MacFarlane, Stacey Nee and Harald Sontheimer. Spinal cord astrocytes display a switch from TTX-sensitive to TTX-resistant sodium currents after injury-induced gliosis in vitro. Primary spinal cord astrocyte cultures were obtained by methods previously described (MacFarlane and Sontheimer 1997). Cultures were >95% positive for glial fibrillary acidic protein by immunocytochemistry and were grown for 8 days in vitro (DIV) before being scarred mechanically with a sterile pipette tip. For time periods at which the scarred region became largely repopulated by cells, coverslips were grid-marked with permanent ink before culturing to identify the region that had been scarred.

**Methods**

**Cell culture**

Primary spinal cord astrocyte cultures were obtained by methods previously described in MacFarlane and Sontheimer (1997). Cultures were >95% positive for glial fibrillary acidic protein by immunocytochemistry and were grown for 8 days in vitro (DIV) before being scarred mechanically with a sterile pipette tip. Time periods at which the scarred region became largely repopulated by cells, coverslips were grid-marked with permanent ink before culturing to identify the region that had been scarred.

**Whole cell patch-clamp recordings**

Recordings were obtained from scar-associated astrocytes (≥6 h postinjury) and age-matched, noninjured control astrocytes using methods previously described (MacFarlane and Sontheimer 1997). Cells were perfused at room temperature with saline solution containing (in mM) 130 NaCl, 5 KCl, 1.2 MgSO₄, 1.6 NaHPO₄, 0.4 NaH₂PO₄, 10.5 glucose, and 32.5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4 with NaOH. Added to this solution just before recording was 1 mM CaCl₂. Electrodes typically had resistances of 4–6 MΩ when filled with a solution containing (in mM) 145 KCl, 1 MgCl₂, 10 ethylene glycol-bis-(β-aminoethyl ether) N,N,N’,N’-tetraacetic acid, and 10 HEPES-sodium salt, adjusted to pH 7.25 with tris(hydroxymethyl)aminomethane. To facilitate analysis of sodium current kinetics, patch pipettes were filled with a solution in which 145 mM...
KCl was replaced with 125 mM N-methyl-D-glucamine titrated to its $p_K$, with HCl, plus 20 mM tetraethylammonium-chloride and 30 mM glucose. For pharmacological analysis, TTX was added via bath perfusion, and $>1.5$ min were allowed before recording. Five-minute washes were used between recording from cells on the same coverslip.

Analysis

Unless otherwise stated, all values are reported as means ± SE with n being the number of cells sampled. For activation plots, normalized peak conductance values were plotted as a function of membrane potential and mean values were fit to the Boltzmann equation by weighted fit. For inactivation, normalized peak current amplitude was plotted versus prepulse potential, and mean values were fit to the Boltzmann equation. For both activation and inactivation plots, the mean ± SE and mean Boltzmann fit are graphed. Activation and inactivation time constant values were well fit by a single exponential function using Origin 4.1 (Microcal, Northampton, MA). The resulting time-constant ($\tau$) values were plotted as a function of the command potential. All statistical evaluations were done with GraphPAD (InStat), using Student’s unpaired, two-tailed t-tests.

RESULTS

We mechanically scarred confluent monolayers of spinal cord astrocytes using a sterile pipette tip, leaving a 200-μm cell-free scratch on the coverslip. As we previously demonstrated, this in vitro scarressing induces a more than threefold increase in proliferation at the scar region. Scar closure occurs within 24 h, is inhibited largely by the mitogenic inhibitor Ara-C, and is associated with marked alterations in $K^+$ channel expression (MacFarlane and Sontheimer 1997). We examined astrocytes associated with such in vitro scars 6–24 h after the insult and analyzed Na$^+$ channel expression. We previously observed a transient, twofold increase in Na$^+$ current densities, albeit without any notable changes in Na$^+$ channel phenotype. We reevaluated this issue with specific attention to potential pharmacological and kinetic differences in the Na$^+$ currents of scar-associated astrocytes versus nonscarred controls.

