Glial and Neuronal Forms of the Voltage-Dependent Sodium Channel: Characteristics and Cell-Type Distribution

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Summary

Two functionally distinct forms of the voltage-dependent sodium channel were observed in glia and in neurons of the mammalian nervous system. Both forms exhibit identical conductance and tetrodotoxin sensitivity and displayed steady-state inactivation, a strongly voltage-dependent rate of activation, and a faster but weakly voltage-sensitive rate of inactivation. However, the glial form had significantly slower kinetics and a more negative voltage dependence, suggesting that it was functionally specialized for glia. This form was found in most glial types studied, while the neuronal form was observed in retinal ganglion cells, cortical motor neurons, and O2A glial progenitor cells. Both forms occurred in type-2 astrocytes. The presence of the glial form correlated with the RAN-2 surface antigen.

Introduction

Mammalian astrocytes express voltage-sensitive sodium channels in culture (Bevan et al., 1985; Nowak et al., 1987; Barres et al., 1985; Soc. Neurosci., abstract; 1988a). As of yet, however, there remains no evidence that mammalian astrocytes express these channels in vivo—that they are not simply an artifact of the culture environment—and no indication of the purpose they serve if they are expressed.

Because astrocytes may have processes in close association with axonal nodes in the CNS (Hildebrand and Waxman, 1984; Raine, 1984; Waxman and Black, 1984; ffrench-Constant and Raff, 1986; ffrench-Constant et al., 1986), it has been hypothesized that astrocytes may synthesize these channels for the purpose of supplying them to neurons (Bevan et al., 1985), as was previously proposed for Schwann cells in the PNS (Shrager et al., 1985). Bevan et al. (1985) argued that astrocyte channels might have properties different from those of nodal channels. In part, this argument was based on a comparison of the characteristics of cultured astrocyte sodium channels with those of nodes, even though the nodal and glial channels compared were studied with different recording techniques, on cells that were at different developmental stages, and from different locations of the nervous system (glia from CNS; nodes from PNS).

We have readdressed this issue by asking whether the properties of the astrocyte sodium channels are identical to their neuronal counterparts when measured under identical experimental conditions. If the properties are identical, then this finding would at least be consistent with the possibility of transfer of channels from glia to neurons. However, if the properties of the glial channel are significantly different, it would suggest that the channels are synthesized for a specific role in the astrocytes.

Here we report the presence of sodium channels in type-1 astrocytes acutely dissociated from optic nerve, suggesting their presence in vivo. We found that glial sodium channels exhibit voltage sensitivity and kinetics that are significantly different from those of the sodium channels found in retinal ganglion cells. Moreover, the properties of the channel seem not to be altered randomly, but instead differ so as to make the channel more likely to be functional in the glial membrane, where resting potential has often been demonstrated to be more negative than in neurons (Kuffler and Potter, 1964).

Results

Astrocytes Express Sodium Channels That Are Qualitatively Similar to Neuronal Sodium Channels

We first recorded from acutely dissociated type-1 astrocytes from postnatal day 7 (P7) optic nerve. Type-1 astrocytes were identified in early experiments by labeling for the RAN-2 surface antigen and for glial fibrillary acidic protein (GFAP) (Figure 1A). They were later identified by their distinct morphology (see Experimental Procedures). The whole-cell, tight-seal recording configuration was used with sodium isolation solution A in the bath and solution B in the pipette (Table 1). These solutions eliminated outward currents almost entirely.

In many astrocytes, we observed a voltage-dependent sodium current, which resembled the classically described voltage-sensitive sodium current in qualitative properties such as voltage-dependent activation and inactivation (Figures 1B and 1C). A series of steps from a prepulse potential of −100 mV to progressively more positive test potentials elicited currents that activated and then declined progressively more rapidly (Figure 1B). A series of steps from a variable prepulse potential to a test potential of −10 mV revealed an inactivation that depended on prepulse potential (Figure 1C). The proportion of astrocytes that expressed sodium current varied among experiments from 20% to 50%. In one experiment, 10 out of 20 astrocytes studied expressed a sodium current, which ranged from −100 to −300 pA peak (inward) current. Of those cells that expressed any sodium current, the average sodium current per astrocyte was found to be −150 ± 50 pA (mean ± SD).

The average capacitance of the glial cells studied was 42 ± 14 pF (10 cells) and that of neurons was 11 ± 3.6 pF (15 cells). The average peak sodium current normalized for surface area (as measured by cell capacitance) was about 4 pA/pF. This number compares with an aver-
Figure 1. Whole-Cell Sodium Currents in Type-1 Astrocytes and Retinal Ganglion Cells

(A) Fluorescence micrograph of type-1 astrocytes in a P7 optic nerve suspension that was labeled with an antibody to GFAP. The astrocytes were also recognized by their characteristic irregular, nonspherical shape. The diameters of the cells shown are about 10–15 μm.

(B) Whole-cell sodium currents in a type-1 astrocyte recorded using solutions A and B (see Table 1). Currents were evoked by a series of test depolarizations ranging from -50 to +40 mV, from a holding potential of -100 mV. (Responses to test steps of +10, +30, and +40 mV are not shown.) The current began to activate at -50 to -40 mV, activated more rapidly as test steps became more depolarized, and, for all steps, completely inactivated.

(C) Voltage-dependence of the inactivation process. Whole-cell current was measured in response to a constant test step depolarization to -10 mV taken from varying prepulse potentials ranging from -100 to -30 mV. As the prepulse potential became more depolarized, the peak inward current diminished, reflecting steady-state inactivation at each prepulse potential. Prepulse duration, 300 ms; repetition rate, 1/s.

(D) Fluorescence micrograph of a retinal ganglion cell in a P7 retinal suspension labeled with antibodies to Thy-1. These cells were also recognized by their large somata and characteristic process-bearing morphology; however, cells with extensive processes were not recorded from (see text). The diameters of the cells shown are about 10–15 μm.

