in the gating of Ca²⁺-selective channels in chicken DRG neurons (Swandulla and Armstrong 1989). In their study, tail currents recorded in the presence of 2 mM Ba²⁺ and 0 mM Mg²⁺ in the presence of external 20 μM Cd²⁺ were demonstrated to result from a rapid unblocking of Ca²⁺ channels by Cd²⁺. The rate of unblock was so rapid at membrane potentials negative to –60 mV, that the tail currents in the presence of 20 μM Cd²⁺ were similar to those recorded in the absence of Cd²⁺. Although it is difficult to entirely exclude Ca²⁺ current contamination in the experiment of Fig. 7A, the magnitude of the tail currents seems excessive given the conditions of the experiment: external 1 mM Ca²⁺, 1 mM Mg²⁺, and 100 μM Cd²⁺. The permeability of TMA⁺ through Ca²⁺ channels has not yet been determined.

Anomalous permeation of Na⁺ through an inwardly rectifying K⁺ channel (Zhu and Ikeda 1993) is a possible cause, but internal 140 mM Cs⁺ did not block the tail current, and its amplitude at a fixed hyperpolarized level varied according to the *prior* depolarization, a property not described in inwardly rectifying channels. Another possibility was that negligible net current at the end of a depolarizing pulse could have resulted from Na⁺ ion accumulation in the vicinity of the membrane, or activation of another current, either of which would cause

transient shifts in the net reversal potential. However, because most experiments, like that shown in this figure, depict current with a minimal sustained component at several positive test voltages, we do not favor this explanation. The residual conductance at the end of the depolarizing pulses was < 1.5 pA/mV, and thus contributed $< 16\%$ of the maximal tail currents with a -100 -mV hyperpolarizing pulse (assuming in the worst case a $+50$ -mV reversal potential of the contaminating ion). Yet another possible explanation that we explore further was that this tail current could have resulted from microscopic kinetic states of the Na^+ channel in which hyperpolarization pulled channels from the inactivated state to an open state before closure.

These possibilities were examined in the experiment shown in Fig. 7B. Here the cell was preconditioned at -140 mV for 150 ms, then depolarized to 0 mV for a variable period before repolarization to -140 mV. Zero millivolts was chosen as the depolarizing level because the amplitude of the inward current remained fairly level over the time course of interest. No tail current was elicited after 100- μ s depolarization. The amplitude of the tail current increased continuously when repolarization occurred after 1.5-, 3.0-, and 5.0-ms depolarizations, even when the absolute current levels at 3.0 and 5.0 ms were approximately the same. If intracellular Na^+ ion accumulation was occurring, these tail currents would be expected to decrease. It seems unlikely that, with 1 mM external Ca^{2+} , tail currents of this magnitude subtended the instantaneous reactivation of a Ca^{2+} selective channel after “unblock” by Cd^{2+} . Activation of another current that would counterbalance the Na^+ current at 0 mV would have to have an unrealistic reversal potential significantly negative to 0 mV, yet would be able to generate 7 nA of inward current at -140 mV.

With regard to the last possibility mentioned above, the increase in tail current amplitude may be explained if over the course of the depolarization, Na^+ channels are entering a relatively unstable inactivated state, while another pool of channels are continuously drawn into the open state. Although the net current remained constant, the tail current amplitude would be expected to increase from the 3- to 5-ms interval, because at 5 ms, inactivated channels were drawn into the open state before closing. This is consistent with a microscopic activation scheme in which weakly voltage-dependent channels are continuously added to the open pool while a rapid, voltage-independent process draws them into an inactivated state (Aldrich et al. 1983).

The width at half-maximum seemed to increase with the right-most tail current. This cannot be specifically accounted for. We speculate that there are at least two contributions to the shape of the tail current: channels instantaneously closing, and those reopening from the inactivated state. Thus the instantaneous component would be expected to primarily form the shape of the tail current at 3 ms when compared with that recorded at 5 ms. Although unreported in Na^+ channels, it is possible that a delay in closure with a hyperpolarizing step may occur. Alternatively, the “absorbency” of the inactivated state(s) could increase with the duration of the depolarization, causing reopening to be a more prolonged, multistep process.

Variations in gating properties among neurons

STEADY-STATE PROPERTIES—Neuron-to-neuron variations in the availability curves (steady-state inactivation) and peak conductance/voltage relationships are shown in Figs. 8 and 9. Figure 8, A, B, D, and E, shows two more examples of families of Na^+ currents recorded from two different neurons with the use of the stimulation protocols given. The peak conductance/voltage relationships are plotted, respectively, in Fig. 8, C and F. When the experiment of Fig. 8, A and B, is compared with that of Fig. 2, A and B, the conditioning potential had a similar effect on the peak conductance. However, whereas activation also began in the -30 -mV vicinity, the peak conductance showed no signs of saturation at voltages up to $+50$ mV (Fig. 8C). Thus there was a dissociated voltage dependence of activation between the Na^+ channels in each neuron. The difference is reversed when the experiment of Fig. 8, D and E, is compared with that of Fig. 2, A and B. A depolarized conditioning potential had a relatively

weak effect on the ensuing peak conductance (Fig. 8 *F*), whereas the voltage dependence of activation formed a similar saturating curve.

The magnitude of the neuron-to-neuron variations in the steady-state properties is better appreciated in Fig. 9. Here, with the use of identical protocols, peak conductance/voltage and availability curves were constructed from five cells, scaled and superimposed. In Fig. 9*A*, at half-maximum, the peak conductances bracket a 20-mV domain among cells. Most conductances began to activate at -30 mV. Yet, whereas some conductances saturated at $+20$ to $+40$ mV (filled circles), others showed no signs of saturation at up to $+50$ mV (filled squares and triangles). Because other cells showed steeper voltage dependence than the left-most curve shown, and because some curves shown are scaled to a nonsaturated peak, 20 mV is an underestimation of the true variation in steady-state voltage dependence that the underlying channel may exhibit. Figure 9*B* shows availability curves constructed from the same cells as in Fig. 9*A*, revealing a far greater variability in the sensitivity of the peak conductance to the conditioning stimulus. Whereas a conditioning potential of -60 mV could remove, in some cells, $>90\%$ of the conductance, in other cells, $<25\%$ of the conductance was removed. In the cell corresponding to the left-most availability curve (filled circles), at -140 mV, the line continues to slope upward to the left, suggesting that not all channels are primed for activation. In three cells, -140 mV did prime all channels for activation. Yet in one of those (filled triangles), the slope of the curve is significantly less steep, such that at the most positive conditioning potential tested (-30 mV), 30% of the peak conductance was still available for activation. These curves taken together reveal a 50-mV variation in their scaled half-maximal conductances. However, because some conductances either do not completely inactivate with a -30 -mV conditioning potential, or maximize the conductance at -140 mV, the true variation is greater.

