Faster Voltage-Dependent Activation of Na^+ Channels in Growth Cones Versus Somata of Neuroblastoma N1E-115 Cells

Jing Zhang,* Leslie M. Loew,# and Robert M. Davidson§
*Neuroscience Program, #Department of Physiology, and §Department of Periodontology, University of Connecticut Health Center, Farmington, Connecticut 06030 USA

ABSTRACT Kinetics of voltage-gated ionic channels fundamentally reflect the response of the channels to local electric fields. In this report cell-attached patch-clamp studies reveal that the voltage-dependent activation rate of sodium channels residing in the growth cone membrane differs from that of soma sodium channels in differentiating N1E-115 neuroblastoma cells. Because other electrophysiological properties of these channels do not differ, this finding may be a reflection of the difference in intramembrane electric field in these two regions of the cell. This represents a new mechanism for channels to attain a range of activities both within and between cells.

INTRODUCTION

Regional differences in the activity of sodium or calcium channels along the cell surface have been described in a number of neuronal cell types, including *Aplysia* neurons (Belardetti et al., 1986), crustacean peptidergic neurons (Meyers, 1993), molluscan neurons (Thompson and Coombs, 1988), mammalian cerebellar Purkinje cells (Usowicz et al., 1992), and sprouting PC12 cells (Streit and Lux, 1989). In developing *Aplysia* neurons, for example, the time course of sodium current decay in the growth cone is 3–4 times faster than in the soma (Belardetti et al., 1986), and in peptidergic neurons found in *Cardisoma carnifex*, the lamellipodia have a greater ability to generate action potentials than the somata (Meyers, 1993). The underlying mechanisms that have been suggested to account for these differences include nonuniform distribution of ion channels and/or regional differences in either intracellular message or cytoplasmic regulatory factors, such as the calcium buffering capacity of a cell.

Because voltage-dependent channels are, fundamentally, sensitive to the intramembrane electric field, it is possible that regional differences in channel behavior could be caused by regional differences in the electrical properties of the cell membrane. Previous work in our laboratory, based on dual-wavelength ratiometric imaging of a voltage-sensitive dye, has shown that the growth cone and soma membrane of differentiating N1E-115 mouse neuroblastoma cells have intrinsically different biophysical properties: the outer leaflet of the growth cone membrane has a more positive intramembrane potential gradient than that of the soma (Bedlack et al., 1994). Variations in transmembrane potential or membrane surface potential in different regions of the cell could not account for the observed variation in intramembrane electric field (Bedlack et al., 1994). We ascribed this to differences in the membrane dipole potential, which originates from the aligned molecular dipoles of lipid ester groups or the water molecules adjacent to them (Honig et al., 1986; Zheng and Vanderkooi, 1992; Franklin et al., 1993).

In this paper we explore the possibility that these previously observed differences in intramembrane electric field between the growth cone and soma of the differentiating N1E-115 cells might be reflected in differences in sodium channel behavior. Fig. 1 illustrates how this might come about. The intense electric fields associated with the dipole potential exist just under the surface of the lipid bilayer, as symbolized by the heavy arrows. The potentiometric dye study revealed that the intensity of this field is about 2 mV/Å more positive in the neurite and growth cone than in the soma (as depicted in the figure by the relative thickness of the heavy arrows). Fig. 1 also speculates that this field may penetrate into the region of gating charges within a channel protein. This would predict that the electrophysiological properties of such a channel would be offset to more depolarized voltages in the growth cone compared to the soma. We report here that the time course of sodium currents in the growth cone membrane indeed differs from that of the soma sodium currents in differentiating N1E-115 neuroblastoma cells. Furthermore, we show that this difference is reflected in a faster rate in the voltage-dependent activation, as opposed to the voltage-independent inactivation, of sodium channels in the growth cone membrane versus the soma membrane.

Received for publication 29 April 1996 and in final form 27 August 1996.

Address reprint requests to Dr. Leslie M. Loew, Department of Physiology, University of Connecticut Health Center, Farmington, CT 06030-3505. Tel.: 860-679-3568; Fax: 860-679-1269; E-mail: les@volt.uchc.edu, or to Dr. Robert Davidson, Basic Science Division, New York University College of Dentistry, 345 East 24th Street, New York City, NY 10012.

0006-3495/96/11/2501/08 $2.00

EXPERIMENTAL PROCEDURES

Cell-attached patch-clamp recordings were obtained from the soma and growth cone of differentiating N1E-115 neuroblastoma cells. Early passage cells were plated on uncoated glass coverslips and allowed to differentiate in Dulbecco's modified Eagle's medium containing 0.5% fetal calf serum and 1% dimethyl sulfoxide for 1–4 days before recording. Only those most