(E and F) Whole-cell currents in a P7 retinal ganglion cell recorded as described above. The neuronal current was diminished to nearly the same amplitude as the glial current by a bath solution containing 10 nM TTX (see Table 1). The whole-cell glial current rose and decayed at a slower rate.

The average neuronal current of about 500 pA/pF in retinal ganglion cells recorded with the same solutions. The average density of current in astrocytes was therefore only about 1% of that found in the retinal ganglion cells.

Sodium Currents of Astrocytes and Neurons Differ Quantitatively

Although astrocytes expressed a sodium current resembling the current in retinal ganglion cells, there were significant quantitative differences between the two. Figure 1 illustrates currents recorded from retinal ganglion cells. The glial current did not peak until long after the neuronal current, and it then decayed with a slower time course. In many such comparisons, the neuronal current was largely inactivated at a time when the glial current was just peaking. This difference could not be accounted for by a simple difference in membrane potential: the traces could not be aligned by superimposing neuronal and glial traces taken at different step potentials. The differences could not be accounted for by a series resistance error, the currents in Figure 1 were obtained from cells with similar magnitudes of current and by electrodes with similar series resistance. Because the cells had either no or only small processes and were small in size, space clamp problems also could not account for this difference.

Table 1. Composition of Solutions

<table>
<thead>
<tr>
<th></th>
<th>External (Solution A)</th>
<th>Internal (Solution B)</th>
<th>High K+ Bath (Solution C)</th>
</tr>
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<tbody>
<tr>
<td>NaOH</td>
<td>140</td>
<td>20</td>
<td>120</td>
</tr>
<tr>
<td>KOH</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃SO₃H</td>
<td>140</td>
<td>120</td>
<td>140</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2</td>
<td>1.0 mM</td>
<td>100 mM</td>
</tr>
<tr>
<td>CdCl₂</td>
<td>0.1</td>
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<td></td>
</tr>
<tr>
<td>HEPES</td>
<td>≥2</td>
<td>≥2</td>
<td>≥2</td>
</tr>
<tr>
<td>Dextrose</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>CsOH</td>
<td>120</td>
<td></td>
<td></td>
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<tr>
<td>CsCl</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGTA</td>
<td>10</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
</tr>
</tbody>
</table>

All concentrations are given in millimolar except where noted.

C TTX (10 μM) was added to solution A for whole-cell recording from neurons.

The free calcium concentration was adjusted to 1 nM to 100 nM, as calculated from an EGTA dissociation constant of 1.02 nM at pH 7.4 (Caldwell, 1970).

Solutions A and C were adjusted to pH 7.4 with NaOH; solution B was adjusted to pH 7.4 with CsOH.
Figure 2. Comparison of Glial and Neuronal Whole-Cell Currents

(A) Peak I-V relationships from the voltage-clamp protocol given in Figure 1. The greatest current occurred at about -10 mV for both cell types, but the glial cell sodium current activated at a value about 10 mV more negative. Data represent currents normalized as percent of peak current and are averaged responses of 15 retinal ganglion cells and 10 type-1 astrocytes.

(B) Voltage dependence of inactivation from Figure 1. The percent of maximum peak inward current was plotted versus the prepulse potential and was fitted by eye with a sigmoidal curve. The glial currents were half-inactivated at a voltage 25 mV negative to that of neurons: type-1 astrocytes, at -80 mV; retinal ganglion cells, at -55 mV.

(C) Decay time constants (t<sub>h</sub>). The decaying phase of current at each test step potential was fitted by eye with an exponential curve. The average time constants for neurons (15 cells) and glia (10 cells) were plotted; these points were fitted by eye with smooth curves generated by an exponential function plus a constant. Standard deviations are given in Table 2.

### Table 2. Properties of Glial and Neuronal Whole-Cell Sodium Currents

<table>
<thead>
<tr>
<th></th>
<th>Type-1 Astrocytes</th>
<th>Retinal Ganglion Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation (mV)</td>
<td>-41.0 ± 1.7</td>
<td>-31.0 ± 2.9</td>
</tr>
<tr>
<td>Peak (mV)</td>
<td>-8.0 ± 4.2</td>
<td>-5.7 ± 4.1</td>
</tr>
<tr>
<td>Half inactivation (mV)</td>
<td>-80.0 ± 5.8</td>
<td>-55.0 ± 4.0</td>
</tr>
<tr>
<td>Equiv charge of inactivation (mC/mV)</td>
<td>4.4 ± 0.5</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>t&lt;sub&gt;h&lt;/sub&gt; (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-30 mV</td>
<td>4.4 ± 0.5</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>-20 mV</td>
<td>2.3 ± 0.3</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>-10 mV</td>
<td>1.7 ± 0.2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>0 mV</td>
<td>1.3 ± 0.2</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>TTX IC50 (nM)</td>
<td>2.8 ± 1.6</td>
<td>2.6 ± 1.1</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation. Temperature was 23°C.

The Voltage Dependence of the Astrocyte Channel Activation and That of Inactivation Are Both Shifted in a Hyperpolarizing Direction

Peak current-voltage (I-V) relationships were determined from voltage-clamp families for neurons and astrocytes. Superimposition of the resulting neuronal and glial curves revealed a consistent difference (Figure 2A; Table 2). Although both neuronal and glial currents reached a maximum at about the same voltage, -10 mV, the glial current consistently activated at values about 10 mV more negative. These differences were quantified by fitting the activation part of each neuronal and glial I-V relationship with a sigmoidal curve. The average threshold voltage of glial current activation, defined as the voltage at which 5% of the peak inward current was elicited, was -41 mV, versus the average neuronal threshold voltage of -31 mV (Table 2).