KINETIC VARIATIONS—Figure 10*A* shows sodium currents recorded from seven different cells with the use of an identical stimulating protocol, scaled and superimposed. The kinetic variability exhibited across neurons studied is seen in Fig. 10*B*, which shows cumulative distributions of the half-times of activation and of inactivation, pooled from 27 experiments on small neurons. In all neurons the conditioning and test pulses were identical, -120 and $+20$ mV, respectively. Activation half-times encountered were virtually continuous over the 620- to 3,200- μ s domain. Inactivation half-times extended over nearly an order of magnitude, from 1.6 to 9.6 ms. Stability of the amplitude and time course is shown in Fig. 10*C*. Four current traces recorded from a cell with the use of identical stimulation protocols at 1.6, 5.5, 8.0, and 12.1 min after attainment of the whole cell clamp are shown. These are single current traces, not local averages, revealing that the amplitude and time course are preserved during this interval. The half-times to peak current varied by 130 μ s over the 12.1 min, a negligible drift compared with the 620- to 3,200- μ s domain over which such half-times were observed to vary among different neurons. Experiments were generally performed within 2–4 min from when the whole cell clamp was achieved, and great effort was made to maintain consistent electrical histories in each cell. Amplitude rundown and/or slow inactivation (Ogata and Tatebayashi 1992b) occurred over the course of tens of minutes (the dashed current trace in Fig. 10*C* was recorded at 17 min) and was best avoided by maintaining the cell at -100 mV resting potential, and using F^- as the major internal anion. Even at longer recording intervals, kinetic parameters remained stable. The baseline at the end of the current relaxation drifted downward. This may have resulted from fluctuations in the leak current over the course of the experiment. Another possibility was dialysis of the internal milieu by F^- ion, thus altering anion conductance. Thus, if the kinetic variations are the result of transitions among gating modes in a single protein, these could not be observed over the time period of a typical experiment. Furthermore, because the cytosolic contents were rapidly dialyzed out in this clamp configuration, the kinetic variations observed were not the result of an unknown soluble factor.

Analyses such as that shown in Fig. 11 were used as a control to further examine the validity of the kinetic and steady-state Na^+ current variations observed from neuron to neuron. Figure 11A shows two currents with similar but not identical on-off rates recorded at +20 mV, scaled and superimposed (solid traces). The slower current recorded at +50 mV, scaled and superimposed (dashed line), was a closer kinetic match to the faster current at +20 mV. If there was a 30-mV error in the recording conditions between experiments, due to, for example, series resistance differences or to an uncompensated junction potential, then the peak conductance/voltage curves constructed for each would be in error by approximately this much. However, when the corresponding conductances are scaled, superimposed, and upshifted to match the equivalent kinetic difference (Fig. 11B), the differences between the two peak conductance/voltage curves are exaggerated, not improved. This result and many others like it indicate that 10- to 30-mV kinetic differences among neurons were not the result of such recording artifacts.

Na^+ currents in large DRG neurons

Neurons in the 44- to 50- μm size range revealed a different repertoire of Na^+ currents. Figure 12 shows Na^+ currents recorded from two neurons that exemplify two typical patterns of current found in this size class. Depolarization to -20 mV in the neuron of Fig. 12A reveals a slowly activating and inactivating inward current. Stronger depolarizations elicit a rapidly activating and inactivating current superimposed on the slower current. Figure 12B shows Na^+ current recorded from another neuron with the use of an identical stimulating protocol, revealing only a fast component of current. Figure 12, C and D, shows Na^+ currents recorded at a fixed test potential preceded by a varying conditioning potential, from the same neurons respectively, as in Figs. 12, A and B. The fast variety of current in Fig. 12D becomes completely unavailable with conditioning potentials positive to -30 mV. Figure 12C reveals, however, that the two components of current have a different sensitivity to the conditioning potential. The fast component is virtually completely removed by conditioning potentials positive to -40 mV, whereas a substantial fraction of the slow current remains. By scaling and superimposing these currents (Fig. 12E), the two current components become visually separable. Although the faster current superimposes upon itself, the slow current comprises an ever increasing fraction of the net current, indicating that it is less sensitive to the conditioning potential. From a -30-mV conditioning potential, inactivation of the slow component is incomplete. Figure 12E may be contrasted to Fig. 6B, which revealed, in a small neuron, a single population of channels based on inactivation kinetics and sensitivity to the conditioning potential. The experiment of Fig. 12, B and D, and 13/13 others like it (Honmou et al. 1994), revealed the consistent presence of kinetically faster currents in larger (and more difficult to voltage clamp) cells. This indicates that the limits of the voltage-clamp capabilities did not contribute to the observed variations in the Na^+ currents among smaller neurons. More importantly, Fig. 12E reveals how, in the absence of pharmacological intervention, it is possible to separate currents in a single neuron on the basis of their kinetic differences. Because no specific blocker of the slow Na^+ channel has been found to our knowledge, such kinetic separations are the only means at hand to reveal multiple channel subpopulations in a single neuron.

Correlations of characteristic parameters

The variability of these conductances led us to examine the correlation of characteristic parameters of currents from different cells. Figure 13A shows that the inactivation half-time strongly correlated with the activation half-time ($r = 0.72$). From this correlation, one can make inferences regarding the microscopic gating scheme. Consistent with the data of Fig. 7B, it appears that a relatively voltage-independent inactivation rate is limited by a voltage-dependent activation rate (Aldrich et al. 1983). Further, the y-inter limit of the voltage-independent channel lifetime. In Fig. 13B, a weak correlation was present between the half-time of inactivation and the midposition voltage of current availability. This is consistent with the idea that conductances whose inactivation rate during a sustained depolarization were slow

exhibited more resilience to positive conditioning voltages. However, this correlation is weak and therefore supports the interpretation of Fig. 6B, in which kinetically separable currents are more likely than not to have a differential sensitivity to the conditioning potential.

A strict lack of correlation (Fig. 13C) was found between the midpoint voltages of steady-state activation and of inactivation ($r = 0.07$). Importantly, this demonstrates that the variations in these parameters from neuron to neuron were not due to local effects of the lipid membrane composition, or to surface charge, but were more likely due to differences in the actual channel molecules.

DISCUSSION

The kinetically slow Na^+ channel studied is within the family of TTX-resistant Na^+ channels known to be present in the membrane of small (predominantly C-type) DRG neurons (Caffrey et al. 1992; Elliott and Elliott 1993; Kostyuk et al. 1981; McLean et al. 1988; Roy and Narahashi 1992; Schwartz et al. 1990; Yoshida and Matsuda 1979). Three previously undescribed features of this Na^+ channel that emerge from these experiments are 1) gating properties are distinctive when compared with other TTX-resistant voltage-dependent Na^+ channels that have been described in DRG neurons, 2) the channel exhibits profound heterogeneity from neuron to neuron, and 3) each small neuron has a single variety of slow Na^+ channel in its somatic membrane (which contrasts with the 44- to 50- μm -diam neuron that can have two varieties of channel in its somatic membrane), in spite of the apparent capability to synthesize a broad library of channel molecules.

Gating properties

The Na^+ conductance studied here revealed voltage dependencies and time courses of activation and of inactivation that suggest that the underlying channel is in the family of S4-containing proteins. To activate the conductance, a conditioning potential negative to -30 mV (Fig. 2B) was necessary. The conductance continued to increase in a near linear fashion as the conditioning potential progressed to more hyperpolarized voltages, even in the -120 to -140 mV range. Activation occurred at stimulating potentials positive to -40 mV and often progressed to saturation in the $+10$ - to $+40$ -mV range. These results indicate that the kinetically slow Na^+ channel is weakly voltage dependent, as an e -fold change in conductance occurred per >20 -mV change in stimulating potential in most cases. In records where conductances did not saturate (Figs. 8C and 9A), the step voltage change required for an e -fold conductance increase was indeterminate, but minimally was ~ 80 mV. This may be inferred because, if we assume that the underlying population of Na^+ channels was uniform, an open probability of 0.5 was not achieved with a test pulse to $+20$ mV (Fig. 5F) wherein the variance did not exhibit a minimum in the vicinity of the peak mean current (Sigworth 1980). This contrasts with the neuron of Fig. 5, C and D, in which the variance did exhibit such a minimum.