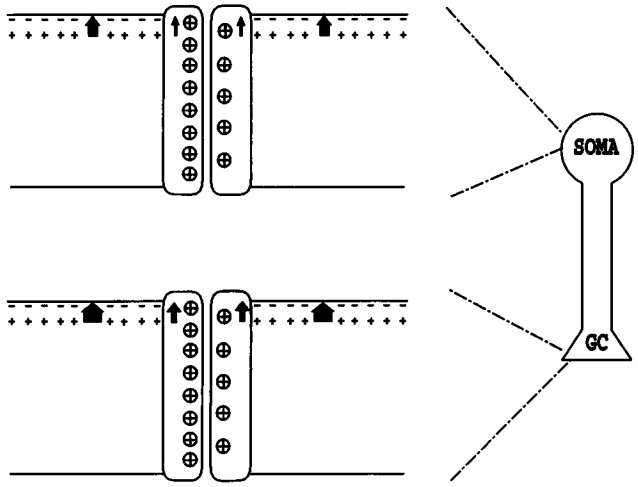

FIGURE 1 Schematic representation of intramembrane dipole electric fields (*arrows*) in the outer leaflet of the soma and growth cone membrane of N1E-115 neuroblastoma cells. The positively charged S4 segments (*rectangles containing the circled plus signs*), presumably from the D4 domain (*left*) and D2 domain (*right*) of a sodium channel, are also shown across the membrane. The circled plus signs represent the basic amino acid residues in the S4 segments, of which the outer three in S4/D4 have been shown to move from the inside to the outside during activation (Yang et al., 1996). The width of the arrows represents the relative strength of the dipole fields. The direction of the dipole fields is the same as the direction of the movement of the gating charges upon membrane depolarization. The dipole electric field in the growth cone membrane is about 1.9 mV/Å higher than that in the soma (Bedlack et al., 1994). The effective dipole electric field at the gating charges within the channel protein is shown to be attenuated but still different in the two regions of the cell (see Discussion).

highly differentiated cells with the longest neurites were chosen for study. All recordings were performed at room temperature (24–26°C).

In the present study the membrane voltage was defined as the negative of the pipette potential. Recording pipettes were pulled from borosilicate glass capillaries using a horizontal microelectrode puller (Model P-87; Sutter Instrument Co.). They were coated with Sylgard resin to reduce electrical capacitance before they were fire-polished. During recording the cells were bathed in the extracellular solution, which contained (in mM) 150 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES (pH 7.35); and the pipette contained the same solution supplemented with 5 mM tetraethyl ammonium chloride. When filled with solution, these pipettes had a resistance ranging from 2 to 5 MΩ. After the formation of a gigaohm seal, repetitive (1-Hz) depolarizations from a holding potential of −80 mV to a given voltage level were generated with a computer and pClamp6 (Axon Instruments, Foster City, CA). In some experiments, NaCl in the bath was replaced by the same concentration of KCl, and a holding potential of −120 mV was used. Resultant currents were amplified and filtered at 2 kHz using an Axopatch 1-C patch-clamp amplifier (Axon Instruments) and digitized for off-line analysis. For kinetic analysis, 64 to 200 such records were averaged at each voltage step, and leak and capacitive currents were digitally subtracted from an average of blank traces or from scaled average records containing no channel openings. Numbers of channels in both the soma and growth cone patches in our study ranged from 0 to 100. Because the number of channels does not affect the channel kinetics, no further quantitative analysis was attempted.

For single-channel analysis, we used pSTAT (Axon Instruments) to construct both open time and amplitude histograms. Single-channel events shorter than 0.34 ms were excluded from analysis, to avoid distortion by the system time constant and the possibility of missed events at very short times. The mean open dwell time was estimated by fitting the open time histograms using a simplex least-squares algorithm (Fig. 3 *a*). This method was appropriate for our patches containing only single-channel openings, but would tend to underestimate the mean open time when patches contained multiple channels with overlapping events (Horn and Standen, 1983). To reduce the inherent error of the method in estimating the mean open time under this condition, we applied a depolarization prepulse with variable duration and amplitude to inactivate most of the channels in the patch, followed by a brief reset holding voltage before the activation voltage step in some experiments. This effectively reduced the number of the overlapping events evoked by activation steps to less than 5% of the total events in some patches containing multiple channels. Single-channel amplitudes were determined by fitting Gaussians to the amplitude histograms. The conductance of the channel was estimated by linear regression analysis of the current-voltage (*I-V*) relation (Fig. 4).

RESULTS

Growth cone Na^+ currents show a faster decay time course than soma currents in N1E-115 neuroblastoma cells

In the present study we obtained cell-attached patch-clamp recordings from both soma and growth cone of differentiating N1E-115 mouse neuroblastoma cells bathed in extracellular saline. Repetitive depolarization steps from a holding potential of −80 mV to a voltage level were applied, and the resultant sodium current records were ensemble-averaged. Fig. 2 *a* shows examples of consecutive records taken from two separate neuroblastoma cells in either growth cone (*left*) or soma (*right*) at 0 mV. Both patches contained multiple channels with overlapping openings, which were clustered within the first 5 ms immediately after the beginning of the depolarization steps. In Fig. 2 *b* we show the average currents derived at each voltage, as indicated to the left of the figure, from these two patches. In each case the declining phase of the average currents was fitted by a single exponential with a time constant, τ_h. The τ_h value obtained from both the soma and growth cone patches was voltage dependent; it decreased as the depolarization step increased. Far more interesting, however, was the finding that, at each voltage step, τ_h was significantly smaller for growth cone currents than for soma currents. This finding was confirmed by our recordings made in a total of 30 separate patches; the mean τ_h value obtained from the growth cone patches was approximately one-half of that obtained from the soma patches at a given voltage ranging from −30 to 0 mV (Fig. 2 *c*; $p < 0.05$ at each voltage, Students's *t*-test).