Steady-state inactivation (or h<sub>∞</sub>) plots were constructed for each of the 15 neurons and 10 astrocytes. Current was elicited by a series of test steps to a constant potential of -10 mV from a series of prepulse potentials. In both the neurons and the astrocytes, the peak inward current at -10 mV diminished as the prepulse potential became more positive (Figure 2B). Each plot was fitted with a sigmoidal curve. The potential at half-inactivation was -55 ± 4 mV for neurons and -80 ± 6 mV for astrocytes—a difference of 25 mV (Table 2). The slopes of the sigmoidal curves were identical and equivalent to about 4 charges moving across the membrane field.

The Kinetics of the Glial Sodium Channel Are Slower

To quantify the kinetic difference between whole-cell currents in astrocytes and neurons, the current decay was fitted with an exponential curve and the time constant was determined. This parameter, t<sub>h</sub>, is traditionally thought to reflect the rate of the inactivation process (Hodgkin and Huxley, 1952) but was found for some cells to correspond to the activation rate (Aldrich et al., 1983). As in neurons, t<sub>h</sub> was highly voltage-dependent in astrocytes, decreasing with membrane depolariza-
neural and glial decay time constants. Glial current decay was significantly slower at all potentials.

**Tetrodotoxin Sensitivity of Glial Channels**

Sodium channels found in cultured astrocytes are reported to be relatively tetrodotoxin (TTX)-insensitive (Bevan et al., 1985). We compared directly the TTX sensitivity of the neuronal and glial sodium channels under identical conditions, since TTX binding can vary with ionic conditions (Henderson et al., 1974) and stimulus presentation rate (Baer et al., 1976; Cohen et al., 1981; Gonoi et al., 1985). A microtube perfusion system was used to determine the percent of inhibition of current with increasing concentrations of TTX (see Experimental Procedures). The two forms of the sodium channel had nearly identical TTX sensitivity (Table 2). The IC50 was found to be 2.8 nM for the glial channel and 2.6 nM for the neuronal channel.

**Glial and Neuronal Single-Channel Currents Are Qualitatively Similar**

To confirm the difference in sodium currents and to understand the basis of the kinetic differences, we next studied the single-channel properties of sodium channels in type-1 astrocytes and retinal ganglion cells. To simplify the analysis, only patches containing 1 channel were studied (see Experimental Procedures). Patches were studied exclusively in the cell-attached configuration, since it has been reported that excised patches may have altered properties (Aldrich and Stevens, 1987). Also, a cytoplasmic difference might underlie the observed whole-cell differences. Because the potential of a cell-attached patch can be controlled only relative to the cell's resting potential, a high potassium bath solution was used to zero the resting potential (see Experimental Procedures).

Single-channel currents were studied over the range of -30 to 0 mV. Below -30 mV, channels had a low probability of opening, and above 0 mV, the single-channel openings became increasingly difficult to differentiate from noise. While more than 1000 patches from each cell type were examined, only 5 patches from each cell type had high-seal resistance, were held stably for sufficient time, and contained only a single channel, thereby proving suitable for detailed analysis.

Examples of typical single-channel openings in type-1 astrocytes and retinal ganglion cells are shown in Figure 3. Averages of single-channel currents closely mimicked the whole-cell currents at corresponding potentials: for both neurons and glia the average current peaked earlier and decayed more quickly with increasing depolarization. Exponential fits of the decay phase of the average currents were comparable to those in whole cells for both type-1 astrocytes and retinal ganglion cells. However, both the time to peak and the rate of decay were slower in glia than in neurons. The average time to peak ranged from 0.46 ms (0 mV) to 1.07 ms (-30 mV) for neurons, but ranged from 0.86 to 5.8 ms for glia.

The amplitudes of the glial and neuronal single channels were identical at each voltage, ranging from 1.8 pA at -30 mV to 1.2 pA at 0 mV. The slope conductances were also identical: 20 pS (Table 3). Three lines of evidence suggest that the high potassium bath solution had adequately zeroed the resting potentials of both glia and neurons. First, glial and neuronal single-channel ampli-
Table 3. Properties of Single Glial and Neuronal Sodium Channels

<table>
<thead>
<tr>
<th></th>
<th>Type-I Astrocytes</th>
<th>Retinal Ganglion Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope conductance (pS)</td>
<td>20.6 ± 2.9</td>
<td>20.0 ± 3.4</td>
</tr>
<tr>
<td>Averaged current</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak probability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-30 mV</td>
<td>0.03 ± 0.02</td>
<td>0.09 ± 0.07</td>
</tr>
<tr>
<td>-20 mV</td>
<td>0.09 ± 0.04</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>-10 mV</td>
<td>0.15 ± 0.08</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.26 ± 0.05</td>
<td>0.34 ± 0.12</td>
</tr>
<tr>
<td>Mean open time (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-30 mV</td>
<td>0.33 ± 0.07</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>-20 mV</td>
<td>0.41 ± 0.06</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>-10 mV</td>
<td>0.40 ± 0.08</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.36 ± 0.07</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Latency distribution (slow time constant; ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-30 mV</td>
<td>4.67 ± 0.5</td>
<td>1.04 ± 0.6</td>
</tr>
<tr>
<td>-20 mV</td>
<td>2.53 ± 0.4</td>
<td>0.43 ± 0.3</td>
</tr>
<tr>
<td>-10 mV</td>
<td>1.24 ± 0.6</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.63 ± 0.4</td>
<td>0.11 ± 0.04</td>
</tr>
</tbody>
</table>

Glia and Neuronal Forms of the Sodium Channel

Glial and neuronal channels were similar in one other way: with strong depolarizations, channels tended to open only once per step, while with weak depolarizations (−30 mV), they reopened 20%−35% of the time. (A difference is that glial openings sometimes occurred as bursts for steps between −30 and −10 mV.)