The inactivation rate was slow (Fig. 6C), dependent on the stimulating voltage, but not on the conditioning pulse (Fig. 6B) or duration of the experiment (Fig. 10C). Results of gating studies in many of our neurons are comparable with what others have found in DRG neurons with respect to activation and inactivation kinetics (Kostyuk et al. 1981; Roy and Narahashi 1992). Voltage dependence of activation tended to be less steep in comparison with those in rat nodose neurons (Bossu and Feltz 1984) and in DRG neurons as reported by others (Elliott and Elliott 1993; Roy and Narahashi 1992). In DRG neurons with kinetically separable currents, McLean et al. (1988) found that a more depolarized conditioning potential removed the faster TTX-sensitive component, in keeping with the results of others (Caffrey et al. 1992; Elliott and Elliott 1993; Roy and Narahashi 1992) and with our findings in 44- to 50- μm -diam neurons (Fig. 12, C and E).

We observed that, when the whole cell patch-clamp configuration was formed and maintained at a hyperpolarized potential, such as -100 mV, the amplitude of the current slightly increased over a period of minutes (e.g., in Fig. 3B, current recorded after TTX exposure was slightly greater). We interpret this result as corresponding to the removal of a slow form of inactivation, as previously described in a similar DRG preparation (Ogata and Tatebayashi 1992b).

Novel closing properties

Our data suggest unique closing mechanisms. The closing properties, in addition to the above gating properties, set this channel apart from the TTX-sensitive Na^+ channel of mammalian axons (gating reviewed by Hille 1992) and give clues to our understanding of the relationship between the microscopic steps of activation and inactivation. Tail currents late in the depolarization, such as those of Fig. 7A, were present in 27/40 small neurons studied in which the slow Na^+ current was also present, and that unambiguously fully inactivated with strong depolarizations. The peaks of the tail currents increased with increasing peak conductance during the test pulse, even if the conductance fully inactivated over the course of the test pulse. We do not have a specific blocker of the underlying channel and cannot entirely rule out the possibility that the observed tail currents arise from the activation of another cation channel. Yet, such tail currents were never observed in 14/14 larger ($44\text{--}50\text{ }\mu\text{m}$ diam) neurons containing only a fast variety of channel (Fig. 12, B and D). Permeation of cations through Ca^{2+} -selective channels, although considered as a possible source of the tail currents, especially in light evidence that Cd^{2+} ion blocks Ca^{2+} channels poorly at hyperpolarized membrane voltages (Lansman et al. 1986; Swandulla and Armstrong 1989), was unlikely given the experimental conditions that very much favored Na^+ permeation. Other possibilities we considered included Na^+ ion accumulation and anomalous permeation through a K^+ channel, which seem unlikely in light of the results of Fig. 7B. Our data are thus consistent with the idea that a substantial fraction of the slow Na^+ channels can pass into the open state from an inactivated state upon membrane hyperpolarization. During a moderate test depolarization (Fig. 7B), when the current was relatively constant, the tail current accumulated amplitude. This, together with the correlation between inactivation half-times and activation half-times, is consistent with a microscopic gating scheme in which both inactivation and activation are limited by the same process, the voltage dependence of activation (Aldrich et al. 1983; Bezanilla and Armstrong 1977; Gonoi and Hille 1987).

Pathways of recovery through an open state have been described for cloned mammalian $I_{\text{K}}(\text{A})$ (RCK4-1) channels (Ruppersberg et al. 1991) and *Shaker* H4 K^+ channels (Demo and Yellen 1991). Ruppersberg et al. (1991) found a relatively slow closing rate (120 ms) in the absence of an inactivating particle ("ball peptide"), but a slower closing rate (730 ms) in the presence of the ball peptide. The time course of the tail currents was thus limited by the reopening of channels from the inactivated state rather than by deactivation. Because the area under the tail currents in both cases was comparable, it was concluded that channels must unbind the peptide and open before deactivation, i.e., they favor a strictly linear gating scheme of the form closed $\leftrightarrow$ open $\leftrightarrow$ inactivated.

Our results (Fig. 7) suggest that a significant fraction of inactivated channels pass through the open state during repolarization. Tail currents late in the depolarization, however, tended to be lower in magnitude than those measured during the peak of the current. It therefore appeared that a fraction of the channels did pass into an inactivated state from which deactivation occurred directly without opening. In the H4 K^+ channel (Demo and Yellen 1991) the time course of recovery from inactivation also limited the time course of the tail currents, although passage from the inactivated state directly to a closed state was permitted in a small fraction of their channels. Closing properties of the H4 K^+ channel are thus more in keeping with ours in favor of a cyclic gating scheme where recovery from inactivation is determined by both the

release of the putative gating particle, thus passing through an open state, and yet another, probably voltage-dependent (?ion dependent, Demo and Yellen 1991) deactivating transition, avoiding the open state. The implication for RCK4-1 K⁺ channel function proposed by Ruppersberg et al. (1991) was the capability of producing a long-lasting after hyperpolarization.

In contrast, the Na⁺ channel central to our study would appear to be capable of supporting a prolonged depolarization after adequate stimulation. This channel would not be expected to react to a single, narrow action potential because the marked delay (Fig. 5B) before opening represents an absorbing state (e.g., no inward or outward current was elicited with the 100- μ s depolarizing pulse in Fig. 7B). Its relatively weak voltage dependence and right-shifted steady-state inactivation curve suggest that the underlying channels could support prolonged, graded, subthreshold inward current responses to graded inputs. A channel of this form could contribute to the delayed depolarization observed in myelinated cutaneous afferent fibers of adult rat, which have been shown to contain both fast and slow components of Na⁺ current (Honmou et al. 1994) (Fig. 12, A and C).

A persistent inward Na⁺-selective current discovered in the membrane of cat neocortical neurons, in combination with a fast, TTX-sensitive current, was shown to participate in subthreshold electrical activities including prolongation of synaptically induced depolarizations (Stafstrom et al. 1982, 1985). Similarly, because the slow Na⁺ channel in small DRG neurons is not present in sufficient density on myelinated sensory axons to support regenerative activity in the presence of TTX (Caffrey et al. 1992; Yoshida et al. 1978), its function may also lie in signal transduction or modulation of impulse activity.

Intraneuronal channel homogeneity

More than one form of channel could not be distinguished in any of the small (18–25 μ m diam) cells analyzed ($n = 27$). Because pharmacological separation could not be accomplished with 1 μ M TTX, we were limited to a kinetic separation of currents in individual neurons. By varying the conditioning potential, one would expect two kinetic varieties to emerge, such as occurred in the 44- μ m-diam neuron shown in Fig. 12, C and E, if two types of Na⁺ channels were present in the membrane. In the neuron of Fig. 12, C and E, because the kinetically faster conductance was more sensitive to the conditioning voltage, scaling and superimposing the current records revealed the slower variety to contribute a greater fraction of the total conductance with successively more positive conditioning potentials. This is representative of our findings in a subpopulation of 44- to 50- μ m-diam DRG neurons, in which multiple voltage-dependent Na⁺ conductances are found (Honmou et al. 1994). Others have demonstrated that TTX may be used to separate currents in large DRG neurons (Roy and Narahashi 1992) such as the neuron shown in Fig. 12, A, C, and E.

Repeated searches for kinetic variations within the small neurons were prompted by the rather unorthodox appearing peak conductance/voltage curves and availability curves (e.g., the biphasic appearance of some availability curves in Fig. 9B), as well as the variance waveforms present in some neurons (Fig. 5, E and F). The weak correlation between the inactivation rate and the midpoint voltage of the availability curves suggest that the technique of Fig. 6B should reveal kinetically separable currents. Thus the most conservative interpretation of the data of Fig. 6B is that if multiple subpopulations of Na⁺ channels were present, their properties were either very similar, or they could not vary by more than one of these parameters: inactivation kinetics, activation kinetics, or sensitivity to the conditioning voltage. Such scaled currents could overwrite each other if one parameter varied, however, they would in turn have to be heterogeneous across all neurons studied.