The faster decay time course in the growth cone could be due to a higher resting potential in this region compared to that of soma (although this was not found in the voltage-sensitive dye study by Bedlack et al., 1994). To test this possibility, we bathed the cells in high potassium solution, where NaCl was replaced by the same concentration of KCl. This should have canceled out any uneven distribution of the resting potential, if present, along the cell surface. Under this experimental condition, a depolarization step of −40 mV from a holding potential of −120 mV was used to generate the sodium current in both regions of the cell. The

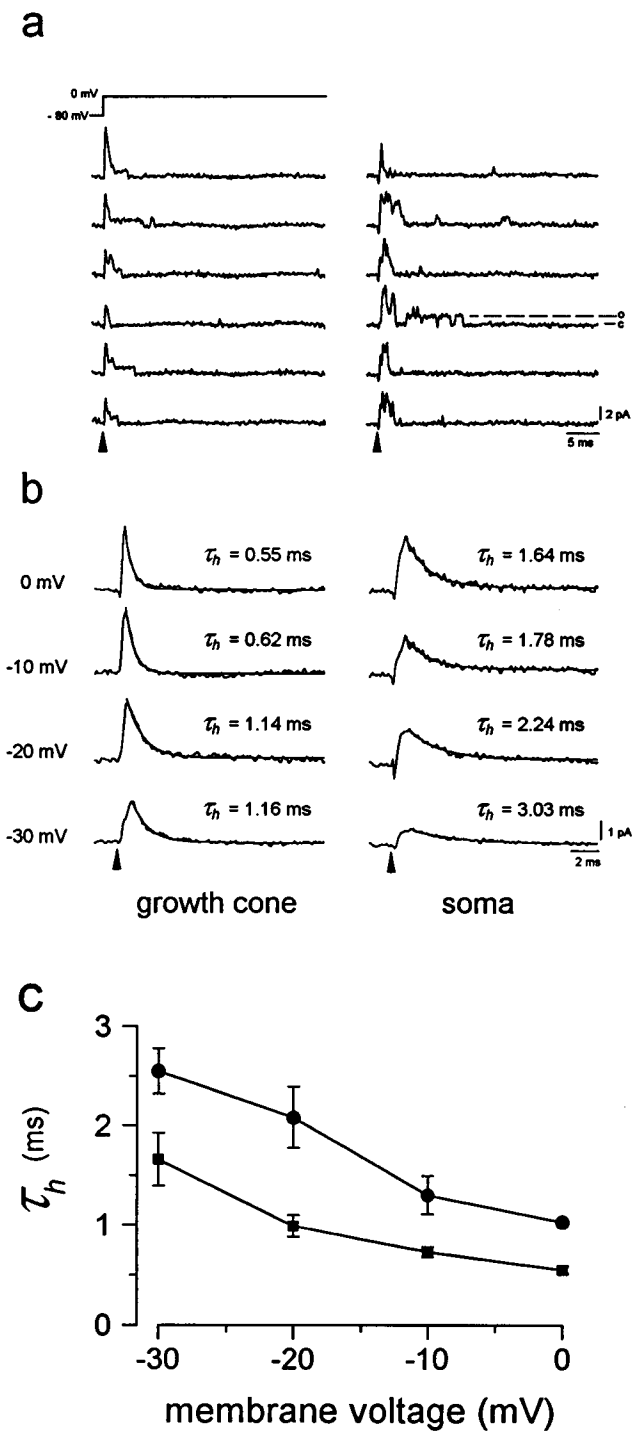

FIGURE 2 Time course of sodium current decay in growth cone and soma of N1E-115 neuroblastoma cells. (*a*) Single-channel records from a growth cone patch (*left*) and a soma patch (*right*), each containing multiple channels. Consecutive traces were evoked by repetitive 40-ms depolarizations to 0 mV from a holding potential of −80 mV. The channel's closed (c) state and unitary open (o) state are indicated to the right of the traces. Arrowheads indicate the beginning of the depolarization steps. (*b*) Ensemble average of 200 consecutive traces at step voltages indicated to the left of the figure. The solid line in each case represents the fit of a single exponential to the falling phase of the average current; the time constant (τ_h) is indicated above each transient. (*c*) Voltage dependence of τ_h for soma (●; n = 12) and growth cone (■; n = 18) patches. Each data point represents a mean ± SEM.

mean τ_h value from the soma and growth cone patches was 3.04 ± 0.43 ms and 1.85 ± 0.15 ms ($p < 0.05$, Student's t-test), respectively. These results indicated that an uneven distribution of the resting potential could not explain the intrinsically different decay time course of the sodium currents between the soma and growth cone of N1E-115 neuroblastoma cells.

Inactivation and deactivation do not contribute to the difference in decay time courses between the growth cone and the soma sodium currents

According to the classic Hodgkin and Huxley model (Hodgkin and Huxley, 1952), τ_h represents inactivation of the channel, which is a first-order voltage-dependent process that is independent of the state of activation. More recently, however, Aldrich and colleagues (Aldrich et al., 1983; Aldrich and Stevens, 1987) have suggested that sodium currents in N1E-115 neuroblastoma cells, in particular, display rapid inactivation with little voltage dependence, and that a slow, voltage-dependent activation process underlies the voltage dependence of τ_h. Our finding that τ_h values for the growth cone and soma were voltage dependent but differed from each other at a given voltage may therefore reflect a difference in activation rather than inactivation of sodium channels in these two regions of the cell. We tested this hypothesis by directly comparing the kinetic properties of single open sodium channels in these two regions.