Scar-associated astrocytes are TTX-resistant

Recordings from unscarred, control astrocyte cultures (>8 DIV) demonstrated voltage-activated Na$^+$ currents in 45% of recorded cells. Figure 1A shows a phase photomicrograph of a control coverslip with representative examples of whole cell current recordings. Inward currents could be induced in response to a series of depolarizing voltage steps from −70 to 80 mV preceded by a 200-ms prepulse to −110 mV. Inward currents were inhibited largely by 10 nM TTX yielding complete block in 56% of control astrocytes. Na$^+$ currents in all remaining cells were completely inhibited by 100 nM TTX (n = 18). Figure 1B shows a phase photomicrograph of scar-associated cells (6-h postinjury) and representative current traces in response to the same voltage protocol. Inward currents activated with depolarizing voltage steps more than −50 mV and were observed in 79% of scar-associated cells. In 83% of these cells (n = 35) currents were insensitive to 10–100 nM TTX. Incidentally, 63% of the cells that were $>300 \mu$m away from the scar but on the injured coverslip also demonstrated TTX-R currents (n = 19), suggesting that a diffusible factor influenced the expression of TTX-R currents in distal cells via the culture media or gap-junction coupling. We examined the TTX sensitivity of scar-associated astrocytes in greater detail and established dose-response relationships over a larger range of concentrations. These data are summarized in Fig. 1C, which compares TTX dose-response curves for both control (n = 12) and scar-associated cells (n = 12). The apparent IC50 for TTX was 314 nM in scar-associated cells versus 8 nM in control cells, suggesting a ~40-fold difference in sensitivity to TTX.

Biophysical properties

In DRG neurons, sensory cranial ganglion cells, and rat skeletal muscle, TTX-R sodium channels demonstrate a positive shift of both the conductance-voltage and inactivation curves of TTX-R sodium currents (for review see Yoshida 1994), whereas in pancake astrocytes, TTX-R currents display a negative shift (Sontheimer and Waxman 1992). We analyzed the inactivation and activation curves for the two current types displayed in control versus scar-associated astrocytes. Figure 2A shows representative recordings using a voltage protocol for steady-state inactivation that involves stepping the cell to varied prepulse voltages ranging from −160 to −40 mV (200 ms) and then stepping to a voltage at which sodium currents were activated maximally (−10 mV). The normalized peak current was plotted as a function of the prepulse voltage, and the mean inactivation curve was fit to the Boltzmann equation. Figure 2B demonstrates the inactivation curves for both TTX-S (n = 14) and TTX-R (n = 12) currents. There is no significant difference between the two curves, and the voltage at which the currents are half maximal ($V_{1/2}$) was −68 mV for TTX-S and −63 mV for TTX-R cells. We similarly determined the voltage-dependence of activation using a protocol (Fig. 2C) that first hyperpolarized cells to a prepulse potential of −110 mV and then stepped for 8 ms to depolarizing voltages (from −70 to 80 mV). The normalized peak conductance then was plotted as a function of command voltage (Fig. 2D) and the mean activation curve fit to the Boltzmann equation. Activation curves were significantly different over the range of −40 to −20 mV (P $\leq$ 0.003). The $V_{1/2}$ values for TTX-S versus TTX-R sodium currents were −33 and −23 mV, respectively.

Kinetic analysis

Because in some published reports the inactivation kinetics of TTX-R sodium currents are slower than those of TTX-S currents (for review see Yoshida 1994), we wanted to see if there were any differences in the kinetics of sodium currents of astrocytes upon injury. Using a pipette solution that allowed isolation of sodium currents, we analyzed the time constants of activation ($\tau_a$) and inactivation ($\tau_i$) versus command potential. Figure 3, A and B, respectively, shows representative examples of the activation and inactivation of TTX-S versus TTX-R currents in response to a hyperpolarizing prepulse followed by a voltage step to −10 mV. Both the activation to peak from baseline and the inactivation to steady-state were fit to a first-order exponential (⋯). The
FIG. 1. Altered tetrodotoxin (TTX) sensitivity upon injury in vitro. A: photomicrograph of control culture and representative Na$^+$ current traces for control cells. In the presence of normal bath solution, Na$^+$ currents are evoked in response to a voltage protocol that hyperpolarized the cell to $-110 \text{ mV}$ for 200 ms and then depolarized the cell for 8 ms to potentials ranging from $-70$ to $80 \text{ mV}$. Sodium currents in control cells are inhibited largely by 10 nM TTX. B: photomicrograph of scar-associated cells and their representative current recordings. In response to the same voltage protocol (above), scar-associated cells exhibit Na$^+$ currents with twofold greater conductance than those of control cells (MacFarlane and Sontheimer 1997). These currents are largely resistant to 10 and 100 nM TTX. C: dose-dependent current inhibition for control vs. scar-associated Na$^+$ currents. Apparent IC$_{50}$ for scar-associated cells was $314 \text{ nM}$, which is $\sim 40$-fold less sensitive than control cells (IC$_{50} = 8 \text{ nM}$). Scale bar indicates $100 \mu\text{m}$.

time constants for activation were identical for TTX-S and TTX-R sodium currents determined for a total of 33 cells (Fig. 3C). However, Fig. 3D demonstrates that TTX-R sodium currents showed significantly slower inactivation kinetics at voltages ranging from $-10$ to $10 \text{ mV}$ ($P < 0.0004$, $P < 0.004$, and $P = 0.03$, respectively).

