Evidence for Persistent Na\(^+\) Current in Apical Dendrites of Rat Neocortical Neurons From Imaging of Na\(^+\)-Sensitive Dye

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Mittmann, Thomas, Shannon M. Linton, Peter Schwindt, and Wayne Crill. Evidence for persistent Na\(^+\) current in apical dendrites of rat neocortical neurons from imaging of Na\(^+\)-sensitive dye. J. Neurophysiol. 78: 1188–1192, 1997. Evidence for a persistent Na\(^+\) current (I_{NaP}) in the apical dendrite of neocortical neurons was sought with the use of fluorescence imaging to measure changes in intradendritic Na\(^+\) concentration. Neurons in neocortical brain slices were filled iontophoretically through an intracellular recording microelectrode with the Na\(^+\)-sensitive dye benzofuran isophthalate (SBFI), and fluorescence images were recorded with a cooled charge-coupled device camera system using 380-nm illumination. In the presence of Ca\(^2+\) and K\(^+\) channel blockers, a short depolarizing current pulse evoked a single action potential followed by a plateau depolarization (PD) lasting >1 s. This tetrodotoxin (TTX)-sensitive PD is known to be maintained by I_{NaP}. A single action potential caused no detectable SBFI fluorescence change, whereas the PD was associated with an SBFI fluorescence change in the soma and apical dendrite indicating increased intracellular Na\(^+\) concentration. Determination of the full spatial extent of the dendritic fluorescence change was prevented by our inability to detect the dim fluorescence signal in the distal regions of the apical dendrite. In each experiment the fluorescence change extended into the apical dendrite as far as dye could be visualized (50–300 \(\mu m\)). A slow, depolarizing voltage-clamp ramp that activated I_{NaP} caused similar fluorescence changes that were eliminated by TTX, indicating that the SBFI fluorescence changes are caused by Na\(^+\) influx due to I_{NaP} activation. We conclude that I_{NaP} can be generated by the apical dendritic membrane to at least 300 \(\mu m\) from the soma.

INTRODUCTION

A noninactivating or “persistent” Na\(^+\) current (I_{NaP}) has been observed during intrasomatic recordings of mammalian neurons from the hippocampus (French et al. 1990), entorhinal cortex (Alonso and Llinás 1989), thalamus (Jahnsen and Llinás 1984), cerebellum (Llinás and Sugimori 1980), and neocortex (Stafstrom et al. 1982, 1985; for review see Crill 1996). In neocortical neurons the generation of I_{NaP} is best explained by a modal change in the inactivation properties of the Na\(^+\) channel (Alzheimer et al. 1993). Recordings from dendrites of neocortical neurons (e.g., Amitai et al. 1993; Huguenard et al. 1989; Kim and Connors 1993; Regge et al. 1993; Stuart and Sakmann 1994) revealed transient sodium channel activity several hundred micrometers away from the soma. Because the same Na\(^+\) channels may generate both the transient and persistent Na\(^+\) current, these results led us to expect that the dendritic membrane also can generate I_{NaP}. This hypothesis was supported by studies showing tetrodotoxin (TTX)- and voltage-sensitive amplification of the axial dendritic current transmitted to the soma (Schwindt and Crill 1995, but see Stuart and Sakmann 1995).

The feasibility of measuring activity-induced changes of intracellular Na\(^+\) concentration ([Na\(^+\)]\(_i\)) in single neurons in a slice preparation was demonstrated by the pioneering work of Lasser-Ross and Ross (1992) in Purkinje neurons and Jaffe et al. (1992) in hippocampal pyramidal neurons with the Na\(^+\)-sensitive fluorescent dye benzofuran isophthalate (SBFI). In the present study we used SBFI to measure a stimulus-evoked rise in [Na\(^+\)]\(_i\) in the soma and the apical dendrite of neocortical layer V neurons in brain slices. The neurons were filled with SBFI; intracellular stimuli were applied that specifically activated I_{NaP}, and the spatial extent of the SBFI fluorescence changes was recorded with the use of a cooled charge-coupled device (CCD) camera. The spatial distribution of the rise in [Na\(^+\)]\(_i\) was expected to indicate the spatial distribution of membrane capable of generating I_{NaP}. Two types of intracellular stimuli were used. One was a long-lasting plateau depolarization (PD) that can be evoked by a small injected current pulse after Ca\(^++\) current (and Ca\(^++\)-dependent K\(^+\) currents) are blocked and voltage-gated K\(^+\) currents are reduced. This TTX-sensitive PD is known to be maintained solely by I_{NaP} (Stafstrom et al. 1985). The large somatic depolarization caused by the PD was expected to depolarize the apical dendrite enough to activate dendritic I_{NaP} if the dendrite was capable of generating such a current. The second type of stimulus was a slow ramp voltage clamp of the soma to a final depolarization that activated I_{NaP} but was subthreshold for action potential initiation. With this stimulus we could verify by direct measurement that I_{NaP} was activated, that its activation resulted in a rise in [Na\(^+\)]\(_i\), and that both responses were abolished by TTX. Using either type of stimulus, we observed a rise of [Na\(^+\)]\(_i\) in the apical dendrite as well as the soma.