To determine if inactivation underlies the difference in τ_h between the soma and growth cone, we measured the dwell time of single-channel openings from both the soma and growth cone membranes. Theoretically, the mean open dwell time reflects both inactivation and deactivation rates, although, at voltages more positive than −40 mV, the contribution of deactivation is negligible for the N1E-115 neuroblastoma sodium channel (Aldrich and Stevens, 1987). Thus if the distribution of open dwell times does not differ between the soma and growth cone, a difference in inactivation and deactivation rates cannot explain the difference in τ_h.

A typical example of open time distribution is illustrated in Fig. 3 *a* for a soma patch recorded at −40 mV; the solid line in the figure represents the fit of a single exponential, with a time constant (τ_o) of 0.8 ms. We have analyzed the open dwell time distribution from 18 patches (nine from the soma and nine from the growth cone) obtained from cells in the extracellular saline at various voltages. At each voltage in both growth cone and soma, the distribution was best described by a single exponential with a τ_o usually less than 1 ms. In Fig. 3 *b* we have plotted the mean τ_o against voltages ranging from −40 mV to 0 mV for both soma and growth cone. Over this potential range, in contrast to τ_h, τ_o did not differ significantly between soma and growth cone. The voltage dependence of τ_o was slightly bell-shaped and could not be used to explain the apparent voltage dependence of τ_h (compare Fig. 2 *c*). Thus it was unlikely that the difference in τ_h between soma and growth cone resulted

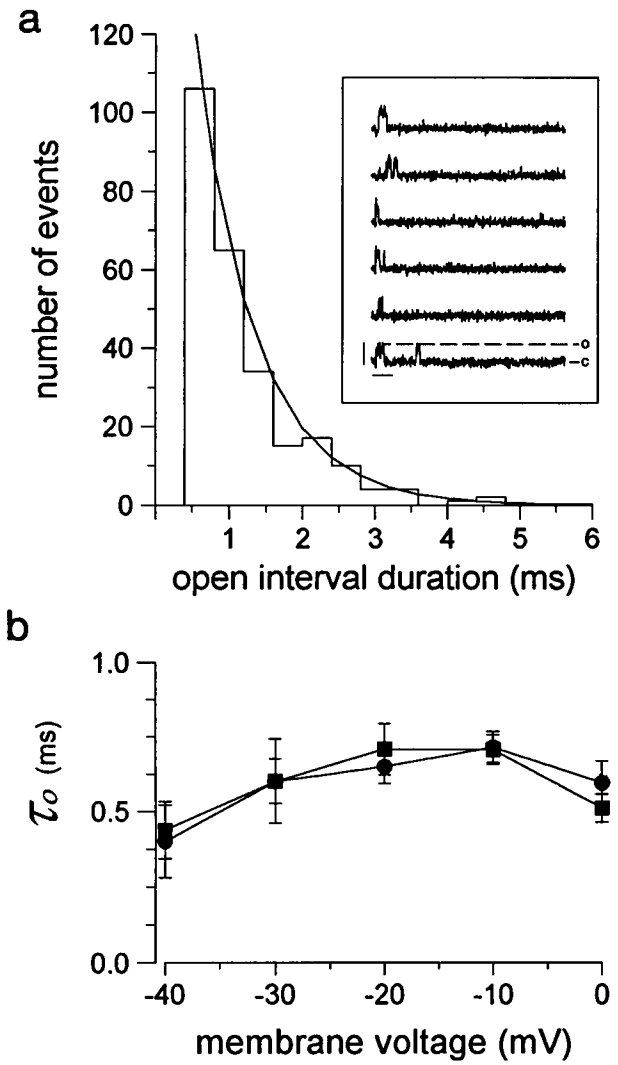

FIGURE 3 Mean open dwell times of sodium channels in growth cone and soma of N1E-115 neuroblastoma cells. (*a*) Distribution of open dwell times for a patch recorded at −40 mV in the soma of a developing N1E-115 neuroblastoma cell. The solid line represents the fit of a single exponential with time constant, τ_o, equal to 0.8 ms. Bin width: 0.4 ms. (*Inset*) A series of representative traces illustrating single-channel openings. Dashed lines indicate the open or closed status of the channel. Vertical scale: 2 pA; Horizontal scale: 10 ms. (*b*) Open dwell time (τ_o) plotted as a function of membrane voltage recorded from separate patches in growth cone (n = 9; ■) and soma (n = 9; ●). Not all patches could be tested through the whole range of voltages. Each data point represents a mean ± SEM.

from a regional difference in the inactivation rate of the channel. The probable source of this difference, therefore, is that growth cone sodium channels show intrinsically faster activation over a given range of voltages than sodium channels found in the soma of N1E-115 neuroblastoma cells.

Growth cone and soma sodium channels have similar conductances

In addition to the similar open dwell time distribution between the soma and growth cone channels, the single-channel conductance between the two regions was similar as well. Fig. 4 shows the current-voltage (*I-V*) relation for 16 patches (eight from the growth cone and eight from the soma) recorded from the cells in the extracellular saline bathing solution. The unitary current amplitude decreased with increasing depolarization for both growth cone (*squares*) and soma (*circles*) channels. A linear regression of these points showed that the slopes of the *I-V* relations were similar, yielding a single-channel conductance of 11.9 pS for the soma and 12.4 pS for the growth cone, respectively. Indeed, comparison of the two regression lines indicated that the single-channel conductance did not differ significantly between the soma and growth cone regions.

