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Persistent Sodium Current in Layer 5 Neocortical Neurons Is Primarily Generated in the Proximal Axon

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In addition to the well described fast-inactivating component of the Na\(^+\) current [transient Na\(^+\) current \((I_{\text{NaT}})\)], neocortical neurons also exhibit a low-voltage-activated, slowly inactivating “persistent” Na\(^+\) current \((I_{\text{NaP}})\), which plays a role in determining neuronal excitability and synaptic integration. We investigated the Na\(^+\) channels responsible for \(I_{\text{NaP}}\) in layer 5 pyramidal cells using cell-attached and whole-cell recordings in neocortical slices. In simultaneous cell-attached and whole-cell somatic recordings, no persistent Na\(^+\) channel activity was detected at potentials at which whole-cell \(I_{\text{NaP}}\) operated. Detailed kinetic analysis of late Na\(^+\) channel activity in cell-attached patches at 36°C revealed that somatic Na\(^+\) channels do not demonstrate “modal gating” behavior and that the probability of single late openings is extremely low \((<1.4 \times 10^{-4}\) or \(<0.02%\) of maximal open probability of \(I_{\text{NaP}})\). Ensemble averages of these currents did not reveal a sustained component whose amplitude and voltage dependence could account for \(I_{\text{NaP}}\) as seen in whole-cell recordings. Local application of TTX to the axon blocked somatically recorded \(I_{\text{NaP}}\), whereas somatic and dendritic currents had little or no effect. Finally, simultaneous current-clamp recordings from soma and apical dendrite revealed that Na\(^+\) plateau potentials originate closer to the axon. Our data indicate that the primary source of \(I_{\text{NaP}}\) is in the spike initiation zone in the proximal axon. The focal axonal presence of regenerative subthreshold conductance with voltage and time dependence optimal to manipulate integration of synaptic input, spike threshold, and the pattern of repetitive firing provides the layer 5 pyramidal neuron with a mechanism for dynamic control of its gain.

Key words: sodium channel; persistent sodium current; axon; excitability; layer 5 pyramidal neuron; neocortex

Introduction

Somata and processes of central neurons contain an inhomogeneously distributed mixture of molecularly distinct voltage-gated Na\(^+\) channels (Migliore and Shepherd, 2002; Yu and Catterall, 2003). Fast-inactivating, transient current through these channels plays a central role in initiation and propagation of action potentials (Hodgkin and Huxley, 1952; Stuart and Sakmann, 1994). In addition, there is a more slowly inactivating, TTX-sensitive, “persistent” Na\(^+\) current \((I_{\text{NaP}})\) (Chandler and Meves, 1966; Gutnick and Grill, 1995; Grill, 1996), the functional importance of which relates to dynamic control of the “gain” (Chance et al., 2002) of the neuronal input–output relationship. \(I_{\text{NaP}}\) operates in the subthreshold voltage range, where other large, voltage-gated conductances are not active (Grill, 1996). It may thus influence both synaptic integration properties of a neuron and the character of its spike output. A precise understanding of the role of \(I_{\text{NaP}}\) requires knowledge of the distribution and the biophysical properties of the ion channels that generate it.

A variety of potential mechanisms are available that might lead different regions of a given neuron to display different amounts of \(I_{\text{NaP}}\). These include regional differences in Na\(^+\) channel density (Catterall, 1981; Migliore and Shepherd, 2002), in channel subunit composition (Westenbroek et al., 1989; Smith et al., 1998; Gong et al., 1999; Caldwell et al., 2000), regional differences in the relationship between the Na\(^+\) channel and intracellular and extracellular matrices (Srinivasan et al., 1998; Qu et al., 1999), and regional differences in neuromodulation (Cantrell and Catterall, 2001). Functional evidence suggests a difference in the propensity of different neuronal compartments to generate \(I_{\text{NaP}}\). Thus, in simultaneous somatic and dendritic recordings from neocortical layer 5 pyramidal neuron, Stuart and Sakmann (1995) found that EPSPs were amplified by \(I_{\text{NaP}}\) that was generated by Na\(^+\) channels located not in the dendrites, but rather, near the soma and axon. Similar findings have also been reported for pyramidal neurons of CA1 area of hippocampus (Andreasen and Lambert, 1999; Yue et al., 2005). On the other hand, evidence for dendritic \(I_{\text{NaP}}\) has also been reported (Lipowsky et al., 1996; Mittmann et al., 1997; Magistretti et al., 1999b).