Glial Sodium Channels Activate More Slowly Than Neuronal Channels

With increasing degree of depolarization, both glial and neuronal channels tended to open earlier after the onset of the step depolarization (the first latency time). To compare the glial and neuronal opening rates, first latency histograms were constructed by tabulating the number of times a channel opened in a given time interval after the onset of a depolarization. The histograms were then integrated to give a first latency distribution, which gives the probability that a channel will have opened by a given time. These distributions were well fitted with a sum of two exponential functions (see Experimental Procedures) and are demonstrated in Figure 4A. The slow time constants dominated the fit and were plotted against voltage for Figure 5A. At each step potential, the glial activation time constant was at least 4 times greater than that of neurons. These points could themselves be well fitted with exponential curves (Figure 5A). However, these curves could not be superimposed by a shift along the voltage axis, providing further evidence that the glial-neuronal first latency differences are not artifically caused by a failure to control membrane potential adequately.

Glial Channels Inactivate More Slowly Than Neuronal Channels

Individual channel openings in glia appeared, on average, to be longer than those in neurons (Figure 3). Open time histograms were constructed for each test potential and were integrated to give open time distributions. The distributions were well fitted by single exponential functions; the time constant gave the mean open time. Because channels mainly opened once per trace (except at the most negative step potentials), the open time reflected the rate of channel inactivation (for example, see Aldrich et al., 1983). Neuronal and glial open time distributions are compared in Figure 4B, and their mean open times in Figure 5B. For both channel types, the...
Figure 5. Voltage Dependence of Activation and Open Times for Glial and Neuronal Sodium Channels

(A) The latency distributions shown in Figure 4 were fitted by a sum of two exponentials (see text). The slow time constant predominated and was plotted relative to the step potential. In both glia and neurons the first latency was observed to be strongly voltage-dependent. Glial first latencies were about 4 times slower than neuronal latencies at all step potentials.

(B) The open time distributions shown in Figure 4 were fitted well by a single exponential function. The time constants were plotted relative to step potential. For both glial and neuronal channels, the mean open times were relatively voltage-independent. Glial open times were about twice as long as neuronal. Values plotted in both (A) and (B) are averages calculated from 5 channels of each type; the standard deviations are given in Table 3.

open times were weakly voltage-sensitive. However, glial channel open times were about twice as long as those of neurons.

The Time Course of Glial and Neuronal Average Single-Channel Currents Is Dominated by the Rate of Channel Activation

Aldrich et al. (1983) demonstrated that in neuroblastoma cells the time course of whole-cell sodium current decay does not reflect the time course of the inactivation process (also see Aldrich and Stevens, 1987). Rather, they found that the time course of activation was slow (and voltage-dependent), while the inactivation rate was much faster (and not particularly voltage-dependent). We found that this applies to sodium channels in both glia and neurons in the CNS. For both, the time course of the average single-channel current—both time to peak and decay phase—largely followed the time course of the latency density at all potentials. The effect was particularly noticeable in the glial cells, in which the activation time course was 4 times slower than in neurons, but the inactivation rate was only 2 times slower.

In Figure 6, the latency histograms were convolved with a relation that is 1 minus the normalized integral of the open time histograms (see Experimental Procedures). The probability of a channel being open, p(t), was calculated by dividing the average single-channel current by the single-channel current amplitude. For both neurons and glia, the convolution integral closely predicted the probability of a channel being open: p(t) could be superimposed on the convolved probability. An exception occurred at very negative potentials, particularly -30 mV, at which the average probability for both neurons and glia decayed more slowly than the convolution. This was caused by the higher incidence of reopening at this potential. A measure of the amount of reopening was given by the difference in area under the two curves. The extent of reopening was another difference between neuronal and glial channels. Neuronal channels reopened less frequently (area differences of 19%, 10%, 10%, and 5% at -30, -20, -10, and 0 mV), while glial channels reopened more frequently (differences of 38%, 34%, 27%, and 15% at the same potentials). It appeared that many of the glial reopenings occurred in bursts associated with the first opening.

Properties of Sodium Channels in Some Other Glial and Neuronal Cell Types

The postnatal rat optic nerve contains three other glial cell types: oligodendrocytes, type-2 astrocytes, and ependymal cells. It also contains a glial progenitor cell, the O2A (Raff et al., 1983b). All of these but the oligodendrocyte express sodium currents. Are the sodium currents in all of these cell types of the glial form?

In ependymal cells, sodium current measured by whole-cell recording was expressed in less than half of these cells; when it was present it was found to have the voltage dependence and kinetics of the type-1 astrocyte (glial) sodium current (Table 4).

Type-2 astrocytes express large sodium currents in culture, usually 2-4 nA (Barres et al., 1988a), but in cell suspensions studied immediately after dissociation, the currents were not present (unpublished data from adult optic nerve suspensions). Using cell-attached, single-
channel recording of type-2 astrocytes in culture, we observed both the glial and the neuronal channel forms, sometimes even in the same patch. The $h_m$ curves obtained with whole-cell recording gave further evidence of a mix of channel types; they could be fitted only by the sum of two sigmoidal curves representing the glial and neuronal forms (Figure 7D). The proportion of each channel type in type-2 astrocytes was approximately determined by the proportion of the glial and neuronal sigmoidal curves used for this fit. The type-2 astrocyte expressed primarily the neuronal form of the channel, with the glial form contributing 8%-18% of the current (10 cells), a proportion that appeared to increase with increasing time of culture.

Type-1 astrocytes found in white matter are thought to be equivalent in some respects to the main astrocyte type found in cortex. Whole-cell recordings of sodium currents in 7 cortical astrocytes in highly purified cultures (see Experimental Procedures) demonstrated their properties to be identical to those of type-1 astrocytes from optic nerve (Table 4). Similar recordings of sodium currents in 15 cortical motor neurons (identified as described in Experimental Procedures) demonstrated their properties to be almost identical to those of the retinal ganglion cells.