**DISCUSSION**

Here we describe that spinal cord astrocytes shift from TTX-S to TTX-R sodium currents upon injury-induced gliosis in vitro. This change is accompanied by a depolarization shift in the inactivation and activation curves of TTX-R sodium currents as well as significantly slower inactivation kinetics as compared with TTX-S currents in control cells. Though it has long been known that glial cells express Na$^+$ channels reminiscent of those in excitatory cells (Bevan et al. 1985; Chiu et al. 1984; Clark and Mobbs 1994), their function in inexcitable cells remains an enigma. Interestingly, subpopulations of astrocytes in optic nerve (Sontheimer et al. 1991), cortex (Bevan et al. 1985; Nowak et al. 1987), hippocampus (Sontheimer et al. 1991), spinal cord (Thio and Sontheimer 1993), and retina (Chao et al. 1993) express Na$^+$ currents that are much less TTX sensitive than typical Na$^+$ currents seen in neurons, with IC$_{50}$ values between 0.5 and $1 \mu\text{M}$. In spinal cord and optic nerve, TTX-S channels are expressed predominantly in stellate astrocytes whereas TTX-R channels predominate in flat astrocytes (Barres et al. 1989; Sontheimer and Waxman 1992; Sontheimer et al. 1991). Some investigators proposed that these currents are mediated by a subclass of TTX-R Na$^+$ channels as opposed to “neuronal” Na$^+$ channels (Barres et al. 1989). Indeed, molecular cloning has identified Na-g, a sodium channel that is presumably TTX-R and is expressed in glial cells and some sensory neurons (Gautron et al. 1992). In situ hybridization studies have demonstrated that facial motor neurons (Iwahashi et al. 1994) and spinal sensory neurons (Waxman et al. 1994) demonstrate a switch to TTX-R channel expression upon axotomy. Ritchie and colleagues have proposed that glial cells may synthesize Na$^+$ channels to be donated to adjacent neurons (Gray and Ritchie 1985; Ritchie 1992). Along these lines, our findings would suggest that upon injury, when some neurons express...
TTX-R channels, associated astrocytes follow suit and also express TTX-R Na⁺ channels. A variety of cell types including skeletal and cardiac muscle cells express TTX-R sodium currents during their development but then switch to TTX-S currents upon maturity (for review, see Yoshida 1994). Upon denervation, skeletal muscles convert TTX-S currents back to TTX-R ones. Likewise, when chick cardiac muscle cells are placed in culture, they exhibit a switch back to the TTX-R channels that are expressed TTX-R Na⁺ channels during embryogenesis. Thus TTX-R channels typically are associated with dedifferentiated cells, and this switch is commonly referred to as the “reverting-back” phenomenon. We previously have demonstrated that our culture model for reactive gliosis causes scar-associated cells to proliferate and

**Kinetic Analysis**

![Image](https://example.com/image1)

**FIG. 3.** Kinetic analysis of TTX-S vs. TTX-R currents. Representative examples of TTX-R (A) and TTX-S (B) currents at the −10 mV command potential. Times of activation to peak and inactivation to steady state were fit to first-order exponential functions (⋯⋯) and mean activation time constants (τ_a) and inactivation time constants (τ_h) ± SE were plotted vs. command voltages. C: activation time constants overlap for both TTX-S (n = 23) and TTX-R (n = 10) currents. D: TTX-R Na⁺ currents display significantly slower inactivation kinetics at more depolarized potentials. At −10 mV the τ_h mean is 0.76 ms for TTX-S currents and 1.128 ms for TTX-R currents (**P < 0.0004). At 0 mV, the τ_h means are 0.66 and 0.93 for TTX-S and TTX-R, respectively (**P < 0.004). At 10 mV, the τ_h means are 0.67 and 0.90, respectively (**P = 0.03).
that this proliferation is associated directly with a recapitulation of electrophysiological current profiles that are seen during gliogenesis (MacFarlane and Sontheimer 1997). Following this reasoning, one might speculate that the change in TTX-sensitivity reflects the dedifferentiated status of scar-associated astrocytes. The exact function that sodium currents might have in astrocytes, and the significance of any changes in their TTX-sensitivity, remains elusive.

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Received 2 December 1997; accepted in final form 23 December 1997.

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