High concentrations of divalent cations have had varying effects on this channel, including blockade (Bossu and Feltz 1984; Kostyuk et al. 1981; Roy and Narahashi 1992). However, because the effects of divalent cations on Na⁺ channel gating are known to be multiple, we elected to maintain divalent cation concentration as constant as possible. Thus, because we did not have a specific blocker for the slow Na⁺ channel, we had to rely on kinetic methods to separate currents in a single neuron. Further, the variance of an ensemble mean of currents recorded from some neurons (Fig. 5, *C* and *D*) had a minimum in the vicinity of the peak of the current, which is consistent with intraneuronal homogeneity (Conti et al. 1984). Although in some neurons the variance peaked then declined monotonically, this is consistent with an underlying population of Na⁺ channels with weaker voltage dependence (Fig. 5, *E* and *F*) than those in other neurons whose variance was of the form of Fig. 5, *C* and *D*, e.g., “interneuronal heterogeneity” may be demonstrated by comparing variance waveforms derived from different neurons. It appears likely that, in any small DRG neuron, there is a relatively homogeneous population of Na⁺ channels.

Interneuronal channel heterogeneity

Although the Na⁺ conductances of a given small neuron revealed homogeneous gating characteristics, considerable variations in the voltage dependencies (Figs. 8 and 9) and kinetics (Fig. 10) were observed among neurons. The fraction of the peak conductance available for activation, following a variable conditioning potential, spanned a >50-mV domain at half-maximum (Fig. 9*B*). All peak conductance versus voltage curves began to rise at −40 to −30 mV ($n = 40$), however, although some achieved saturation at positive voltages, others continued to rise, even at up to +50 mV ($n = 12$; Fig. 9*A*). At half-maximum, they bracketed >20 mV. Slow inactivation (Ogata and Tatebayashi 1992*b*) does not affect channel kinetics and is a static property of a given neuron. Under maintained hyperpolarization, our recordings were stable with respect to voltage dependence and amplitude (Fig. 10*C*), and therefore slow inactivation did not account for the heterogeneity we observed among neurons. Variations in the steady-state properties observed among neurons was unlikely to result from series resistance artifact, local surface charge variations, or an uncompensated junction potential because 1) all conductances began to rise over a rather narrow window (−40 and −30 mV; Fig. 9*A*); 2) the midposition voltages of peak conductance did not correlate with those of inactivation (Fig. 13*C*); and 3) attempts to correct the position of the peak conductance/voltage curves by equivalent kinetic shifts (Fig. 11) tended to exaggerate rather than reduce the differences.

Considerable kinetic variations were observed as well, illustrated by the cumulative distributions of activation and inactivation half-times (Fig. 10*B*). The kinetic properties were stable over the course of the experiments (Fig. 10*C*) and were unaffected by replacement of the cytoplasm with the inert pipette solutions. Activation half-times varied up to fivefold, and inactivation half-times varied over nearly an order of magnitude. The differences observed among neurons were not the result of inadequate space clamp, because kinetically faster conductances could consistently be recorded from larger neurons under less favorable space-clamp conditions (Fig. 12, *B* and *D*). Moreover, the differences observed were unlikely to be due to the dissociation procedure including enzymatic treatments. Howe and Ritchie (1990) observed a reduction in saxitoxin-binding capacity and peak Na⁺ current in Schwann cells exposed to *extracellular* collagenase plus Dispase. There were no significant differences in voltage-dependent parameters or times-to-peak of the Na⁺ currents in control versus enzyme-treated cells. Non-membrane-permeant chemical agents that modify Na⁺ channel gating generally require *intracellular* exposure (reviewed by Catterall 1980; Hille 1992). In our experiments, many DRG neurons whose Na⁺ conductances were compared were on the same culture dish and subject to the same *extracellular* exposure to enzymes. One would have to make the unlikely hypothesis that all Na⁺ channels in a given neuron were uniformly affected, but in a different way from those in adjacent neurons. Others have identified multiple channel

populations on the basis of kinetics in similar cells (Caffrey et al. 1992; Schwartz et al. 1990). It thus appears that the neuron-to-neuron heterogeneity of the Na⁺ conductances that we observed is the result of heterogeneity of the channel molecules.

There is precedent for electrophysiological heterogeneity of Na⁺ channels in invertebrate and vertebrate preparations: electroplax channels studied in planar lipid bilayers (Moczydlowski et al. 1984; Recio-Pinto et al. 1990), squid axon (Gilly and Armstrong 1984; Matteson and Armstrong 1982), immature rat skeletal muscle (Weiss and Horn 1986), cat sensorimotor cortical neurons (Stafstrom et al. 1985), and rat striatum, hippocampus (Hoehn et al. 1993), and neocortex (Huguenard et al. 1988). Two questions that then arise are as follows: what structural variations of the Na⁺ channel molecule are capable of conferring physiological heterogeneity and why? Many differences in Na⁺ channel structure have been identified among phyla, among different tissues within a species, and within a single mammalian tissue. Our results in DRG neurons fall into the latter category. Structural heterogeneity has been shown to arise from 1) expression of different genes, 2) differential splicing of RNA transcripts, 3) heteromultimeric assembly, 4) the extent of glycosylation, and 5) phosphorylation. It appears that established mechanisms for structural heterogeneity within a species of ion channel could contribute to the behavioral heterogeneity among Na⁺ channels that we observed in small DRG neurons.

Mechanism 2 seems plausible in light of the fact structural alterations in the primary amino acid structure of the α -subunit can lead to differential toxin sensitivity (Noda et al. 1989), shifts of steady-state voltage dependence of activation and/or of inactivation (Auld et al. 1990; Stühmer et al. 1989), and kinetic slowing (Stühmer et al. 1989; Vassilev et al. 1988). Differential splicing of RNA transcripts has been shown to be operant in the transient K⁺(A) channel of the *Shaker* mutant of *Drosophila* (Papazian et al. 1987; Schwarz et al. 1988) where the behavioral consequences were found to be the heterogeneity in the inactivation kinetics (Timpe et al. 1990). Diversity of the transient K⁺(A) channel properties in central neurons of rat included variation in the inactivation rate, and in the midposition voltage of the availability curves (Rizzo and Nonner 1992), which comprised some of our findings in the Na⁺ conductance of small DRG neurons.

Alternative splicing has recently been found to confer remarkable structural heterogeneity upon *Drosophila* sodium channels as identified from polymerase chain reaction (PCR) amplification of the *para* transcript (Thackeray and Ganetzky 1994); the number of possible isoforms was minimally 48. Structural heterogeneity conferred by the differential splicing of RNA transcripts has been described as well for Na⁺ channels in rat skeletal muscle (Schaller et al. 1992) and in rat brain (Gustafson et al. 1993; Schaller et al. 1992), although so far has not been shown to be as extensive. This mechanism may invoke even a single charged amino acid substitution, causing marked changes in ion permeation or voltage dependence (Gustafson et al. 1993). Differential splicing of RNA transcripts therefore seems to be a likely candidate for the mechanism by which neurons could confer Na⁺ channel structural heterogeneity.

Mechanism 3 is plausible, because it has been established that, when the Na⁺ channel β -subunit is coexpressed with the α -subunit, the steady-state inactivation curve is shifted to more hyperpolarized voltages (Isom et al. 1992). However, few β -subunits have been identified (reviewed by Barchi 1988), and they alone are not adequate to explain the extent of the heterogeneity observed. One would have to invoke, in addition, one of the first two mechanisms.

Mechanism 4 is less plausible. The diffuse appearance on SDS-PAGE of the 260-kDa band purified from *Electrophorus electricus* was interpreted by Miller et al. (1983) as resulting from variations in the extent of glycosylation of the channel, i.e., in structural microheterogeneity.