Previous studies of the relationship between Na\(^+\) channels and \(I_{\text{NaP}}\) have all involved experiments in enzymatically isolated neurons (Alzheimer et al., 1993b; Magistretti et al., 1999b; Tadese and Bean, 2002) or in heterologous systems (Smith et al., 1998; Cummins et al., 2001; Lossin et al., 2002; Mantegazzza et al., 2005; Rush et al., 2005). In this study, we investigated \(I_{\text{NaP}}\) and Na\(^+\) channel activities in layer 5 pyramidal neurons in slices maintained at physiological temperature. We report that the somatic and dendritic Na\(^+\) channels play a minimal role in generation of \(I_{\text{NaP}}\), which is almost entirely generated by axonal Na\(^+\) channels.
Materials and Methods

Slice preparation and maintenance. Experiments were performed in coronal slices of somatosensory cortex of postnatal day 14 (P14) to P24 CD-1 mice and P14–P30 Wistar rats, using procedures that are standard in our laboratory (Fleidervish et al., 1998). Animals of either sex were deeply anesthetized with Nembutal (60 mg/kg i.p.), killed by decapitation, and their brains were rapidly removed and placed in cold (6°C), oxygenated (95% O2–5% CO2) artificial CSF (aCSF). Coronal slices (300 μm) from a region corresponding to the primary somatosensory cortex were cut on a vibratome (Series 1000; Pelco International, Redding, CA) and placed in a holding chamber containing aCSF at room temperature; they were transferred to a recording chamber after >1 h of incubation.

Patch-clamp recording. Whole-cell or cell-attached recordings from layer 5 neurons were either made blindly (Hamill et al., 1991; Blanton et al., 1989) or under infrared differential interference contrast (IR-DIC) microscopic control (Stuart et al., 1993). For blind recording, the slices were maintained in a small (300 μl) interface-type recording chamber (Haas et al., 1979); for visually controlled recording, slices were held submerged in a chamber on the fixed stage of an Axioskop FS microscope (Carl Zeiss, Oberkochen, Germany). Single Na+ channel openings were recorded in cell-attached configuration, and whole-cell current was recorded in whole-cell configuration using an Axopatch 200A or Axopatch 200B amplifier (Molecular Devices, Foster City, CA). Simultaneous current-clamp voltage recordings from the soma and an apical dendrite in whole-cell configuration were obtained using an Axoclamp-2B amplifier in bridge mode and Axopatch 200B amplifier in fast current-clamp mode. Simultaneous whole-cell and cell-attached recordings were obtained from the soma using an Axoclamp-2B amplifier in bridge mode and Axopatch 200B amplifier in voltage-clamp mode. Patch pipettes were manufactured from thick-walled borosilicate glass capillaries (outer diameter, 1.5 mm; Hilgenberg, Malsfeld, Germany) and had resistances of 1.5–3.5 MΩ for somatic recordings and 7–9 MΩ for dendritic recordings. For cell-attached recordings, pipettes were coated to within ~100 μm of the tip with Sylgard (Dow Corning, Midland, MI). All recordings were made at 36 ± 1°C.

Command voltage protocols were generated and single-channel data were acquired on-line with a Digidata 1320A analog-to-digital interface. Data were low-pass filtered at 2–5 kHz (−3 dB, four-pole Bessel filter) and digitized at 10–20 kHz. For Na+ channel recordings, capacitive and leak currents were reduced before data acquisition using the built-in circuits of the amplifier. Null traces for digital subtraction of remaining capacitative and leak current components were produced by stepping from a depolarized membrane potential, at which all Na+ channels in the patch were inactivated. For simultaneous whole-cell and cell-attached recordings, null traces were produced by holding the patch at depolarizing voltage while applying the same current command through the whole-cell pipette. Before digital subtraction, the traces were aligned at half-amplitude of an action potential upstroke. For whole-cell voltage-clamp recording, care was taken to maintain membrane access resistance as low as possible (usually 3–4 MΩ and always <10 MΩ); series resistance was 80% compensated using the built-in circuitry of the amplifier. Data were low-pass filtered at 2 kHz (−3 dB, four-pole Bessel filter) and sampled at 5–10 kHz digitalization frequency. For whole-cell current-clamp recordings, data were low-pass filtered at 10 kHz (−3 dB, single-pole Bessel filter), stored on videotape, and digitized off-line at up to 20kHz.

Apparent cell capacitance was estimated from recordings of capacitive current elicited by 40 ms, 10 mV step hyperpolarizations from a holding potential of ~−70 mV. Pipette capacitive currents were nulled before break-in. In some neurons, apparent cell capacitance was estimated in current-clamp recordings by applying small hyperpolarizing pulses and determining the time constant and the amplitude of the resulting voltage deflection.