**Discussion**

Two Functionally Distinct Forms of the Voltage-Sensitive Sodium Channel

Two functionally different forms of the voltage-dependent sodium channel occur in the mammalian CNS. One form was predominantly found in neurons, while the other form was predominantly found in glia. Al-
though qualitatively similar, the two channels were quantitatively distinct in both voltage dependence and kinetics: the glial form activated more than 4 times more slowly than the neuronal form and inactivated twice as slowly, while the voltage dependence of activation and that of steady-state inactivation were shifted 10 and 25 mV, respectively, in a hyperpolarizing direction. In addition, the glial channel reopened more frequently.

Could these differences be artifactual? Several possible artifacts appear unlikely: antibody labeling of the cell surface did not account for the differences, as channels exhibited identical whole-cell properties regardless of labeling of the cell. Enzyme effects on the channel could also not explain the difference: each cell type was exposed to the same enzymes for identical lengths of time. Also, whole-cell and single-channel records from cortical astrocytes and type-2 astrocytes in culture demonstrated the same properties as those observed in acutely dissociated cells. Could a differing developmental time course of assembly of sodium channels between neurons and glia account for the differences (for instance, see Wollner et al., 1988)? This is also unlikely: acutely dissociated adult optic nerve type-1 astrocytes had whole-cell sodium currents with properties identical to those in P7 type-1 astrocytes (although our preliminary observations indicate that these adult glial cells have a much higher density of sodium current).

Relationship of Single-Channel Properties to the Whole-Cell Current
Our results confirm several of the findings of Aldrich et al. (1983), who showed that voltage-dependent sodium channels in neuroblastoma cells usually open once per depolarization epoch and then close to an inactivated state from which they do not reopen. In neuroblastoma this inactivation rate is rapid and not very voltage-dependent; the rise and decline in current during a voltage step is instead accounted for by the time and voltage dependence of the slower activation process. Our data indicate that both the glial and the neuronal forms of the sodium channel in rat brain share this basic behavior. When corrected for bath temperature (using a \(Q_10\) of 2; Bezanilla and Taylor, 1978), the activation and inactivation rates of the neuronal rat brain channel correspond nearly exactly with those in neuroblastoma. Because the difference between activation and inactivation rates was greater for the glial channel than for the neuronal channel or the neuroblastoma channel, the glial channel offered a more conspicuous demonstration of the dominance of the activation process in determining overall time course of the whole-cell current.

(D) Both forms of the channel were present in type-2 astrocytes. Steady-state inactivation curves determined from whole-cell records from type-2 astrocytes in culture (solid line) were well fitted by the sum of two sigmoidal curves with midpoints near those of the glial and neuronal steady-state inactivation curves. Here 85% of a sigmoid with a midpoint at \(-50\) mV (dashed line) and 15% of a sigmoid with a midpoint at \(-80\) mV (dashed line) have been added to fit the steady-state inactivation curve of a type-2 astrocyte.
Gonoi and Hille (1987) have recently confirmed these basic conclusions of Aldrich et al. (1983) by showing that proteolytic enzymes which remove inactivation prolong the rising phase of macroscopic sodium currents in neuroblastoma cells. Since a different result was found in squid axons, frog node, and frog muscle, they concluded that sodium currents in neuroblastoma cells have a microscopic behavior different from that in these traditional preparations. Our results demonstrate that sodium channels in mammalian CNS neurons and glia share the microscopic properties of sodium channels in neuroblastoma (also see Shraga et al., 1985).

**Comparison with Other Types of Sodium Channels**

There is evidence for a multiplicity of sodium channel types in several other preparations, including squid axon (Matteson and Armstrong, 1982; Gilly and Armstrong, 1984), frog node of Ranvier (Benoit et al., 1985), cerebellar Purkinje cells (Llinas and Sugimori, 1980), cardiac muscle (Patlak and Ortiz, 1985), neuroblastoma (Nagy et al., 1983), and skeletal muscle (Frelin et al., 1983; Nagy et al., 1984; Gonoi et al., 1985; Haimovich et al., 1986). Yet the glial sodium channel has properties that clearly distinguish it from most of these other sodium channel types. Certain endocrine-derived cells, including GH3 cells and pancreatic β islet cells, have TTX-sensitive sodium channels with a very negative steady-state inactivation curve (Vandenberg and Horn, 1984; Hiriart and Matte-
adult type-1 and type-2 astrocytes are excitable. Our data suggest that the O2A progenitor cell may be excitable. It is equally possible that glial sodium channels either play no role in cell function or function in some capacity other than excitability.

What Causes the Difference between Glial and Neuronal Forms of the Sodium Channel?

Membrane Environment

It is possible that the protein structures of the channels are identical, but the local membrane environment is different. If so, it cannot be just a difference in local surface charge: the differences are not accounted for by a simple voltage shift or by changing the energy of any one state or energy barrier. Moreover, both glial and neuronal forms of the channel have been found in the type-2 astrocyte membrane, even within the same patch.

Posttranslational Modification

Perhaps the channels have the same primary amino acid structure but differ because of a posttranslational modification. Recent results suggest that phosphorylation may alter function of sodium channels (Coombs et al., 1988, Biophys. J., abstract; Sigel and Baur, 1988). Neither of these modifications appears to account for the alterations in properties we have observed.

Subunit Difference

Although only the α subunit of the sodium channel appears to be necessary to construct a normally functioning sodium channel (Goldin et al., 1986; Noda et al., 1986b), the function of the β1 and β2 subunits in rat brain is not known. A switch of subunits has been shown to explain the different kinetic properties of the adult and fetal acetylatehine receptors (Schuetze, 1986); it is a possibility here, too.

Primary Structure

Numa and co-workers (Noda et al., 1986a) isolated three different cDNAs for the sodium channel from rat brain. Two were expressed in oocytes and were characterized physiologically (Noda et al., 1986b; Stuhmer et al., 1987; Suzuki et al., 1987); the other remains to be characterized. One of these cDNAs may in fact represent the message for the glutamate channel. However, comparison of our data with those from oocyte injection experiments would not provide a definitive answer, since sodium channels composed of α subunits alone have altered properties (Auld et al., 1988).