Parallel shift of activation rate along the voltage axis between the growth cone and soma channels

Because τ_h is an empirical value reflecting both activation and inactivation processes, it cannot be used to compare activation kinetics of soma and growth cone sodium channels. Thus we derived rate constants for activation (k_a) of the sodium channel using the following three-state, unidirectional kinetic model:

$$C \xrightarrow{k_a} O \xrightarrow{k_i} I, \qquad (1)$$

where C represents the proportion of channels in a closed state(s), O the proportion of channels in the open state, I the proportion of channels in the inactivated state, and k_i the rate constant for inactivation. It should be noted that this model assumes that I is an "absorbing" state, and that no deactivation occurs at the voltages studied. As noted above, this assumption is based on the observation that in N1E-115

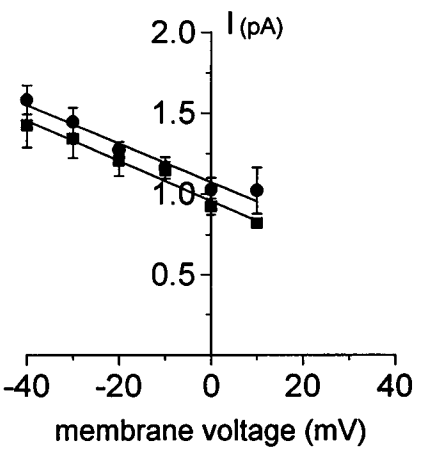

FIGURE 4 Single-channel conductance of the sodium channel. Current-voltage (*I-V*) relation of the sodium channel recorded in growth cone (■; n = 8) and soma (●; n = 8) patches. Each data point represents a mean ± SEM. The single-channel conductance was given by the slope of the solid lines, which were obtained from a least-squares fit through the data points (see text).

cells, at voltages more positive than −40 mV, inactivation of sodium channels far outweighs deactivation (Aldrich and Stevens, 1987). Thus k_i is taken as the inverse of the mean open lifetime ($k_i = 1/\tau_o$). The transition from $C \rightarrow I$ is also ignored, because it has no effect on the time course of the sodium current (Godoy and Cukierman, 1994). The differential equations describing this model, therefore, are as follows:

$$\frac{dC}{dt} = -k_a C \tag{2}$$

$$\frac{dO}{dt} = k_a C - k_i O. \tag{3}$$

Assuming that all sodium channels are in the closed state ($C = 1$) at the time point when a depolarizing step begins ($t = 0$), integration of these equations yields the following expression, which reflects the probability that a channel is open at time t:

$$O = \frac{k_a}{k_i - k_a}(e^{-k_a t} - e^{-k_i t}). \tag{4}$$

The time course of the average current, $F(t)$, is given by simply multiplying this expression by a scale factor, A, which represents the single-channel amplitude times the number of channels in the patch available for activation.

$$F(t) = \frac{Ak_a}{k_i - k_a}(e^{-k_a t} - e^{-k_i t}). \tag{5}$$

Because $F(t)$ and k_i at a given voltage are experimentally measurable values, k_a can be determined by using this expression to fit the falling phase of the average current. One such analysis is illustrated in Fig. 5 *a*, and in Fig. 5 *b* we show the relation between k_a, plotted as $[\ln(k_a)]$, and membrane potential for growth cone and soma at voltages ranging from −30 mV to 0 mV. The slopes of the best linear fits to these data were quite similar in both regions of the N1E-115 neuroblastoma cell, with a ~20 mV change in membrane potential corresponding to an e-fold change in k_a. However, at a given voltage, k_a for growth cone channels was significantly faster than k_a for soma channels (Fig. 5 *b* and Table 1), representing a shift in the threshold potential of approximately 30 mV. The relatively slow activation of soma sodium channels in response to a depolarization signal may lead to relatively late openings (comparing the records from the soma and growth cone in Fig. 2 *a*), which underlie the slower decay time course of the average current.

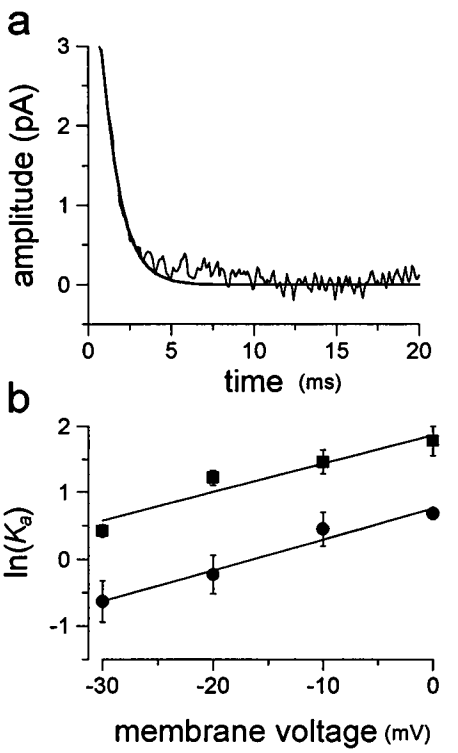

FIGURE 5 Activation rate (k_a, ms^{-1}) of N1E-115 neuroblastoma cells. (*a*) Nonlinear regression of the falling phase of the average current obtained at −20 mV from the same growth cone patch as shown in Fig. 2 *b*, using Eq. 5. For this record, the calculated k_a value was 3.59 ms^{-1}. (*b*) Relation of voltage and k_a, plotted as $[\ln(k_a)]$, in growth cone (■) and soma (●). Each data point represents the mean ± SEM and is calculated from three to five patches. Solid lines represent a least-squares fit through the data points.