Exposure to neuraminidase of purified, batrachotoxin-treated electroplax Na⁺ channels studied in planar lipid bilayers (Recio-Pinto et al. 1990) caused a significant (+30 mV) shift in the peak open probabilities, and interestingly, a dispersion of the distribution of the midpoint voltages of steady-state activation and of inactivation. The authors felt that either complete removal of the sialic acid residues influenced the local electric field, or random partial desialidation led to a large variety of stable positional changes in the associated channel domains. The first hypothesis contrasts with the interpretation of our results that argues against a purely electrostatic effect. Our observations would be more in keeping with the second hypothesis but would mandate a highly controlled system for uniform sialidation in a given neuron. Yet it appears that existing data regarding the effects of glycosylation on Na⁺ channel gating does not allow for the magnitude of the independent differences we observed in some of the characteristic parameters, such as the order of magnitude variations of the inactivation half-time.

There is not enough information regarding the consequences of channel phosphorylation (*mechanism 5*) to say if or not this could explain our observations. Mammalian Na⁺ channels contain a 200-amino acid peptide between internal repeats I and II that contains six to eight potential phosphorylation sites (Rossie et al. 1987). Phosphorylation by adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinases reduces the peak Na⁺ conductance (Li et al. 1992) and slows inactivation (Numann et al. 1991) in cloned type IIA Na⁺ channels and in native Na⁺ channels of cultured embryonic rat brain. The correlation exhibited between the half-times of inactivation and of activation in DRG neurons (Fig. 13A) argue against phosphorylation, a mechanism so far shown to confer isolated slowing of inactivation. This and the fact that the channel properties remained stable in spite of dialysis of the cytosol (Fig. 10C) make *mechanism 5* less tenable than 2.

We interpret our observation of significant diversity of Na⁺ conductances as suggesting structural diversity of Na⁺ channel molecules within the different neurons comprising a single class of DRG neurons. However, it appears that each neuron deploys a single population of channel to its membrane. Thus each neuron displays channel homogeneity, whereas the neuron population as a whole displays channel heterogeneity. Because the gating properties are consistent with a channel that could respond in a graded fashion to excitatory stimuli, this favors the idea that the entire neuronal membrane can respond in a synchronized manner, uniformly conditioning the neuron for subsequent depolarization. Alternatively, if this Na⁺ channel occupies a presynaptic site, it could support a prolonged depolarization of graded duration, permitting release of neurotransmitter in proportion to axonal discharge frequency. According to this scheme, each neuron would be tuned by virtue of the type of channel expressed. Moreover, neuron-to-neuron variability in slow Na⁺ channel properties would be expected to confer, on the entire population of sensory neurons, a dynamic response range that is far broader than that of a single neuron.

Acknowledgements

We are grateful to H.-F. Mi for providing and maintaining neuronal cultures.

This work was supported in part by grants from the National Institute of Neurological Disorders and Stroke (NINDS), the National Multiple Sclerosis Society, and by the Medical Research Service, Department of Veterans Affairs. M. A. Rizzo was supported in part by a Clinician/Investigator Development Award from the NINDS, and by a Spinal Cord Research Fellowship from the Eastern Paralyzed Veterans Association.

References

- Aldrich RW, Corey DP, Stevens CF. A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature Lond* 1983;306:436–441. [PubMed: 6316158]

- Auld VJ, Goldin AL, Krafte DS, Catterall WA, Lester HA, Davidson N, Dunn RJ. A neutral amino acid change in segment IIS4 dramatically alters the gating properties of the voltage-dependent sodium channel. *Proc Natl Acad Sci USA* 1990;87:323–327. [PubMed: 1688658]
- Barchi RL. Probing the molecular structure of the voltage-dependent sodium channel. *Annu Rev Neurosci* 1988;11:455–495. [PubMed: 2452597]
- Bezanilla F, Armstrong CM. Inactivation of the sodium channel. I. Sodium current experiments. *J Gen Physiol* 1977;70:549–566. [PubMed: 591911]
- Bossu JL, Feltz A. Patch-clamp study of the tetrodotoxin-resistant sodium current in group C sensory neurones. *Neurosci Lett* 1984;51:241–246. [PubMed: 6096775]
- Brown AM, Lee KS, Powell T. Voltage-clamp and internal perfusion of single rat heart muscle cells. *J Physiol Lond* 1981;318:455–477. [PubMed: 7320901]
- Caffrey JM, Eng DL, Black JA, Waxman SG, Kocsis JD. Three types of sodium channels in adult rat dorsal root ganglion neurons. *Brain Res* 1992;592:283–297. [PubMed: 1280518]
- Campbell D. Large and small vertebrate sensory neurons express different Na and K channel subtypes. *Proc Natl Acad Sci USA* 1992;89:9569–9573. [PubMed: 1329102]
- Carbone E, Lux HD. Kinetics and selectivity of a low-voltage-activated calcium current in chick and rat sensory neurones. *J Physiol Lond* 1987;386:547–570. [PubMed: 2445968]
- Catterall WA. Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu Rev Pharmacol Toxicol* 1980;20:15–40. [PubMed: 6247957]
- Conti F, Hille B, Nonner W. Non-stationary fluctuations of the potassium conductance at the node of Ranvier of the frog. *J Physiol Lond* 1984;353:199–230. [PubMed: 6090640]
- Demo SD, Yellen G. The inactivation gate of the *Shaker* K⁺ channel behaves like an open channel blocker. *Neuron* 1991;7:743–753. [PubMed: 1742023]
- Elliott AA, Elliott JR. Characterization of TTX-sensitive and TTX-resistant sodium currents in small cells from adult rat dorsal root ganglia. *J Physiol Lond* 1993;463:39–56. [PubMed: 8246189]
- Frelin C, Cognard C, Vigne P, Lazdunski M. Tetrodotoxin-sensitive and tetrodotoxin-resistant Na channels differ in their sensitivity to Cd and Zn. *Eur J Pharmacol* 1986;122:245–250. [PubMed: 2423344]
- Fukuda J, Kameyama M. Tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in tissue cultured spinal ganglion neurons from adult mammals. *Brain Res* 1980;182:191–197. [PubMed: 7350989]
- Gilly WF, Armstrong CM. Threshold channels—a novel type of sodium channel in squid giant axon. *Nature Lond* 1984;309:448–450. [PubMed: 6328313]
- Gonoi T, Hille B. Gating of Na channels. Inactivation modifiers discriminate among models. *J Gen Physiol* 1987;89:253–274. [PubMed: 2435840]
- Gustafson TA, Clevinger EC, O'Neill TJ, Yarowsky PJ, Krueger BK. Mutually exclusive exon splicing of type III brain sodium channel α -subunit RNA generates developmentally regulated isoforms in rat brain. *J Biol Chem* 1993;268:18648–18653. [PubMed: 8395514]
- Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch* 1981;391:85–100. [PubMed: 6270629]
- Harris JB, Thesleff S. Studies on tetrodotoxin-resistant action potentials in denervated skeletal muscle. *Acta Physiol Scand* 1971;83:382–388. [PubMed: 5134176]
- Heinemann SH, Terlau H, Stühmer W, Imoto K, Numa S. Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature Lond* 1992;356:441–443. [PubMed: 1313551]
- Hille, B. *Ionic Channels of Excitable Membranes*. 2. Sunderland, MA: Sinauer; 1992. p. 472–503. p. 445–471. p. 321–326.
- Hoehn K, Watson TWJ, MacVicar BA. A novel tetrodotoxin-insensitive, slow sodium current in striatal and hippocampal neurons. *Neuron* 1993;10:543–552. [PubMed: 8461141]
- Honmou O, Utzschneider DA, Rizzo MA, Bowe CM, Waxman SG, Kocsis JD. Delayed depolarization and slow sodium currents in cutaneous afferents. *J Neurophysiol* 1994;71:1627–1637. [PubMed: 8064338]