Because highly purified populations of retinal ganglion cells (Barres et al., 1988b) and astrocytes can be obtained, it should be possible to extract sufficient message to compare the molecular structures of the neuronal and glial forms of the sodium channel. If the functional differences are based on variations in primary sequence, those variations may point to functional domains of the channel and thus to rational design of in vitro mutagenesis experiments.

Experimental Procedures

Preparation of Cell Suspensions

Retinal Ganglion Cells

P7 retinas were dissected and then dissociated enzymatically to make a suspension of single cells, essentially as described by Huetter and Baughman (1986). Briefly, the tissue was incubated at 37°C for 75 min in a papain solution (30 U/ml; Worthington) equilibrated with 95% O2 and 5% CO2. This solution also contained Earle's balanced salts, magnesium, EDTA, sodium bicarbonate, glucose, and L-cysteine. The tissue was then triturated sequentially with a 1 ml pipette in a solution containing ovomucoid (0.2%); Boehringer Mannheim) and bovine serum albumin (BSA: 0.1%; Sigma) to yield a suspension of single cells. After centrifugation at 800 × g, cells were resuspended in Eagle's minimum essential medium. To avoid toxic effects, cells were never exposed to glutamate, aspartate, or glutamine and were never cooled below room temperature during this procedure. The total time for this dissociation procedure was only about 2 hr.

This dissociation procedure was selected because it results in larger yields than standard trypsin dissociation techniques and because the resulting cells are in excellent condition for immediate electrophysiological recording. We found that it was easier to obtain long-lasting and stable seals on these cells when the membranes were further cleaned by a brief exposure to trypsin. Residual ovomucoid (which inhibits trypsin) was washed out with PBS, and cells were incubated in trypsin (0.125%) in PBS (containing normal calcium and magnesium concentrations) for 4 min. The trypsin was then inhibited with soybean trypsin inhibitor (Sigma) and the cells were resuspended in Eagle's medium containing 0.1% BSA.

Cortical Motor Neurons

Newborn Long-Evans rats (P1 or P4) were injected with rhodamine-conjugated latex microspheres (Katz et al., 1984) into the corticospinal tract at the cervico-medullary junction according to the technique of Swearengen and Chun (unpublished data). Two days were allowed for retrograde transport of the microspheres into the cells bodies of motor neurons in the CNS. At day P5 or P6, the animal was sacrificed by decapitation and the motor cortex was dissected out. This cortical tissue was then dissociated enzymatically with papain as described above.

Astrocytes

Optic nerves from P6 Long-Evans rats were dissected. The nerves were taken from just posterior to the optic foramen through to the anterior one-third of the optic chiasm. (Although the optic nerve itself does not contain neuronal cell bodies, the posterior chiasm may contain neuronal cell bodies originating from the supraoptic nucleus.) Dissociated cell suspensions were then prepared with papain and trypsin by a procedure identical to that described for neurons except that 21 and 23 gauge syringe needles were used for trituration. We found that this dissociation technique not only resulted in significantly higher cell yield and viability than the trypsin/colagenase procedure for optic nerve (Raff et al., 1983a), but also provided better preservation of cell membranes. This facilitated seal formation for patch-clamp recording and identification of cell types because of the excellent degree of morphological preservation.

Preparation of Cultures

Purified Cortical Astrocytes

Cultures of cerebral cortical astrocytes were prepared from P1 rats using the technique of McCarthy and deVellis (1980). Primary cultures of astrocytes were grown to confluence in medium containing DMEM and 10% fetal calf serum. The top layer of cells was shaken off manually, and the remaining cells were treated with 25 μg/mL cytosine arabinoside for 2 days. Finally, these purified cells were trypsinized from the flask and plated on 13 mm diameter round coverslips coated with poly-l-lysine. These could be directly transferred to the recording chamber. The final cultures were demonstrated to be greater than 95% pure astrocytes on the basis of immunohistochemical labeling with markers to GFAP, A2B5, and galactocerebroside.

Type-2 Astrocytes

Cultures were prepared according to the procedures of Raff and co-workers (Raff et al., 1983a), (1983b). Briefly, type-2 astrocyte cultures were prepared from suspensions of optic nerves from P7 rats. The type-2 astrocytes were cultured in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin, 0.01% streptomycin, and 2 mM L-glutamine. The cells were plated at a density of 20,000 cells per 13 mm round glass coverslip (precoated with poly-l-lysine) and

Experimental Procedures

Preparation of Cell Suspensions

Retinal Ganglion Cells

P7 retinas were dissected and then dissociated enzymatically to
were cultured at 37°C in a humidified atmosphere of 5% CO2 and 95% air.

Identification of Cell Types in Suspensions

**Retinal Ganglion Cells**

In initial experiments, retinal ganglion cells were identified by labeling suspensions with antibodies to the Thy-1 surface antigen using indirect immunofluorescence (Barnstable and Drager, 1984; 2G12 ascites was generously provided by Dr. Colin Barnstable). However, because these cells were easily recognized by morphology alone and the largest somata present and a characteristic dendritic morphology, we performed most experiments without the use of antibody labeling. Control experiments demonstrated that the presence of antibody labeling did not alter the properties of the whole-cell sodium currents. To minimize problems with spatial control of membrane potential, cells with small residual dendrites were selected for whole-cell recording experiments.

**Cortical Motor Neurons**

Motor neurons were identified during electrophysiological recording by viewing with an epifluorescence illumination. The retrogradely transported beads were brightly fluorescent with a rhodamine filter set. Although most cells had retained long processes and dendrites (apical and basal), some cells had lost all of their processes during the dissociation procedure and were nearly spherical. These cells were exclusively selected for electrophysiological recording to eliminate the problem of spatial control of membrane potential. To rule out the possibility that these spherical cells could be astrocytes that had engulfed beads released from labeled motor neurons, a cell suspension was labeled with a rabbit antisera to GFAP (Accurate) and incubated with a fluorescein-conjugated goat anti-rabbit immunoglobulin (Cappel). This procedure, which normally labels all astrocytes, revealed no doubly labeled cells and thus demonstrated that no bead-containing cells were astrocytes.