DISCUSSION

The major finding in the present study is that the activation rate of the sodium channel in the growth cone membrane is faster than in the soma. This might be explained by the more positive intramembrane dipole field in the outer leaflet of the growth cone membrane in N1E-115 neuroblastoma cells (Bedlack et al., 1994; Fig. 1). The results in Fig. 5 *b* suggest that the soma sodium channels are behaving simply as if they are experiencing an offset voltage relative to the growth cone.

The dipole potential is the potential difference (several hundred millivolts) between the interior of the bilayer and the membrane/bulk solution interface (Honig et al., 1986; Zheng and Vanderkooi, 1992; Franklin et al., 1993). The oriented dipoles in the lipid molecules and in the water molecules adjacent to the interface are the origin of the potential. Because the dipole potential is highly dependent on the lipid composition (Szabo, 1974), the difference in the dipole potential between the soma and the growth cone membrane probably originates from different cholesterol-to-phospholipid ratios in these two regions (Igarashi et al., 1990). The membrane dipole potential can account for a 1000-fold difference in the rate of movement of hydrophobic anions versus cations across lipid bilayers (Liberman and Topaly, 1969; Szabo, 1974; Flewelling and Hubbell, 1986; Franklin and Cafiso, 1993; Franklin et al., 1993). Because the intramembrane electric field associated with the dipole potential can be 50 times stronger than the electric field produced by the transmembrane potential

TABLE 1 Comparisons by voltage of activation rate constants (k_a) (ms^{-1}) in growth cone and soma

	−30 mV	−20 mV	−10 mV	0 mV
GC	1.54 ± 0.13* (n = 3)	3.42 ± 0.36** (n = 3)	4.53 ± 0.86* (n = 4)	6.34 ± 1.30* (n = 4)
Soma	0.62 ± 0.21 (n = 4)	0.94 ± 0.28 (n = 5)	1.73 ± 0.46 (n = 4)	2.00 ± 0.17 (n = 4)

Values represent mean ± SEM. GC, Growth cone.
*$p < 0.05$; **$p < 0.01$.

(Gross et al., 1994), it can be reasonably expected that the dipole potential may influence voltage-gated channels.

Activation of the sodium channel is believed to be associated with the movement of either gating charges or dipoles within the channel protein, resulting in a conformational change from closed states to an open state (Patlak, 1991; Catterall, 1992). Site-directed mutagenesis experiments have provided evidence that the highly conserved S4 segment in the α-subunit of the sodium channel may bear these gating charges (Stühmer et al., 1989) and serve as the voltage sensor. A recent study has shown that three charged S4 residues in domain 4 of a muscle sodium channel can move outwardly across the membrane upon depolarization (Yang and Horn, 1995; Yang et al., 1996). Thus these charges must pass through the region of the dipole potential and should experience at least a portion of the associated electric field, as suggested in Fig. 1. The outward movement of the gating current upon depolarization of the membrane may thus be accelerated by this higher intramembrane dipole field in the growth cone membrane, leading to faster conformational changes from the closed states to the open state, relative to the soma. As a consequence, at a given transmembrane voltage, the sodium channels in the growth cone membrane show faster activation kinetics than those in the soma membrane.

Within the context of intramembrane electric field rather than voltage differences, one can translate the 30-mV offset in the activation energies for gating between the growth cone and soma (Fig. 5 *b*) into an electric field difference of 0.75 mV/Å, assuming a 40-Å membrane thickness. The difference in the intramembrane electric field determined in our previous study using voltage-sensitive dye was 1.9 ± 0.4 mV/Å (Bedlack et al., 1994). Thus the gating charges sense only 1/2 to 1/3 of the field sensed by the dye (as indicated by the thinner electric field arrows within the protein compared to the adjacent membrane in Fig. 1). This attenuation can be attributed to the decay of the field as it penetrates both laterally into the channel protein and toward the interior of the membrane, consistent with the model calculations of Jordan (1987) for the very small effect of the dipole potential on channel conductance.

In addition to the dipole potential, surface potential and transmembrane potential also contribute to the intramembrane electric field. Asymmetrical surface charge density along the inner and outer surface of the membrane (on the lipids or the channel protein itself) creates an intramembrane electric field that may affect the channel's gating position along the voltage axis (Cukierman, 1991; Ji et al., 1993). A difference in resting potential between the soma and growth cone regions might also affect the gating behavior of the channel. However, neither the surface potential nor the transmembrane potential can account for the difference in intramembrane electric field found in the soma and growth cone regions of this cell line (Bedlack et al., 1994). The τ_h values from these two areas differed from each other even when the cells were bathed in high potassium solution in the present study. Although these values were slower than expected from bathing the cells in extracellular saline, they clearly indicated that the difference in τ_h could not be explained by an uneven distribution of the resting potential along the cell surface.