The aCSF contained the following (in mM): 124 NaCl, 3 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 glucose, pH 7.3 at 37°C when bubbled with a 95% O2–5% CO2 mixture. The pipette solution for Na+ channel experiments contained the following (in mM): 130 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 10 glucose, 4 tetraethylammonium chloride, 1 4-AP, and 10 HEPES (sodium salt), pH 7.3. The pipette solution for whole-cell voltage-clamp experiments contained (in mM): 135 CsCl, 2 MgCl2, and 10 HEPES (cesium salt), pH 7.25. The pipette solution for whole-cell current-clamp experiments contained (in mM): 130 Cs gluconate, 6 CsCl, 2 MgCl2, and 10 HEPES (cesium salt), pH 7.25. TTX (0.1–1 μM) was either added to the bath solution or puff-applied (5–10 ms, 20 psi pressure pulses) through the patch pipette. All chemicals were obtained from Sigma (St. Louis, MO).

Data analysis. Data averaging, digital subtraction of null traces, and current peak detection were made using pClamp 9.0 (Molecular Devices) and Serf 1.78 software kindly adjusted to fit our needs by Dr. J. de Weille (Institut de Pharmacologie Cellulaire et Moleculaire, Valbonne, France). Data were fitted using Origin 6.0 (OriginLab, Northampton, MA). If not otherwise noted, values are given as mean ± SD. For statistical analysis, Student’s t test was performed.

Results

Whole-cell persistent Na+ current in layer 5 pyramidal neurons

Figure 1 illustrates the properties of whole-cell I NaP elicited by slow (35 mV/s) depolarizing voltage ramps in visually identified layer 5 pyramidal neurons (Fig. 1A) with K+ and Ca2+ conductances blocked pharmacologically. To ascertain that the current generated by the ramp was indeed persistent, rather than reflecting very slow inactivation of transient Na+ current (I NaT) (Cummins et al., 2001), the rising rate of the ramps was adjusted to entirely inactivate even the “slowest” known transient Na+ chan-
nels (Cummins et al., 2001) while causing minimal slow inactivation of $I_{NaP}$ (Fleidervish and Gutnick, 1996). As shown previously (Alzheimer et al., 1993a; Fleidervish and Gutnick, 1996), current was inward from around $-65$ mV, reflecting $I_{NaP}$ activation. At potentials more positive than $-35$ mV, $I_{NaP}$ was superimposed on a large outward cationic current, cationic current ($I_{NaT}$) (Alzheimer, 1994; Fleidervish and Gutnick, 1996). Digital subtraction of traces in the absence and presence of TTX yielded an instantaneous $I-V$ curve for $I_{NaP}$, which peaked at voltages between $-30$ and $-20$ mV (Fig. 1B).

Additional evidence against contamination by $I_{NaP}$ was obtained in five neurons by comparing currents elicited by depolarizing and hyperpolarizing voltage ramps. As illustrated in Figure 1, C and D, the amplitudes and voltage dependences of TTX-subtracted currents evoked by ramps of opposite polarity were not different, although the hyperpolarizing ramp was preceded by a 200 ms step to $-35$ mV to completely inactivate transient $I_{NaT}$. Thus, for eight neurons, the $I_{NaP}$ magnitude at $-35$ mV for hyperpolarizing ramps was $311 \pm 48$ pA, half activation voltage was $-43 \pm 0.9$ mV, and maximal steepness of the activation curve was $4.6 \pm 0.2$ mV$^{-1}$, whereas for depolarizing ramps, these values were $313 \pm 47$ pA, $-41 \pm 1.2$ mV, and 4.6 $\pm 0.1$ mV$^{-1}$, respectively ($p>0.05$, paired Student’s t test).

Although the maximum amplitude of $I_{NaP}$ in slices varied from cell to cell over a fairly wide range (8–1600 pA; $n=177$), the median was 385 pA, which is significantly higher than values reported for acutely dissociated neurons (Alzheimer et al., 1993a; Hammarstrom and Gage, 1999; Taddese and Bean, 2002) or for non-neural mammalian cells expressing human neuronal Na$^+$ channels (Lossin et al., 2002). These rarely exceed 100 pA. At first, we thought to attribute the difference between experimental preparations to the fact that much of the dendritic membrane, which has been shown to possess Na$^+$ channels (Huguenard et al., 1989; Stuart and Sakmann, 1994), remains intact in the brain slice. This conclusion seemed to be supported by the finding that the apparent $I_{NaP}$ density calculated for the 67 neurons in which capacitance was measured (see Materials and Methods) was 0.3–14.3 pA/PF (median, 1.9 pA/PF), which is comparable with or smaller than values in acutely dissociated cells (Alzheimer et al., 1993a; Hammarstrom and Gage, 1998). The calculation, however, is based on the assumption that channels responsible for $I_{NaP}$ are homogenously distributed throughout the neuron; in the next series of experiments, we found this assumption to be wrong.