**Type-1 Astrocytes**

In initial experiments, we labeled optic nerve suspensions with antibodies directed against RAN-2 (Bartlett et al., 1981; RAN-2 is preserved on optic nerve type-1 astrocytes after papain dissociation) and we identified type-1 astrocytes using indirect immunofluorescence during electrophysiological recording. As we found for neurons, recordings of astrocytes were facilitated by a brief incubation in trypsin. Unfortunately, trypsin destroys the RAN-2 marker (Bartlett et al., 1981). Nevertheless, we found that RAN-2-positive cells in P6 optic nerve suspensions have a unique morphology after the papain dissociation that distinguishes them from the other main cell types present: such as oligodendrocytes and O2A progenitor cells. These other cell types had smooth spherical membranes, but the type-1 astrocytes were often irregularly shaped and always had a rough-appearing membrane. Therefore, for our later experiments, it was necessary to use other markers for astrocytes.

**Type-2 Astrocytes**

Type-2 astrocytes were often irregularly shaped and always had a rough-appearing membrane. Therefore, for our later experiments, it was necessary to use other markers for astrocytes.

**Ependymal Cells**

The ependymal cell was recognized by its characteristic morphology: it was large and vacuolated, and the beating cilia were a dead giveaway.

**O2A Progenitors**

The O2A was recognized in P7 suspensions by prelabeling with antibodies to the surface antigen A2B5. In P7 optic nerve suspensions, about 25% of the cells are O2A cells (Raff et al., 1983b), recognized by both their small size and their A2B5-positive phenotype. They constitute about 5% of the A2B5-positive cells at this age and cannot be confused with type-2 astrocytes (which are also A2B5-positive) because type-2 astrocytes do not develop until after P7 (Miller et al., 1985).

Preparation of the RAN-2 Antibody

We obtained the RAN-2 cell line from American Type Culture Collection. Supernatants prepared from this cell line stained optic nerve cell suspensions and cultured cells only weakly. The cell line was subcloned, and each clone was screened for the presence of an antibody that would provide stronger labeling. Several such clones produced significantly stronger staining, and one in particular, RAN-2 clone 1/5, was excellent. This antibody clearly retained its specificity, as judged by its ability to label type-1 astrocytes, meningeal cells, and ependymal cells. The labeling retained the characteristic speckling pattern (Bartlett et al., 1981) on type-1 astrocytes.

**Immunofluorescence Staining**

Monoclonal RAN-2 supernatant was used at a 1:1 dilution to label optic nerve suspensions for identification of type-1 astrocytes. 2G12 ascites was used at a dilution of 1:1000 to label retinal suspensions for identification of retinal ganglion cells. A2B5 (obtained from American Type Culture Collection) supernatant was used at a dilution of 1:10 to label O2A glial progenitor cells in P7 optic nerve suspensions. Galactocerebroside supernatant (generously provided by Barbara Ranscht, Ranscht et al., 1982) was used at a dilution of 1:50 to label oligodendrocytes in P7 optic nerve suspensions. The binding of primary antibodies was detected with a rhodamine- or fluorescein-conjugated goat anti-mouse immunoglobulin (Flah), fragment antibody (Cappel), fragment-specific diluted 1:50 (Cappel). No significant staining was observed when control monoclonal supernatant was used in place of the primary monoclonal antibodies.

**Electrophysiological Recording**

**Tight-Seal, Whole-Cell Recording**

A small aliquot of cells (10 µl) was placed in the recording chamber that contained an appropriate bath solution (volume 500–750 µl). Standard procedures for pipette preparation, seal formation, and whole-cell recording were used (Hamill et al., 1981; Corey et al., 1984). Micropipettes were drawn from hard borosilicate capillary glass (VWR), coated with Sylgard to reduce their capacitance, and fire-polished to a bubble diameter of 4.0–4.5 µm (corresponding to an internal tip diameter of about 0.6–0.8 µm; Corey and Stevens, 1983; Mittman et al., 1988; corrected to inner diameter as per our unpublished data). Pipette capacitance and series resistance were electronically compensated by the patch clamp. A Yale Mark V. All experiments were done at room temperature, approximately 23°C.

Voltage stimuli were generated, and responses were recorded with a PDP 11/73 computer (INDEC). Analog signals were filtered with an 8-pole, low-pass Bessel filter before being digitized and recorded by the computer. In each experiment, linear capacitive and leakage currents were measured and subtracted before storage of data. The BCLAMP program set was used for acquisition and analysis of whole-cell data.

The need for an agar bridge to connect the electrode with the headstage of the patch clamp was eliminated by the addition of 5 mM CSCI to the whole-cell internal solutions (Table 1). Junction potentials between the ground and the input were electronically nulled. Because the bath and pipette solutions were filtered with a 8-pole, low-pass Bessel filter before being digitized and recorded by the computer, in each experiment, linear capacitive and leakage currents were measured and subtracted before storage of data. The BCLAMP program set was used for acquisition and analysis of whole-cell data.