Consistent with the studies of Aldrich et al. (Aldrich et al., 1983; Aldrich and Stevens, 1987), little or no voltage dependence was observed for the inactivation of the sodium channels in the present study. This is not surprising, because the channel structure responsible for inactivation is thought to be located in the cytoplasmic side of the membrane and may not be able to respond to the voltage changes across the membrane. The chemical compounds that can destroy inactivation, such as pronase and *N*-bromoacetamide, are only effective when applied internally (Armstrong et al., 1973; Oxford et al., 1978). Site-directed mutagenesis and site-specific antipeptide antibody experiments have detected a highly conserved intracellular loop between the homogeneous domain III and IV of the α-subunit of the sodium channels as the structure responsible for inactivation (Vassilev et al., 1988, 1989; Stühmer et al., 1989). Furthermore, no gating current was found to be associated with the time course of inactivation (Armstrong, 1981), suggesting that the inactivation itself may not necessarily be voltage dependent. This view is supported by our finding that inactivation rates of sodium channels in the growth cone and in the soma membrane do not differ from each other, even though the growth cone and soma membranes have intrinsically different intramembrane electric fields.

An alternative explanation postulating two distinct populations (or isoforms) of sodium channels in soma and growth cone, respectively, each with distinct kinetic properties, is unlikely. This is because, aside from the 30-mV shift of k_a versus voltage (Fig. 5 *b*), the channel behaved identically in these two regions. Neither τ_o nor the single-channel conductance differed significantly in growth cone versus soma. The parallel shift of k_a along the voltage axis actually represents the intrinsically same voltage dependence of activation but can be explained by an offset of intramembrane electric field between the soma and growth cone.

Protein glycosylation is known to play a significant role in the functional expression of the sodium channel in developing neurons. For example, application of an inhibitor of protein *N*-glycosylation into the cell culture of rat neocortical neurons decreased the sodium currents of the growing cells, although the same treatment did not affect the sodium currents of the mature neurons (Zona et al., 1990). However, the sodium channel kinetics were not affected by the inhibitor in that study. This implies that a differential glycosylation cannot account for our findings. Furthermore, protein phosphorylation may be able to modulate sodium channel activity but, based on several recent studies, a differential phosphorylation of the sodium channels in the soma versus growth cone is an unlikely explanation of our results. Neither the time course nor the voltage dependence of the type IIA brain sodium channel can be altered by activation of adenosine 3′,5′-cyclic monophosphate-dependent protein kinase (Li et al., 1992). Activation of protein kinase C in rat brain neurons has been reported to increase the τ_h value of the same type of sodium currents (Numann et al., 1991); however, the increase in τ_h by protein kinase C phosphorylation was explained by a slowed inactivation but not activation process, because the lifetime of single sodium channel openings was found to be increased in that study. This is consistent with the cytoplasmic locus of the inactivation gate, but cannot explain the modulation of activation observed here. The most direct evidence that the phosphorylation by protein kinase C cannot explain our experimental data is that the activation of protein kinase C in N1E-115 neuroblastoma cells has no effect on the time course of the sodium currents, although it enhances the transition from *C* to *I* (Godoy and Cukierman, 1994).

Our finding suggests that, relative to the soma, sodium channels in the growth cone may have increased sensitivity to a depolarizing signal. This increased sensitivity could be critical in generating localized intracellular events in the growth cone that are important in neurite outgrowth and pathfinding (Connor, 1986; Bedlack et al., 1992; Davenport and Kater, 1992). This mechanism may be very important, therefore, in the developing nervous system. In a broader sense, these results suggest that differences in intrinsic membrane electrical properties can provide a previously unappreciated mechanism for regional difference in channel activity within and between cells containing voltage-dependent channels. In addition, because the intramembrane dipole field can be modulated by cholesterol, this mechanism could also provide a new perspective for investigations of cholesterol-related diseases.

We thank Dr. Mei-de Wei for providing us with N1E-115 neuroblastoma cells and Drs. Hermes Yeh, Mary Ann Epstein, Barbara Ehrlich, and Lawrence B. Cohen for helpful comments.

This study was supported by grants GM 35063 and ESO5973 (to LML) and DE09662 (to RMD) from the National Institutes of Health.

REFERENCES

Aldrich, R. W., D. P. Corey, and C. F. Stevens. 1983. A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature.* 306:436–441.

Aldrich, R. W., and C. F. Stevens. 1987. Voltage-dependent gating of single sodium channels from mammalian neuroblastoma cells. *J. Neurosci.* 7:418–431.

Armstrong, C. M. 1981. Sodium channels and gating currents. *Physiol. Rev.* 61:644–683.

Armstrong, C. M., F. Bezanilla, and E. Rojas. 1973. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* 62:375–391.

Bedlack, R. S., M.-d. Wei, S. H. Fox, E. Gross, and L. M. Loew. 1994. Distinct electric potentials in soma and neurite membranes. *Neuron.* 13:1187–1193.

Bedlack, R. S., M.-d. Wei, and L. M. Loew. 1992. Localized membrane depolarizations and localized intracellular calcium influx during electric field-guided neurite growth. *Neuron.* 9:393–403.

Belardetti, F., S. Schacher, and S. A. Siegelbaum. 1986. Action potentials, macroscopic and single channel currents recorded from growth cones of *Aplysia* neurons in culture. *J. Physiol.* 374:289–313.

Catterall, W. A. 1992. Cellular and molecular biology of voltage-gated sodium channels. *Physiol. Rev.* 72:S15–S48.

Connor, J. A. 1986. Digital imaging of free calcium changes and of spatial gradients in growing process in single, mammalian central nervous system cells. *Proc. Natl. Acad. Sci. USA.* 83:6179–6183.

Cukierman, S. 1991. Asymmetric electrostatic effects on the gating of rat brain sodium channels in planar lipid membranes. *Biophys. J.* 60: 845–855.