**Somatic Na$^+$ channels do not open at subthreshold voltages**

Because the functionally relevant consequences of $I_{NaP}$ are primarily related to its activation at membrane potentials just below the spike threshold (Crill, 1996), we looked for somatic Na$^+$ channel late openings in this voltage range. This could not be accomplished with channel recordings alone because the absolute value of the membrane potential is unknown and varies from cell to cell (Fleidervish et al., 1996, 1998). Therefore, while one pipette in cell-attached configuration recorded from Na$^+$ channels, an additional whole-cell pipette in the same neuron was used to record membrane potential and inject current (Fig. 2).

Na$^+$ channels in cell-attached patches were identified on the basis of their characteristic voltage dependence and conductance, as described in detail by Fleidervish et al. (1996). Briefly, Na$^+$ channel openings first appeared at test pulse amplitudes between $V_r$ + 10 and $V_r$ + 25 mV, and a progressive increase in test potential amplitude caused openings to cluster at the beginning of the pulse and late openings to become rarer. Slope conductance, as determined from a linear fit to the $I-V$ curve of the single open channel in the range of $V_r$ + 20 to $V_r$ + 70 mV, was $15 \pm 2$ pS; the extrapolated reversal potential was $V_r + 156 \pm 12$ mV ($n=5$). Assuming that $V_r$ is near $-77$ mV (Fleidervish et al., 1996, 1998), we estimate from the Nernst equation an internal Na$^+$ activity of $\sim 7$ mm.

As illustrated in Figure 2A, depolarizing voltage pulses applied to the patch pipette and action potentials elicited by suprathreshold current steps were associated with transient Na$^+$ channel openings. However, depolarization of the membrane to voltages just below threshold elicited no detectible Na$^+$ channel openings. In the same recording, no channel openings were detected when the cell was depolarized by 250-ms-long current steps to the subthreshold potentials at which $I_{NaP}$ begins to operate. Shown are 20 whole-cell current (bottom) and voltage (middle) sweeps along with the corresponding patch current traces (top).

**Probability of late Na$^+$ channel openings in somatic patches**

To determine whether probability of late openings ($P_o$) of somatic Na$^+$ channels could account for whole-cell $I_{NaP}$, we re-
corded Na⁺ channel currents from somata of 19 neurons. Individual openings were clearly resolvable with a filter bandwidth open to 2–3.5 kHz (see Fig. 4). All patches contained multiple channels, as indicated by the appearance of overlapping opening events during the peak of the currents elicited by depolarizing steps. Although it would be possible to directly assess \( P_o \) for late openings of an individual channel if the patch contained only one Na⁺ channel, in our multichannel patches, the late Na⁺ channel activity had to be normalized to the number of channels, \( N \), a value which had to be explicitly determined.

In most previous studies (Alzheimer et al., 1993b; Magistretti et al., 1999a), the number of Na⁺ channels has been estimated by determining the maximal current amplitude in a long series of repetitive depolarizations divided by the unitary channel current \( i \). Considering that the peak \( P_o \) value is low even at depolarizing voltages, as evident from significant trial-to-trial fluctuations of Na⁺ currents, this method can only provide a lower limit to-trial fluctuations of Na⁺ voltages, as evident from significant trial-to-trial fluctuations of Na⁺ currents elicited by depolarizing steps. All Na⁺ channel openings that overlap during the peak of the current vary from a minimum of three to maximum of nine. Left, Fluctuations in Na⁺ current peak amplitudes during repetitive depolarizations. Seven consecutive current peaks are plotted as a function of the mean current. Right, Fluctuations in Na⁺ current peak amplitudes during a series of 750 depolarizing steps as in the left panel. The right axis is calibrated in single-channel unitary amplitudes, and dashed lines mark multiples of these. Note that in a series of repetitive depolarizations, the number of Na⁺ channel openings that overlap during the peak of the current varies from a minimum of three to maximum of nine. Middle column, Differences between each individual trace and the mean current (Fig. 3C, thin line). The straight line represents a linear fit of the portion of the current ratio, \( \sigma^2(t)/I(t) \), to the mean current, \( \mu(t) \). Right column, The squares of differences between the variance and the mean current, \( \sigma^2(t)/I(t) \), and the peak to mean current ratio, \( \sigma^2(t)/I(t) \), plotted as a function of the mean current, \( \mu(t) \). The relationship was linear, as predicted by Equation 5. The unitary single-channel current amplitude, \( i \), was estimated from the y-intercept of the extrapolated linear regression line [e.g., at \( I(t) = 0 \), was 1.36 pA, which is near the \( i \) value directly measured from well resolved single channel events (1.37 pA). The \( x \)-intercept of the extrapolated linear regression line [the imaginary state that would occur when all channels are open; \( P_o = 1 \), \( \sigma^2(t)/I(t) = 0 \) and \( I(t) = iN \)] was 12.2 pA. Division of this value by \( i \) gave \( N \) = 9 channels. Good agreement between the values of \( N \) determined by peak current fluctuation analysis and by current decay fluctuation analysis was obtained in 24 additional patches. In most cases (\( n = 18 \)), the number of Na⁺ channels, as determined by fluctuation analysis, was equal to the maximal peak current amplitude divided by the unitary current amplitude. However, in the other six patches, all of which contained >16 channels, the “traditional” method yielded a lower \( N \) value; this suggests that with a large number of channels and a finite series of depolarizations, no single step causes all channels to open simultaneously.