- Howe JR, Ritchie JM. Sodium currents in Schwann cells from myelinated and non-myelinated nerves of neonatal and adult rabbits. *J Physiol Lond* 1990;425:169–210. [PubMed: 2170628]
- Huguenard JR, Hamill OW, Prince DA. Developmental changes in Na⁺ conductances in rat neocortical neurons: appearance of a slowly inactivating component. *J Neurophysiol* 1988;59:778–795. [PubMed: 2452862]
- Ikeda SR, Schofield GG. Tetrodotoxin-resistant sodium current of rat nodose neurones: monovalent cation selectivity and divalent cation block. *J Physiol Lond* 1987;389:255–270. [PubMed: 2445974]
- Isom LL, De Jongh KS, Patton DE, Reber BFX, Offord J, Charbonneau H, Walsh K, Goldin AL, Catterall WA. Primary structure and functional expression of the $\beta 1$ subunit of the rat brain sodium channel. *Science Wash DC* 1992;256:839–842.
- Jan LY, Jan YN. How might the diversity of potassium channels be generated? *Trends Neurosci* 1990;13:415–419. [PubMed: 1700515]
- Kayano T, Noda M, Flockerzi V, Takahashi H, Numa S. Primary structure of rat brain sodium channel III deduced from the cDNA sequence. *FEBS Lett* 1988;228:187–194. [PubMed: 2449363]
- Kostyuk PG, Veselovsky NS, Tsyndrenko AY. Ionic currents in the somatic membrane of rat dorsal root ganglion neurons. I. Sodium currents. *Neuroscience* 1981;6:2423–2430. [PubMed: 6275294]
- Lansman JB, Hess P, Tsien RW. Blockade of current through single calcium channels by Cd⁺⁺, Mg⁺⁺, and Ca⁺⁺. *J Gen Physiol* 1986;88:321–347. [PubMed: 2428920]
- Leybaert L, De Hemptinne A. A voltage-clamp study of calcium currents in neurons freshly isolated from the dorsal root ganglion of adult rats. *Arch Int Physiol Biochim Biophys* 1993;101:315–323. [PubMed: 7508292]
- Li M, West JW, Lai Y, Scheuer T, Catterall WA. Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. *Neuron* 1992;8:1151–1159. [PubMed: 1319185]
- Matteson DR, Armstrong CM. Evidence for a population of sleepy sodium channels in squid axon at low temperature. *J Gen Physiol* 1982;70:739–758. [PubMed: 6284858]
- McLean MJ, Bennett PB, Thomas RM. Subtypes of dorsal root ganglion neurons based on different inward currents as measured by whole-cell voltage clamp. *Mol Cell Biochem* 1988;80:95–107. [PubMed: 3173341]
- Miller JA, Agnew WS, Levinson SR. Principle glycopeptide of the tetrodotoxin/saxitoxin binding protein from *Electrophorus electricus*. Isolation and partial chemical and physical characterization. *Biochemistry* 1983;22:462–470. [PubMed: 6186277]
- Moczydlowski E, Hall S, Garber SS, Strichartz GS, Miller C. Voltage-dependent blockade of muscle Na⁺ channels by guanidinium toxins. *J Gen Physiol* 1984;84:687–704. [PubMed: 6096479]
- Neumcke B. Diversity of sodium channels in adult and cultured cells, in oocytes and in lipid bilayers. *Rev Physiol Biochem Pharmacol* 1990;15:1–49. [PubMed: 2158141]
- Noda M, Ikeda T, Kayano T, Suzuki H, Takeshima H, Kurasaki M, Takahashi H, Numa S. Existence of distinct sodium channel messenger RNAs in rat brain. *Nature Lond* 1986a;320:188–192. [PubMed: 3754035]
- Noda M, Ikeda T, Suzuki H, Takeshima H, Takahashi T, Kuno M, Numa S. Expression of functional sodium channels from cloned cDNA. *Nature Lond* 1986b;322:826–828. [PubMed: 2427955]
- Noda M, Suzuki H, Numa S, Stühmer W. A single point mutation confers tetrodotoxin and saxitoxin insensitivity on the sodium channel II. *FEBS Lett* 1989;259:213–216. [PubMed: 2557243]
- Numann R, Catterall WA, Scheuer T. Functional modulation of brain sodium channels by protein kinase C phosphorylation. *Science Wash DC* 1991;254:115–118.
- Ogata N, Tatebayashi H. Ontogenic development of the TTX-sensitive and TTX-insensitive Na⁺ channels in neurons of the rat dorsal root ganglia. *Dev Brain Res* 1992a;65:93–100. [PubMed: 1312915]
- Ogata N, Tatebayashi H. Slow inactivation of tetrodotoxin-insensitive Na⁺ channels in neurons of rat dorsal root ganglia. *J Membr Biol* 1992b;129:71–80. [PubMed: 1328647]
- Papazian DM, Schwarz TL, Tempel BL, Jan YNJ, Jan LY. Cloning of genomic and complementary DNA from *Shaker*, a putative potassium channel gene from *Drosophila*. *Science Wash DC* 1987;237:749–753.
- Pappone PA. Voltage-clamp experiments in normal and denervated mammalian skeletal muscle fibres. *J Physiol Lond* 1980;306:377–410. [PubMed: 6257898]

- Ransom BR, Holz RW. Ionic determinants of excitability in cultured mouse dorsal root ganglion and spinal cord cells. *Brain Res* 1977;136:445–453. [PubMed: 922495]
- Recio-Pinto E, Thornhill WB, Duch DS, Levinson SR, Urban BW. Neuraminidase treatment modifies the function of *Electroplax* sodium channels in planar lipid bilayers. *Neuron* 1990;5:675–684. [PubMed: 2171591]
- Rizzo MA, Nonner W. Transient K current in the somatic membrane of cultured central neurons of embryonic rat. *J Neurophysiol* 1992;68:1708–1719. [PubMed: 1479440]
- Rizzo MA, Waxman SG, Kocsis JD. Heterogeneity of voltage-dependent Na⁺ conductance in dorsal root ganglion neurons of adult rat. *Soc Neurosci Abstr* 1993;19:1528.
- Rogart RB, Cribbs LL, Muglia K, Kephart DD, Kaiser MW. Molecular cloning of a putative tetrodotoxin-resistant rat heart Na⁺ channel isoform. *Proc Natl Acad Sci USA* 1989;86:8170–8174. [PubMed: 2554302]
- Rose RD, Koerber HR, Sedivec MJ, Mendell LM. Somal action potential duration differs in identified primary afferents. *Neurosci Lett* 1986;63:259–264. [PubMed: 3951752]
- Rossie S, Gordon D, Catterall WA. Identification of an intracellular domain of the sodium channel having multiple cAMP-dependent phosphorylation sites. *J Biol Chem* 1987;262:17530–17535. [PubMed: 2447073]
- Roy ML, Narahashi T. Differential properties of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in rat dorsal root ganglion neurons. *J Neurosci* 1992;12:2104–2111. [PubMed: 1318956]
- Ruppersberg JP, Frank R, Pongs O, Stocker M. Cloned neuronal I_{K(A)} channels reopen during recovery from inactivation. *Nature Lond* 1991;353:657–660. [PubMed: 1922383]
- Schaller KL, Krzemien DM, McKenna NM, Caldwell JH. Alternatively spliced sodium channel transcripts in brain and muscle. *J Neurosci* 1992;12:1370–1381. [PubMed: 1313493]
- Schwartz A, Palti Y, Meiri H. Structural and developmental differences between three types of Na channels in dorsal root ganglion cells of newborn rats. *J Membr Biol* 1990;116:117–128. [PubMed: 2166163]
- Schwarz TL, Tempel BL, Papazian DM, Jan YN, Jan LY. Multiple potassium channel components are produced by alternative splicing at the *Shaker* locus in *Drosophila*. *Nature Lond* 1988;331:137–142. [PubMed: 2448635]
- Sigworth FJ. The variance of sodium current fluctuations at the node of Ranvier. *J Physiol Lond* 1980;307:97–129. [PubMed: 6259340]
- Sommer B, Seeburg PH. Glutamate receptor channels: novel properties and new clones. *Trends Pharmacol Sci* 1992;13:291–296. [PubMed: 1380742]
- Spitzer NC. Ion channels in development. *Annu Rev Neurosci* 1979;2:363–397. [PubMed: 395884]
- Stafstrom CE, Schwindt PC, Chubb MC, Crill WE. Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex in vitro. *J Neurophysiol* 1985;53:153–170. [PubMed: 2579215]
- Stafstrom CE, Schwindt PC, Crill WE. Negative slope conductance due to a persistent subthreshold sodium current in cat neocortical neurons in vitro. *Brain Res* 1982;236:221–226. [PubMed: 6279236]
- Stühmer W, Conti F, Suzuki H, Wang X, Noda M, Yahagi N, Kubo H, Numa S. Structural parts involved in activation and inactivation of the sodium channel. *Nature Lond* 1989;339:597–603. [PubMed: 2543931]
- Suzuki H, Beckh S, Kubo H, Yahagi N, Ishida H, Kayano T, Noda M, Numa S. Functional expression of cloned cDNA encoding sodium channel III. *FEBS Lett* 1988;228:195–200. [PubMed: 2449364]
- Swandulla D, Armstrong CM. Calcium channel block by cadmium in chicken sensory neurons. *Proc Natl Acad Sci USA* 1989;86:1736–1740. [PubMed: 2537985]
- Thackeray JR, Ganetzky B. Developmentally regulated alternative splicing generates a complex array of *Drosophila para* sodium channel isoforms. *J Neurosci* 1994;14:2569–2578. [PubMed: 8182428]
- Timpe LC, Schwarz TL, Tempel BL, Papazian DM, Jan YN, Jan LY. Expression of functional potassium channels from *Shaker* cDNA in *Xenopus* oocytes. *Nature Lond* 1990;331:143–145. [PubMed: 2448636]
- Trimmer JS, Agnew WS. Molecular diversity of voltage-sensitive Na⁺ channels. *Annu Rev Physiol* 1989;51:401–418. [PubMed: 2540698]