METHODS

Sprague-Dawley rats (19–35 days postnatal) were anesthetized with ketamine (150 mg/kg) and xylazine (10 mg/kg) and killed by carotid section. Brain slices 200–250 \(\mu m\) thick were cut with the use of a vibratome (Oxford) and transferred to a recording chamber. A No. 1 glass coverslip formed the bottom of the recording chamber (volume ~0.25 ml). One face of the submerged slice rested against this coverslip, and the slice was held down by a U-shaped platinum wire. The slices were perfused at ~1.5 ml/min with artificial cerebrospinal fluid (ACSF) maintained at 31°C. ACSF consisted of (in mM) 130 NaCl, 3 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 1.25 NaHPO\(_4\), 26 NaHCO\(_3\), and 10 glucose saturated with 95% O\(_2\)-5% CO\(_2\), pH 7.4. In current-clamp experiments optical and...
electrical recordings were made after change to an ACSF in which Mn$^{2+}$ (2 mM) was substituted for Ca$^{2+}$. NaH$_2$PO$_4$ was omitted to avoid precipitation, and 20 mM tetroethylammonium chloride (TEA) was substituted for 20 mM NaCl. In voltage-clamp experiments the cells were recorded in the ACSF. In some of these experiments TTX (1 μM) was added to the ACSF.

An Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) was used to inject current in active bridge mode or to control somatic membrane potential in single-electrode voltage-clamp mode with the use of a switching rate of 4–6 kHz with a 30% duty cycle. Membrane potential, membrane current and iontophoretic current were monitored, filtered at 0.1–10 kHz, amplified, and recorded on a multichannel video cassette recorder with pulse code modulation (Neuro-Data, New York, NY). Resting potential was taken as the difference between the intracellular and extracellular potentials recorded on a chart recorder. Recorded data were digitized to analyze evoked responses with the use of a computer program.

Cells were impaled with sharp microelectrodes made from standard borosilicate tubing (1.0 μm OD). The tip of the recording microelectrode was filled with the Na$^+$-sensitive, membrane-impermeant dye SBFI (Molecular Probes, Eugene, OR) at a concentration of 10–12 mM dissolved in 0.2 M KCl and 30 mM 3-(N-morpholino)propanesulfonic acid (pH 7.2) for a distance of ~1 mm. The rest of the electrode was filled with 2.7 M KCl. DC resistance was 25–35 MΩ. Neurons located near the bottom surface of the slice were impaled and loaded iontophoretically with SBFI by passing a DC current of ~1 nA through the intracellular electrode for 30 min to allow time for diffusion of the dye from the soma to the apical dendrite.

The recording chamber was fixed on top of an inverted microscope (Nikon Diaphot 200) equipped with x10 and x20 objectives (Nikon Fluor). Epi-illumination was obtained from a 75-W Xenon lamp supplied by a stabilized power supply (Ludl Electronics, Hawthorne, NY) with the use of an excitation filter of 380 ± 8 nm (Chroma, Brattleboro, VT). Emitted fluorescence at 510 ± 20 nm was recorded with a cooled CCD camera (Princeton Instruments, Trenton, NJ). The camera chip (EEV 512 × 1024 pixels) was controlled by Winview software (Princeton Instruments) working in frame transfer mode. Full-frame acquisitions were controlled by Metaflour software (Universal Imaging, West Chester, PA). Pixel binning (2 × 2) was used to increase both acquisition speed and signal-to-noise ratio (Lasser-Ross et al. 1991).

Recording of fluorescence signals commenced after SBFI loading. Just before an electrical stimulus was given, 5–10 images of the resting fluorescence signals were acquired. Starting at the onset of the electrical stimulus, a series of images was collected for a period of 10–20 s by taking repeated exposures (200 ms each) with the use of the frame transfer mode or by taking an exposure of 200 ms repeated each 1 s. The exposure time of 200 ms was the minimum resulting in a satisfactory signal-to-noise ratio with the use of SBFI with our system.