The mean current \( I(t) \) was calculated by averaging 750 consecutive Na⁺ channel sweeps. \( \sigma^2(t) \) was calculated as the average of the squares of the differences between each individual current trace and the mean current (Fig. 3B). In Figure 3C, the variance to current ratio, \( \sigma^2(t)/I(t) \), was plotted as a function of the current, \( I(t) \). The relationship was linear, as predicted by Equation 5. The unitary single-channel current amplitude, \( i \), estimated from the y-intercept of the extrapolated linear regression line [e.g., at \( I(t) = 0 \)], was 1.36 pA, which is near the \( i \) value directly measured from well resolved single channel events (1.37 pA). The \( x \)-intercept of the extrapolated linear regression line [the imaginary state that would occur when all channels are open; \( P_o = 1 \), \( \sigma^2(t)/I(t) = 0 \) and \( I(t) = iN \)] was 12.2 pA. Division of this value by \( i \) gave \( N \) = 9 channels. Good agreement between the values of \( N \) determined by peak current fluctuation analysis and by current decay fluctuation analysis was obtained in 24 additional patches. In most cases (\( n = 18 \)), the number of Na⁺ channels, as determined by fluctuation analysis, was equal to the maximal peak current amplitude divided by the unitary current amplitude. However, in the other six patches, all of which contained >16 channels, the “traditional” method yielded a lower \( N \) value; this suggests that with a large number of channels and a finite series of depolarizations, no single step causes all channels to open simultaneously.

Figure 4 illustrates a series of experiments in which the char-
characteristics of late Na\(^+\) channel openings were studied using 500-ms-long depolarizing voltage steps from \(V_r = -40\) mV to a voltage just above the threshold for Na\(^+\) channel activation (\(V_r + 25\) mV) (Fig. 4A, left) and to \(V_r + 60\) mV (Fig. 4A, right). In previous studies in acutely dissociated neurons (Alzheimer et al., 1993b; Magistretti et al., 1999a) or in 100-μm-thick neocortical slices (Alzheimer et al., 1993b) at room temperature, the most prominent form of late Na\(^+\) channel activity was described as a temporary failure of Na\(^+\) channels to inactivate. The resultant sustained bursts of openings, commonly interpreted as transient entrance of the channel into a persistent or “noninactivating” gating mode, has been reported to happen in ~1% of depolarizations (Patlak and Ortiz, 1985; Alzheimer et al., 1993b; Magistretti et al., 1999a) in patches that contain ~4–12 channels. Surpris-ingly, this phenomenon was never observed in our recordings from >100,000 depolarizations and >500 patches at physiological temperature. Sporadic, brief, late Na\(^+\) channel opening, an additional form of late channel activity (Alzheimer et al., 1993b), was observed, albeit rarely, at various times during a sustained depolarization (Fig. 4A). Calculation of the per channel open state probability corresponding to these events gave values of 0.8 ± 0.3 × 10\(^{-4}\) for \(V_r + 25\) mV (\(n = 10\) patches) and 1.3 ± 0.5 × 10\(^{-4}\) for \(V_r + 60\) mV (\(n = 14\) patches). This is too low to account for the magnitude of \(I_{\text{NaP}}\) in whole-cell recordings. Similar results were obtained in experiments in which late Na\(^+\) channel openings were studied using 500-ms-long depolarizing voltage ramps from \(V_r\) to \(V_r + 70\) mV. For example, in the representative patch in Figure 4B, which contained 14 Na\(^+\) channels and was held at \(V_r\) (approximately −77 mV), the incidence of brief late opening during the slow voltage ramps was rare at all voltages, although transient channels openings were seen at the beginning of the depolarizing steps to \(V_r + 70\), which were applied before each ramp to ensure the stability of the recording and the neuronal resting potential. In the voltage range tested, \(P_o\) of the brief late openings followed a Boltzmann-type pattern with a maximum of 1.0–1.4 × 10\(^{-4}\) (≤0.02% of maximal \(P_o\) of the transient Na\(^+\) current) at voltages positive to \(V_r + 45\) mV (Fig. 4C).