- Trimmer JS, Cooperman SS, Tomiko SA, Zhou J, Crean SM, Boyle MB, Kallen RG, Sheng Z, Barchi RL, Sigworth FJ, Goodman RH, Agnew WS, Mandel G. Primary structure and functional expression of a mammalian skeletal muscle sodium channel. *Neuron* 1989;3:33–49. [PubMed: 2559760]
- Vassilev P, Scheuer T, Catterall WA. Identification of an intracellular peptide segment involved in sodium channel inactivation. *Science Wash DC* 1988;241:1658–1661.
- Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B. Structural determinants of ion flow through recombinant glutamate receptor channels. *Science Wash DC* 1991;252:1715–1718.
- Weiss RE, Horn R. Functional differences between two classes of sodium channels in developing rat skeletal muscle. *Science Wash DC* 1986;233:361–364.
- White G. Heterogeneity in EC_{50} , and n_H of $GABA_A$ receptors on dorsal root ganglion neurons freshly isolated from adult rats. *Brain Res* 1992;585:56–62. [PubMed: 1324774]
- Yoshida S, Matsuda Y. Studies on sensory neurons of the mouse with intracellular-recording and horseradish peroxidase-injection techniques. *J Neurophysiol* 1979;42:1134–1145. [PubMed: 479922]
- Yoshida S, Matsuda Y, Samejima A. Tetrodotoxin-resistant sodium and calcium components of action potentials in dorsal root ganglion cells of adult mouse. *J Neurophysiol* 1978;41:1096–1106. [PubMed: 702189]
- Zhu Y, Ikeda SR. Anomalous permeation of Na^+ through a putative K^+ channel in rat superior cervical ganglion neurones. *J Physiol Lond* 1993;468:441–461. [PubMed: 8254517]

FIG. 1.

Adult rat dorsal root ganglion (DRG) neurons, as visualized by video photomicroscopy, after 1 day in culture. Scale bar is 100 μm . Several small, spherical 18- to 25- μm -diam neurons without processes can be seen in the center of this field (arrows). These neurons, used for most experiments, had a predominant tetrodotoxin (TTX)-resistant, slow Na^+ conductance, the subject of this paper. Larger neurons (44–50 μm diam, *) predominantly contained TTX-sensitive, fast Na^+ conductances.

FIG. 2.

Na⁺ currents recorded from a 22-μm-diam DRG neuron. *A*: from a holding potential of -60 mV, the cell was preconditioned at -120 mV for 150 ms, then depolarized to -50 to +50 mV in steps of 10 mV at $t = 0$. *B*: same cell was preconditioned for 150 ms from -140 to -30 mV in steps of 10 mV, then depolarized to +20 mV. *C*: corresponding peak conductance/voltage relationship (■) and availability curves (steady-state inactivation, ●) are plotted. *Solutions EI/II*. Temperature 19°C.

FIG.3.

Sensitivity to TTX of Na^+ current in (A) a 48- μm -diam neuron, and (B) a 20- μm -diam neuron. A: cell was preconditioned at -100 mV for 150 ms, then depolarized to $+20$ mV at $t = 0$ before and after exposure to $1 \mu\text{M}$ TTX. Solutions E2/I2; temperature 20°C . A small amount of remaining outward current probably represents noninactivating voltage-dependent K^+ current persisting as a result of incomplete dialysis of the intracellular contents by Cs^+ ion from the pipette. In contrast to the cell in A, B reveals a TTX-resistant, kinetically slower Na^+ current. B: neuron was preconditioned at -100 mV, then depolarized to $+20$ mV, before and after exposure to $1 \mu\text{M}$ TTX. Solutions E3/I3; temperature 20°C .

FIG. 4.

Na^+ selectivity. Peak currents, recorded at the indicated test potential from a conditioning potential of -120 mV, are plotted with external 150 mM Na^+ (triangles, solution E1), then with 30 mM Na^+ and 110 mM TMA^+ ($\circ$, solution E3). Internal solution I3 (5 mM Na^+). The reversal potential shifts -30 mV with external TMA^+ , consistent with a permeability ratio $P_{\text{TMA}^+}/P_{\text{Na}^+} = 0.086$.

FIG. 5.

Activation kinetics. *A*: currents of Fig. 2*A*, at test depolarizations to -20 to $+50$ mV in steps of 10 mV, are scaled and superimposed. *B*: corresponding delays (●; time to 10% peak) and half-times (○; time to 50% peak) are plotted against test potential. *C* and *D*: time courses of the (inverted) mean Na⁺ current (—) and ensemble variance (+++) at 2 different time scales. A DRG neuron was subjected to 136 stimulations of a single pulse protocol applied every 5 s. Holding potential -100 mV; conditioning potential -120 mV; test potential $+20$ mV. *Solutions E3/I3*. Low-pass filter setting, 20 kHz. A background variance of 24 pA^2 was subtracted before scaling. The variance was scaled to superimpose the mean current at the end of the pulse with the use of a scale factor of $0.38/\text{pA}$. *E* and *F*: the same pulse protocol was applied 100 times to a different cell, yielding a qualitatively different result in that the variance did not exhibit a minimum near the peak of the mean. *Solutions E3/I3*. Low-pass filter setting, 20 kHz. A background variance of 18 pA^2 was subtracted before scaling. Scale factor was $0.05/\text{pA}$.

FIG. 6.