Davenport, R., and S. Kater. 1992. Local increases in intracellular calcium elicit local filopodial responses in Helisoma neuronal growth cones. *Neuron.* 9:405–416.

Flewelling, R. F., and W. L. Hubbell. 1986. The membrane dipole potential in a total membrane potential model. Applications to hydrophobic ion interactions with membranes. *Biophys. J.* 49:541–552.

Franklin, J. C., and D. S. Cafiso. 1993. Internal electrostatic potentials in bilayers: measuring and controlling dipole potentials in lipid vesicles. *Biophys. J.* 65:289–299.

Franklin, J. C., D. S. Cafiso, R. F. Flewelling, and W. L. Hubbell. 1993. Probes of membrane electrostatics: synthesis and voltage-dependent partitioning of negative hydrophobic ion spin labels in lipid vesicles. *Biophys. J.* 64:642–653.

Godoy, C. M. G., and S. Cukierman. 1994. Diacylglycerol-induced activation of protein kinase C attenuates sodium currents by enhancing inactivation from the closed state. *Pflügers Arch. Eur. J. Physiol.* 429: 245–252.

Gross, E., R. S. Bedlack, and L. M. Loew. 1994. Dual-wavelength ratiometric measurement of the membrane dipole potential. *Biophys. J.* 67:208–216.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117:500–544.

Honig, B. H., W. L. Hubbell, and R. F. Flewelling. 1986. Electrostatic interactions in membranes and proteins. *Annu. Rev. Biophys. Biophys. Chem.* 15:163–193.

Horn, R., and N. B. Standen. 1983. Counting kinetic states: the single channel approach. *In* The Physiology of Excitable Cells. A. Grinnell and W. Moody, editors. Liss, New York. 181–189.

Igarashi, M., H. Waki, M. Hirota, Y. Hirabayashi, K. Obata, and S. Ando. 1990. Differences in lipid composition between isolated growth cones from the forebrain and those from the brainstem in the fetal rat. *Dev. Brain Res.* 51:1–10.

Ji, S., J. N. Weiss, and G. A. Langer. 1993. Modulation of voltage-dependent sodium and potassium currents by charged amphiles in cardiac ventricular myocytes: effects via modification of surface potential. *J. Gen. Physiol.* 101:355–375.

Jordan, P. C. 1987. How pore mouth charge distributions alter the permeability of transmembrane ionic channels. *Biophys. J.* 51:297–311.

Li, M., J. W. West, Y. Lai, T. Scheuer, and W. A. Catterall. 1992. Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. *Neuron.* 8:1151–1159.

Liberman, E. A., and V. P. Topaly. 1969. Permeability of biomolecular phospholipid membranes for fat-soluble ions. *Biophysics.* 14:477–487.

Meyers, D. E. R. 1993. Distribution of ionic currents in the soma and growing region of an identified peptidergic neuron in defined culture. *J. Neurophysiol.* 69:406–415.

Numann, R., W. A. Catterall, and T. Scheuer. 1991. Functional modulation of brain sodium channels by protein kinase C phosphorylation. *Science.* 254:115–118.

Oxford, G. S., C. H. Wu, and T. Narahashi. 1978. Removal of sodium channel inactivation in squid giant axons by *N*-bromoacetamide. *J. Gen. Physiol.* 71:227–247.

Patlak, J. 1991. Molecular kinetics of voltage-dependent sodium channels. *Physiol. Rev.* 71:1047–1080.

Streit, J., and H. D. Lux. 1989. Distribution of calcium currents in sprouting pc12 cells. *J. Neurosci.* 9:4190–4199.

Stühmer, W., F. Conti, H. Suzuki, X. Wang, M. Noda, N. Yahagi, H. Kubo, and S. Numa. 1989. Structural parts involved in activation and inactivation of the sodium channel. *Nature.* 339:597–603.

Szabo, G. 1974. Dual mechanism for the action of cholesterol on membrane permeability. *Nature.* 252:47–49.

Thompson, S., and J. Coombs. 1988. Spatial distribution of calcium currents in molluscan neuron cell bodies and regional differences in the strength of inactivation. *J. Neurosci.* 8:1929–1939.

Usowicz, M. M., M. Sugimori, B. Cherksey, and R. Llinás. 1992. P-type calcium channels in the somata and dendrites of adult cerebellar Purkinje cells. *Neuron.* 9:1185–1199.

Vassilev, P. M., T. Scheuer, and W. A. Catterall. 1988. Identification of an intracellular peptide segment involved in sodium channel inactivation. *Science.* 241:1658–1661.

Vassilev, P. M., T. Scheuer, and W. A. Catterall. 1989. Inhibition of inactivation of single sodium channels by a site-directed antibody. *Proc. Natl. Acad. Sci. USA.* 86:8147–8151.

Yang, N., A. L. George, and R. Horn. 1996. Molecular basis of charge movement in voltage-gated sodium channels. *Neuron.* 16:113–122.

Yang, N., and R. Horn. 1995. Evidence for voltage-dependent S4 movement in sodium channels. *Neuron.* 15:213–218.

Zheng, C., and G. Vanderkooi. 1992. Molecular origin of the internal dipole potential in lipid bilayers: calculation of the electrostatic potential. *Biophys. J.* 63:935–941.

Zona, C., F. Eusebi, and R. Miledi. 1990. Glycosylation is required for maintenance of functional voltage-activated channels in growing neocortical neurons of the rat. *Proc. R. Soc. Lond.* 239:119–127.