**Axonal origin of \(I_{\text{NaP}}\)**

The above experiments indicate that the \(I_{\text{NaP}}\) evident in whole-cell recordings does not originate at the soma. Na\(^+\) channels that might give rise to \(I_{\text{NaP}}\) are present with more or less uniform density (Stuart and Sakmann, 1994; Colbert and Johnston, 1996; Migliore and Shepherd, 2002) in the membrane of other neuronal compartments, including apical and basal dendrites and the proximal axon. We tested their possible involvement in whole-cell \(I_{\text{NaP}}\) by locally applying TTX while recording ramp currents with a somatic whole-cell pipette. To minimize the spread of toxin, we used a TTX concentration of 10\(^{-7}\) M, which is 10-fold lower than usual. TTX was delivered to various sites by gentle pressure ejection (5–10 ms pressure pulses of 20 psi) through a patch pipette with tip diameter of 1 μm. In a control experiment to estimate the area of toxin spread, glutamate at a concentration of 100 μM (to achieve a concentration ratio similar to that in TTX experiment), when applied under similar conditions, only produced detectable NMDA receptor-mediated current if applied within 20–25 μm of the dendritic membrane of the neuron under study. In the representative recording shown in Figure 5A, local application of TTX to the apical dendrite (top trace and image) and to the soma (middle) had little or no effect on \(I_{\text{NaP}}\). However, appli-
Axonal origin of $I_{NaP}$ underlying Na plateau potentials

The simplest interpretation of these observations is that $I_{NaP}$ is generated by Na$^+$ channels located in the proximal axon, with little, if any, participation of somatic and dendritic channels. We therefore performed a series of experiments in which a current clamp manifestation of $I_{NaP}$, the Na plateau potential (Fleidervish and Gutnick, 1996), was used to test whether its behavior is compatible with a focal, axonal origin for $I_{NaP}$. Figure 6A shows a current-clamp recording from a representative layer 5 neuron in which K$^+$ currents were blocked by using Cs$^+$ as the main intracellular cation and Ca$^+$ currents were blocked by adding 200 μM Cd$^+$ to the bath. Under these conditions, a brief suprathreshold depolarizing current pulse delivered to the soma (Fig. 6A) or to the dendrite (data not shown) elicited a prolonged, all-or-none Na plateau that persisted after the initial fast spike. As described previously (Fleidervish and Gutnick, 1996), during the plateau, which lasted 0.5–5 s, the gradual decline in membrane potential reflected the changing balance between slowly inactivating $I_{NaP}$ and $I_{Na}$. When the plateau reached a value of about −35 mV, regenerative repolarization ensued. The decline of the plateau was usually associated with a crescendo of voltage oscillations (7–25 Hz), which gradually grew to trigger slowly rising action potentials of increasing amplitude (Fig. 6A, B). In simultaneous, double recordings from soma and proximal apical dendrite (−300 μm), the depolarizing envelope of the Na plateau initiated near the soma and spread into the dendrite almost without attenuation (Fig. 6B). This is not surprising considering the electrotonic compactness of a Cs$^+$-dialyzed pyramidal cell (apparent input resistance, 1.5 ± 0.3 GΩ; $n = 12$). Indeed, most of the dendritic tree of a pyramidal neuron perfused with Cs$^+$ is probably isopotential for near DC events (Spruston et al., 1994). In contrast, we expect the electrotonic spread of the high-frequency events, such as oscillations and action potentials, to be constrained by the Cs$^+$ dialysis, which causes a significant increase in membrane time constant (34 ± 2 ms; $n = 12$). With events generated by a focal current sink, the extent of dendritic filtering and the phase lag are expected to increase with increasing distance from the active region. In eight simultaneous recordings from soma and dendrites (Fig. 6C), the dendritic oscillations were delayed and had lower amplitude than the somatic ones. This accords well with the hypothesis that the $I_{NaP}$ that provides the inward current for the oscillations is concentrated in the axons, and the oscillation spread passively into the dendrites.

Figure 6, D and E, illustrates a different approach to determine the location of the $I_{NaP}$ sink that underlies the Na plateau. The Na plateau can be terminated by injecting a hyperpolarizing current pulse whose magnitude and duration are large enough to bring the membrane potential below a critical value (Fig. 6D). If simultaneous recording from soma and dendrite, comparison of the minimal duration of the hyperpolarizing pulse of fixed amplitude that causes a regenerative repolarization (Fig. 6E) revealed that this duration was always shorter at the soma than in the dendrite. Moreover, the apparent voltage threshold of regenerative repolarization was significantly more negative for dendritic injections than for somatic ones (Fig. 6E) ($−69 ± 8$ mV vs $−60 ± 8$ mV, $n = 43$; $p < 0.01$). This is a reflection of larger electrotonic distance between the current injection site and the place where $I_{NaP}$-generating channels are located.