Inactivation kinetics. *A*: currents of Fig. 2*A*, at test potentials from -20 to $+50$ mV, were scaled and superimposed to reveal the relaxation phases of the current. *B*: currents of Fig. 2*B* were scaled to their peaks and are seen to superimpose with respect to time course. *C*: half-times to inactivation are plotted against test potential.

FIG. 7.

Closing properties. *A*: Na^+ currents recorded in a DRG neuron at test potentials from -50 to $+60$ mV in steps of 10 mV, from a 100 -ms conditioning potential of -140 mV. Tail currents were recorded at -100 mV. The gradually increasing magnitude of the inward tail current occurred with increasing prior depolarization. Bold trace was recorded at $+60$ -mV depolarization, indicating how in this and many other experiments, maximal inward tail current followed what appeared to be a fully unavailable condition of the underlying channels. *Solutions* E3/I3; temperature 19°C ; low-pass filter setting 10 kHz; each record is the average of 4 traces. *B*: tail currents recorded from another small neuron. In this experiment, the neuron was repolarized to -140 mV at 100 μs , 1.5 , 3 , and 5 ms into depolarizing pulses to 0 mV.

Conditioning potential: -140 mV. No inward current is observed after the $100\text{-}\mu\text{s}$ pulse. Repolarization from the ensuing 3 pulses results in a gradual increase in the amplitude of the tail current. *Solutions* E3/I2; temperature 19°C ; low-pass filter setting 20 kHz; each record is the average of 4 traces.

FIG. 8.

Heterogeneity: peak conductance/voltage relationships. Activation and availability protocols, analogous to those of Fig. 2, applied to 2 other neurons. In the 1st neuron (A–C) the peak conductance/voltage relationship gradually increases with increasing depolarization (●, C). In the 2nd neuron (D–F) the peak conductance was saturable (●, F). The Na^+ conductance in the 2nd neuron was considerably less sensitive to the conditioning potential (E, ▲ in F) than the 1st (B, ▲ in C). In both experiments the solutions were *E1/I1*; temperature 18°C; low-pass filter setting 10 kHz.

FIG. 9.

Heterogeneity: peak conductance/voltage relationships in 5 neurons. Identical stimulation protocols applied to 5 different neurons yielded the 5 peak conductance/voltage relationships (A) and availability curves (B) shown. Curves were scaled to their peaks. Matching symbols in A and B correspond to the same neuron. Neurons were held at -60 mV, and in A, conditioned at -120 mV for 150 ms, then depolarized to the test pulse shown (abscissa). In B the cells were depolarized to $+20$ mV from a variable prepulse (abscissa). *Solutions E1/II*; temperature 18 – 20°C ; low-pass filter setting 10 kHz.

FIG. 10.

Heterogeneity: kinetics. *A*: current records from 7 different neurons recorded under identical conditions and protocols. Cells were held at -60 mV, preconditioned at -100 mV for 150 mS, then depolarized to $+20$ mV. Each record is the average of 4 traces and is scaled to the peak inward current. *Solutions E1/I1*; temperature 18 – 20°C ; low-pass filter setting 10 kHz. *B*: distributions of activation ($\circ$) and inactivation ($\square$) half-times recorded at $+20$ -mV test potential from 27 different small DRG neurons, plotted as cumulative distributions. A virtual continuum of half-times was encountered. Note that the activation and inactivation half-times at the same level were not obtained from the same neuron; examples of half-times derived from the same neuron are shown with connecting lines. *C*: kinetics were stable in a given cell. Four individual current traces (not local averages) in a single neuron recorded at 1.6, 5.5, 8.0, and 12.1 min from when the whole cell clamp configuration was obtained are shown. Holding potential, -100 mV; conditioning potential, -120 mV; test potential, $+20$ mV. *Solutions E3/I3*; temperature 18°C ; low-pass filter setting 20 kHz. Dashed trace was recorded at 17 min, indicating the beginning of Na^{+} current rundown.

FIG. 11.

Differences in the peak conductance/voltage relationship between neurons cannot be corrected for by equivalent kinetic corrections. *A*: Na^+ currents recorded from 2 cells (*cell 27* and *cell 33*) with similar but not identical kinetics are compared. Solid traces are scaled currents, recorded at +20 mV test potential from -120 mV conditioning potential. Dashed trace represents the Na^+ current recorded from *cell 33* at +50 mV, a closer kinetic match to *cell 27* at +20 mV. *B*: absolute peak conductance/voltage relationships in *cell 27* (■) and *cell 33* (●). The peak conductance of *cell 27* was upscaled (□) to match the peak of *cell 33*, then shifted by 30 mV (Δ) to match the kinetic correction from *A*. The difference in the peak conductance/

voltage relationship becomes exaggerated, not improved upon. *Solutions E1/I1*; temperature 19°C; low-pass filter setting 10 kHz; each record is the average of 4 traces.

FIG. 12.

Na⁺ currents recorded from 2 larger (44–50 μm diam) neurons. *A*: inward Na⁺ currents were recorded from a 44- μm -diam neuron at -40 to $+20$ mV in steps of 10 mV, from a conditioning potential of -100 mV. The inward current appears to have 2 kinetic components. The same stimulation protocol in another neuron (48 μm diam) reveals a singular, kinetically faster Na⁺ current. *C* and *D*: records obtained from the respective neurons by varying the 150-ms conditioning potential from -120 to -30 mV in steps of 10 mV, to a $+20$ -mV test potential. *E*: currents of *C* are scaled and superimposed to reveal how the kinetically slow current comprised a relatively greater fraction of the current trace with positive conditioning potentials. Current traces recorded from a -30 -mV conditioning potential are so labeled. *Solutions E2/II*; temperature 19°C ; low-pass filter setting 10 kHz; each record is the average of 4 traces.

FIG. 13.

Correlograms among characteristic parameters. *A*: half-times to Na^+ current inactivation correlated positively with those of activation ($r = 0.72$). Inactivation half-times were calculated as the difference between the time to half-inactivation and the time to peak to avoid biasing the data. Without this correction, the coefficient of correlation was slightly higher. The line is a linear regression with a slope of 1.4 and y-intercept 0.42. Data for *A–C* were pooled from 27 experiments in which identical stimulation protocols and recording conditions were utilized. The conditioning pulse was -120 mV; the test pulse was $+20$ mV. *B*: inactivation half-times correlated weakly with the voltages of half-inactivation ($r = 0.40$). The line is a linear regression whose slope corresponds to an e -fold change of half-time per 143 mV. *C*: midpoint voltage of the availability curves did not correlate with midpoint of the peak conductance/voltage relations ($r = 0.08$). Solutions E1/II; temperature 19°C .

TABLE 1

Solutions

	I1	I2	I3	E1	E2	E3
NaCl [*]						
NaF [†]		5		154	30	30
KCl [*]			5	3	3	3
TMACl [†]					110	110
CsCl	144	139				
CsF [†]			134			
CaCl ₂ [*]				1	1	1
MgCl ₂ [*]	2	2		1	1	1
CdCl ₂ [†]				0.1	0.1	0.1
4-AP [‡]	1	1	1	1	1	
EGTA ^{†,‡}	10	10	10	10	10	10
HEPES [†]						

Values are expressed in millimolar. The prefixes E and I refer to external and internal solutions, respectively. For some experiments, 1 μm tetrodotoxin (TTX, Sigma, St. Louis, MO) was added to external solutions. TMACl, tetramethylammonium chloride; 4-AP, 4-aminopyridine; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

^{*} J. T. Baker Chemical, Phillipsburg, NJ.

[†] Aldrich Chemical, Milwaukee, WI.

[‡] Ethylene glycolbis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) buffers were prepared with the hydroxide of the main cation; this added an additional 5 mM of the main cation that is included in the table.