Of interest (RIs) of the SBFI-labeled neurons were analyzed off-line. These RIs included the soma and serial 50-μm-long sections of the apical dendrite (Fig. 2A). In the soma, the RI (not shown) consisted of the largest circle (20–40 μm diam) that could be fit within the imaged soma cross section. The average fluorescence of each RI was calculated with the use of the software Metaflour. The stimulus-linkage change in fluorescence (ΔF) of each RI was computed as ΔF = Fₜ - F₀ after application of a bleaching correction, where Fₜ is the fluorescence following stimulation and F₀ is the prestimulation resting fluorescence of the RI. The fluorescence signals were corrected for bleaching by recording a sequence of the same number of images obtained at the same rate and exposure time but without the electrical stimulus. These signals also were corrected for background fluorescence. Background fluorescence was determined at the end of each experiment by recording images from the slice at a spot away from the fluorescent neuron. Data are presented as percent change in fluorescence (ΔF/Fₜ), where ΔF/Fₜ = (Fₜ - F₀)/(Fₜ - F₀) and F₀ is background fluorescence. No attempt was made to calibrate ΔF/Fₜ in terms of [Na$^+$]. On the basis of spectral data (Minta and Tsien 1989), ΔF/Fₜ is monotonically related to intracellular sodium concentration. Increased [Na$^+$] causes a decrease of SBFI fluorescence at 380-nm excitation (Minta and Tsien 1989).

**RESULTS**

Intracellular recordings were made from 25 layer V pyramidal neurons located in areas HL, FL, or PAR1 (Zilles and Wree 1985) of neocortex. When viewed with 380-nm excitation after SBFI loading (see METHODS), it could be verified that the recorded neurons had a soma in layer V and an apical dendrite extending toward the pial surface (see Fig. 2A). Two impalements were in the apical dendrite 60–80 μm from the soma; the rest were in the soma. Resting potential of these neurons averaged −70.5 ± 2.6 mV. Input resistance measured by a 100-ms-duration hyperpolarizing injected current pulse averaged 27.9 ± 5.1 MΩ. Each neuron responded to a 1-s injected current pulse with regular, tonic repetitive firing for the duration of the pulse.

In 17 of the neurons loaded with SBFI, a PD was used to activate the persistent Na$^+$ current (IₚNa). Blockade of voltage-gated Ca$^{2+}$ current (and thus Ca$^{2+}$-dependent K$^+$ current) was obtained by substitution of Mn$^{2+}$ for Ca$^{2+}$ in the ACSF, and voltage-gated K$^+$ currents were reduced by the addition of 20 mM TEA (see METHODS). About 10 min...
after this solution change, a small depolarizing current pulse evoked a single action potential followed by a PD that could last \( > 3 \) s. To limit the duration of the PD to a shorter, fixed value, we terminated it prematurely by injecting a hyperpolarizing current pulse (Fig. 1, A and B). Its duration was fixed at 300 ms in about half the experiments and at 1 s in the other half. PD amplitude above resting potential averaged 54.2 ± 5.5 mV. The PD is known to be maintained by TTX (Stafstrom et al. 1985), and we verified that it was abolished by the addition of 1 mM TTX in the present experiments (data not shown). In some neurons the membrane current during the PD was not net inward, because a small depolarizing injected current was required to maintain the PD (Fig. 2B). In other cells the PD outlasted the brief injected current pulse that was used to trigger it (Fig. 1B).

Changes in \([\text{Na}^+]\), were signaled by a decrease of the normalized SBFI fluorescence signal (\(\Delta F/F\)) obtained at 380-nm excitation (see methods). No change in \(\Delta F/F\) was detectable after a single action potential (Fig. 1A). A detectable change in \(\Delta F/F\) required a PD lasting \( \geq 300 \) ms (Fig. 1B). This rather low sensitivity of the optical signal to \(\text{Na}^+\) influx may result from the low affinity of the dye for \(\text{Na}^+\) (\(K_d = 17–19\) mM) (Minta and Tsien 1989) and from the small rise in \([\text{Na}^+]\), caused by \(\text{Na}^+\) influx accompanying a single action potential when averaged over the volume of the soma or a dendritic segment. Apparently, the continuous \(\text{Na}^+\) influx associated with \(I_{\text{Na}^+}\) was required to cause a detectable rise of \([\text{Na}^+]\).

**FIG. 2.** SBFI fluorescence changes associated with \(I_{\text{Na}^+}\) activation are visible in soma and along apical dendrite. A: representative fluorescence image of layer V neuron filled with the \(\text{Na}^+\)-sensitive dye SBFI. Box around dendrite (length: 50 \(\mu\)m; distance to soma: 150 \(\mu\)m): region of interest (RI). Scale bar: 50 \(\mu\)m. B: when \(\text{Mg}^2+\) was substituted for \(\text{Ca}^2+\) in perfusate and 20 mM TEA was added, 1-s depolarizing current pulse (top trace) evoked single action potential followed by PD (middle trace) in same neuron. C: PD caused fluorescence decrease (\(\Delta F/F\)) in soma and along apical dendrite up to 250 \(\mu\)m from soma. Arrow in C: time of electrical stimulation. Note different time scales for electrical and optical recordings.