Discussion

The main conclusion from these data are that the Na$^+$ channels responsible for $I_{NaP}$ generation are primarily located in the axon. Thus, direct TTX application to the proximal axon consistently

Figure 5. Local application of TTX to the proximal axon blocks whole-cell $I_{NaP}$, whereas somatic and dendritic applications have little or no effect. A, Whole-cell somatic voltage-clamp recording of $I_{NaP}$ and the effect of application of 0.1 μM TTX near the apical dendrite (75 μm), near the soma, and near the proximal axon (−10 μm). Superimposed are current traces before (gray) and after TTX application (black) at a time indicated by arrow. Currents were elicited by applying the 2-s-long voltage ramps from −70 to 0 mV. Note that blockade of $I_{NaP}$ by TTX applied near the axon was partially reversible. B, The graph showing the relative effect of TTX on $I_{NaP}$ amplitude as a function of application site. The dots are pooled data from nine neurons including that shown in A. Each dot represents a ratio of $I_{NaP}$ amplitude at −35 mV before and after TTX application.

cation of TTX to the proximal axon of the same neuron rapidly and reversibly blocked $I_{NaP}$ by −65%. As evident in the graph in Figure 5B, which shows pooled data from the nine experiments in which this protocol was implemented, the same fundamental result was always found: TTX application near the initial portion of the axon, 10–40 μm from the soma, caused $I_{NaP}$ to decrease by as much as 97%, whereas application to the proximal portion of apical dendrite (<300 μm) (Fig. 5B) or to the basal dendrites (<70 μm; data not shown) had no effect on $I_{NaP}$. In three cells, application directly to the soma caused $I_{NaP}$ to decrease by 10–15%. In all cases, vertical or lateral movement of the application pipette by 20–25 μm completely abolished the TTX effect.
Na plateau potentials are underlaid by axonal \( I_{\text{NaP}} \). A, Current-clamp manifestation of \( I_{\text{NaP}} \) in layer 5 neuron: a prolonged, regenerative Na plateau potential triggered by a brief, just suprathreshold somatic current pulse (10 ms, 200 pA). K\(^+\) currents were blocked by using Cs\(^+\) as the main intracellular monovalent cation, and Ca\(^{2+}\) currents were blocked by adding 200 \( \mu\)M Cd\(^{2+}\) to the bath. Membrane potential was kept at about −65 mV by injection of holding current of −50 pA. B, Plateau potentials recorded simultaneously from the soma (thin traces) and the dendrite (thick traces; distance, 80 \( \mu\)m). Note that the slow decline of the plateau is associated with a crescendo of voltage oscillations that grew to trigger action potentials of increasing upstroke velocity and amplitude. C, Voltage oscillations are generated close to soma and spread into dendrites. Two cycles of voltage oscillation recorded simultaneously from the soma (thin traces) and the dendrite (thick traces; distance, 80 \( \mu\)m) to show that the dendritic oscillation is phase-shifted (−16\(^\circ\)) and smaller in amplitude compared with the somatic one. In a different pyramidal cell (dendritic recording distance, 220 \( \mu\)m), this phase shift is larger (−30\(^\circ\)), consistent with hypothesis that the oscillations initiate around soma and spread passively into the dendrites. D, Hyperpolarizing current pulses (120 pA) of incrementing duration delivered through the somatic pipette during Na plateau, to test the threshold of all- or-none repolarization. Pulses of >40 ms were effective, and the voltage threshold, as determined from six trials, was approximately −58 mV. E, Simultaneous recordings from the soma (thin traces) and the dendrite (thick traces; distance, 200 \( \mu\)m) to show that the minimal duration of hyperpolarizing current pulses required to elicit regenerative repolarization is longer in dendrite than in the soma. The apparent voltage threshold of all-or-none repolarization is significantly lower in the dendrite (−69 ± 8 mV; \( n = 24 \)) compared with the soma (−60 ± 8 mV; \( n = 43 \); \( p < 0.01 \)).

Our evidence is based, in part, on whole-cell voltage-clamp recordings of \( I_{\text{NaP}} \), elicited by very slow voltage ramps in neurons in which Cs\(^+\) has replaced K\(^+\). It has been suggested that voltage control of neuronal processes may be inadequate with this approach (Cantrell and Catterall, 2001). However, input resistance of Cs\(^+\)-dialyzed neurons was always >1 GΩ. A theoretical analysis by Spruston et al. (1994) of attenuation into dendrites of near-DC (but not transient) voltage signals under these conditions predicts satisfactorily (~98%) control of voltage at distance of up to 1000 \( \mu\)m from a somatic pipette.