To obtain the cell-attached patch configuration, the pipette was positioned in contact with the cell surface and mild suction was applied, resulting in a gigohm seal with a resistance of 1–100 GΩ (typically, 10–50 GΩ). Capacitive transients were then electronically compensated, for experiments using the whole-cell patch recording configuration. Further suction was applied, resulting in rupture of the patch of membrane beneath the pipette. The solution in the pipette was then continuously replaced with a solution that is isosmotic to the cell and contains a 100 mM KCl. Capacitive transients were then electronically compensated, for experiments using the whole-cell patch recording configuration. Further suction was applied, resulting in rupture of the patch of membrane beneath the pipette. The solution in the pipette was then continuously replaced with a solution that is isosmotic to the cell and contains a 100 mM KCl.
of these experiments. Although series resistance was compensated electronically, typically a residual series resistance of 4-6 MΩ remained. In the presence of a very large whole-cell sodium current (up to 6 nA), as was observed in neurons, this amount of resistance could produce a large voltage drop across the electrode, thereby altering the voltage control of the cell. To minimize artifactual differences from this problem, neuronal sodium current was decreased by the addition of 10 nM TTX (Sigma) to the bath solution, thus approximating that found in the astrocytes (see Table 2). Finally, it has frequently been reported that many channels undergo apparent shift in their I-V relationship over time during whole-cell recording (Marty and Neher, 1983; Corey et al., 1984). Although we have observed large voltage shifts in our studies of calcium channels, we have detected only small shifts with the sodium currents. Control experiments in both neurons and astrocytes established that such shifts were usually less than 10 mV and were complete after the first 5 min in the whole-cell configuration. Therefore, all data were collected after an obligatory 5 min wait. It was rare to record stably from either neurons or glia in the whole-cell configuration for longer than 15 min.

**Single-Channel Recording**

All recordings were performed using the cell-attached recording configuration. Patch currents were filtered at 3 kHz and were sampled at a rate of 5 or 10 kHz. Noise averaged about 0.25 pA Hz at 3 kHz.

Only patches containing 1 channel were recorded from and analyzed. Because of the use of a high potassium bath solution that depolarized the cells, it was necessary to hold patch potentials negative for several minutes to reach currents in the patch that had undergone slow inactivation. In the case of astrocytes, whose membranes contained a low density of sodium channels, pipettes with bubble numbers of 1 or 4.5 were used (corresponding to an internal pipette diameter of 0.7 μm). For neurons, in which the density of channels was high, bubble numbers of about 2 were used (internal tip diameter of about 0.2 μm). We found World Kwik-Fil electrode glass (Cat. 1B105-04) made by Pyrex glass (Corning 7740 or its Kilmber equivalent KG-33) allowed both low noise, high resistance seals (50-200 MΩ) and easy filling even with bubble numbers less than 2. Typically only 1 of about 100 astrocyte patches contained a single sodium channel and was otherwise adequate for study. The majority of patches contained no sodium channels. In the case of retinal ganglion cells, about 1 in 50 patches could be studied. Most patches contained multiple sodium channels; although many patches contained no channels. For both astrocytes and neurons, patches containing potassium channels were not studied.

**Analysis of Single-Channel Data**

Between 256 and 512 voltage steps were presented at each test voltage (–30, –20, –10, and 0 mV). Secondary leak subtraction was done by subtracting the average of all traces with no openings from the average of all traces containing openings. Open probability as a function of time was computed by averaging all traces and dividing by the average single-channel current amplitude at that test step potential. The peak probabilities were maximum at 0 mV and tanged among all cells studied at this voltage from 0.20 to 0.50. These values indicate that if 2 channels were present in a patch, from 4 to 25 traces per hundred should contain 2 channels simultaneously open. Therefore we collected sufficient traces to detect patches containing 2 or more channels.

Open durations were measured at the half-single-channel-current level, and open time histograms were constructed from these values. First latency histograms were constructed from measurements of the time from the onset of the command voltage to the first opening of a channel in a trace, minus a 0.17 ms delay that occurred at the filter. Open time histograms were integrated to form open time distributions, and these were fitted with single exponential curves to obtain mean open times. Estimates of missed events were obtained from the intersection of this curve with the y axis. Latency histograms were integrated to give latency distributions and were fitted by the function:

$$F(t) = 1 - (P_0 \exp(-RT) - R \exp(-R/\tau - R))$$

as given in Aldrich et al. (1983). Convolutions of first latency histograms and open time distributions were calculated as described in Aldrich et al. (1983).

**Solutions**

The solutions were designed so that only currents carried by sodium would be observed (Table 1). Solution A was the bath solution for whole-cell recording and the pipette solution for cell-attached, single-channel recording. TTX (10 nM) was added to solution A for whole-cell recording of neurons (see above). Solution B was used in the pipette for whole-cell recording. All cell-attached recordings were done with solution C in the bath (Table 1). This solution contained a high concentration of potassium, approximately equal to that within the cells, in order to zero the cell's resting potential so that the command potentials would be known absolutely (Heis et al., 1986).

Calcium (2 mM) was present in the external solutions to maintain a relatively normal surface charge, but current that could be carried by calcium was blocked by the addition of 100 μM calcium. However, calcium currents were not observed in the astrocytes even in the presence of 10 mM calcium and the calcium channel agonist BAYK-8644 (10 μM; Miles). Small calcium currents could be observed in the neurons, but only in the presence of 10 mM calcium. Because a calcium-blockable sodium channel has recently been reported to occur in bullfrog autonomic axons (Bowers, 1985), we looked for the presence of a calcium-sensitive sodium current in the astrocytes and found none.

**TTX Dose-Response Determination**

The TTX dose-response relationship was obtained using a microtube perfusion system (Yellen, 1982). The microtube system design we used was provided by David Nriel; it consists of a series of 1 ml microcapillary tubes (Drummond) epoxied together in a linear array. Each tube was about 64 mm long and 60 μm in internal diameter and was connected through narrow tubing to a 10 ml syringe containing its respective perfusion solution. The microtube assembly was lowered into the bath prior to recording, with all tubes clamped shut. After establishment of the whole-cell configuration, the cell was successively exposed directly in front of each tube, the tubing to that tube was unclamped, and the whole-cell sodium current was recorded. Because a stable whole-cell configuration could only be maintained for about 15 min, we limited each dose-response experiment to four concentrations of TTX in order to allow a 1-2 min exposure time for each concentration of TTX. This was sufficient time to reach an equilibrium current. Each set of four points was plotted on a log concentration scale and fitted with a sigmoidal binding curve. The concentration that blocked half of the current was determined from the curve (IC50).

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