Although our findings strongly point to the axon as the place where most \( I_{\text{NaP}} \) originates, it remains unclear whether this reflects higher channel density, higher probability of late openings of the individual channel, or both. If axonal and somatic channels have the same properties, then the experimentally observed \( I_{\text{NaP}} \) magnitude requires the axonal density to be 500–1000-fold higher than the somatic density. Such very high densities have been hypothesized in a theoretical study (Mainen et al., 1995), but they have not been confirmed experimentally (Catterall, 2000) Na\(_{\text{V1.6}}\) channels enter the noninactivating gating mode more frequently and produce more persistent current than do channel isoforms localized in soma and dendrites (Raman and Bean, 1997; Smith et al., 1998; Goldin, 2001; Rush et al., 2005).

Our finding that the origin of \( I_{\text{NaP}} \) is axonal appears to conflict with the data of Mittmann et al. (1997). These authors used a Na\(^+\)-sensitive dye to image ion accumulation during Na\(^+\) plateau potentials in layer 5 pyramidal neurons and found Na\(^+\) influx in the apical dendrites, which they attributed to \( I_{\text{NaP}} \). Although our data do not rule out a small \( I_{\text{NaP}} \) in the dendrites, the results of our focal TTX applications indicate that its contribution to the \( I_{\text{NaP}} \) we record during slow voltage ramps is relatively insignificant. It is important to note that in addition to Na\(^+\) channels, there is another route for Na\(^+\) entry during the Na\(^+\) plateau, which is not active at subthreshold voltages: the nonspecific \( I_{\text{NaP}} \). That the membrane potential during the Na\(^+\) plateau is around −20 mV reflects a balance between inward \( I_{\text{NaP}} \) and outward \( I_{\text{cat}} \) (Fleidervish and Gutnick, 1996). Although the molecular identity of the channels that produce \( I_{\text{cat}} \), is still unknown, ion substitution experiments have shown it to be permeable to Na\(^+\) (Alzheimer, 1994). Interestingly, a nonspecific cationic conductance similar to that which underlies \( I_{\text{NaP}} \) was reported recently in somata, dendrites, and axons of hippocampal pyramidal cells and identified to be dependent on background activation of Na\(^+\)-permeable channels of the TRP family (Strubing et al., 2001). Activation of dendritic \( I_{\text{NaP}} \) by the TTX-sensitive Na\(^+\) plateau could explain the increase in dendritic \([\text{Na}^+]_i\), without dendritic \( I_{\text{NaP}} \).

The probability of late openings of somatic Na\(^+\) channels we find in slices is significantly lower than that of heterologously...
expressed Na⁺ channels (Mantegazza et al., 2005; Rush et al., 2005) and of native Na⁺ channels in the proximal dendrites of isolated stellate cells from entorhinal cortex (Magistretti et al., 1999a,b). The reason for the differences between the different experimental preparations has yet to be elucidated. It might reflect a direct or indirect influence of different experimental manipulations on channel characteristics. For example, we cannot completely rule out the possibility that somatic Na⁺ channels do undergo late openings that are so brief as to go undetected in our recording conditions, yet so prevalent as to underlie \( I_{\text{NaP}} \). However, the finding that TTX application to the soma was minimally effective if at all mitigates against this.

It has been shown that under most conditions layer 5 pyramidal neurons are strongly biased to initiate action potentials in the axon rather than in the dendrites, where the EPSP amplitude is largest (Stuart et al., 1997; Waters et al., 2005). Thus, despite the fact that dendrites are excitable, dendritic EPSPs usually generate axonal spikes, which then backpropagate. Thus, the threshold for spike initiation is so much more negative in the axon than in the dendrite that it is still reached first despite TTX attenuation caused by conduction from a distant site (Stuart et al., 1997). Our findings point to specialized properties of axonal Na⁺ channels, which confer on them a far greater propensity generate \( I_{\text{NaP}} \). This is not only helps to establish the axon as the zone of spike initiation, but also directly affects input attenuation by “boosting” EPSPs. The focal axonal presence of large, regenerative subthreshold conductance with voltage and time dependence optimal to manipulate integration of synaptic input, spike threshold, and the pattern of repetitive firing, could provide the pyramidal neurons with a mechanism for dynamic control of their gain.

Enhancement or suppression of \( I_{\text{NaP}} \) by regulatory systems (Cantrell and Catterall, 2001; Carr et al., 2003; Mantegazza et al., 2005) or electrical uncoupling of the axonal \( I_{\text{NaP}} \) source from the input areas of the cell by GABAergic inhibition that targets axonal hillock and initial segment (Jones and Powell, 1969) might temporarily shift the spike initiation site and thereby modify integration and output properties of a given pyramidal neuron. Moreover, ectopic appearance of \( I_{\text{NaP}} \) in dendrites, attributable, for example, to a Na⁺ channelopathy that affects inactivation (Losin et al., 2002), might enhance positive feedback within the recurrent cortical circuitry, and thus be epileptogenic.

References


Magistretti J, Ragsdale DS, Alonso A (1999a) High conductance sustained