Figure 2A shows the soma and a portion of the apical dendrite of an SBFI-injected layer V pyramidal neuron. In the same cell an \(I_{\text{Na}^+}\)-associated decrease in \(\Delta F/F\) was visible at the soma and in each of the 50-\(\mu\)m-long RIs along the apical dendrite out to 250 \(\mu\)m from the soma (Fig. 2C).

In each neuron tested, the \(I_{\text{Na}^+}\)-induced reductions of fluorescence in the apical dendrites were detectable as far as the dye could be visualized (distance from soma: 50–300 \(\mu\)m, \(n = 12\)). The fluorescence change was detectable beyond 200 \(\mu\)m in four of these cells and beyond 100 \(\mu\)m in six of the cells. In no case in which we observed an adequate resting dye signal did we not see an \(I_{\text{Na}^+}\)-induced decrease of that signal.

Our inability to obtain a signal that was sufficiently above background at some limiting distance from the soma was probably a function of several factors. The resting fluorescence was dim and fluorescence decreased during \(\text{Na}^+\) influx. Resting fluorescence is proportional to the volume of the imaged structure for fixed dye and \(\text{Na}^+\) concentrations, and the volume of a distal dendritic segment is far less than that of the soma. Resting \([\text{Na}^+]\), is expected to be low, the dye’s \(K_d\) is relatively large (see above), and we were unable to adequately compensate for these factors by increasing dye concentration by 30 min of dye loading. Thus we were incapable of determining the maximum distance over which an \(I_{\text{Na}^+}\)-induced increase of \([\text{Na}^+]\), occurred, but we observed that it can occur to at least 300 \(\mu\)m from the soma.

To ensure that the recorded changes in fluorescence reflected an \(I_{\text{Na}^+}\)-induced rise in \([\text{Na}^+]\), nine other neurons were depolarized in standard ACSF (see methods) with a slow ramp voltage-clamp command 0.5 or 1 s in duration that rose from resting potential (about −70 mV) to a value of membrane potential just below spike threshold (about −50 mV). Within this voltage range \(I_{\text{Na}^+}\) is the sole or dominant voltage-gated current. Membrane potential was then held constant at the final value for durations of 1–5 s (usually, 1 or 2 s) in different experiments (Fig. 3). In control solution this subthreshold depolarization evoked both \(I_{\text{Na}^+}\), as signaled by the inward rectification of the membrane current (Fig. 3, A1 and B, top trace) and by the negative slope in the corresponding current-voltage relationship (Fig. 3B, top), and a decrease in \(\Delta F/F\) in the soma (Fig. 3, A1 and B, bottom traces) and at least 100 \(\mu\)m along the apical dendrite (Fig. 3B, bottom traces). To confirm that the inward rectification observed in voltage clamp was caused by \(I_{\text{Na}^+}\), TTX (1 \(\mu\)M) was added to the ACSF after the control recordings were made in four of the experiments. In each cell tested, TTX abolished both the inward rectification (Fig. 3A2, top trace) and the decrease in \(\Delta F/F\) (Fig. 3A2, bottom trace).

**DISCUSSION**

Our main finding is that \(I_{\text{Na}^+}\)-induced rises of \([\text{Na}^+]\), as deduced from changes in SBFI fluorescence, occur in the apical dendrite out to at least 300 \(\mu\)m from the soma, which constituted the limit of detectability in our experiments. Because the only \(\text{Na}^+\) current evoked by the stimuli used was \(I_{\text{Na}^+}\), we suppose that the rise of \([\text{Na}^+]\), was caused by \(\text{Na}^+\) influx due to \(I_{\text{Na}^+}\) activation in the dendritic membrane. We considered two other possibilities that could lead to a rise
of dendritic $[\text{Na}^+]$, by a mechanism other than dendritic $I_{\text{NaP}}$ activation. Could it be that $I_{\text{NaP}}$ was generated only in the soma, and the rise of $[\text{Na}^+]$, in the apical dendrite was due simply to diffusion of $\text{Na}^+$ from the soma? Our data are incompatible with this idea. Using the one-dimensional diffusion equation and considering a 1-s constant influx of $\text{Na}^+$ into the base of the apical dendrite (as might occur during constant $I_{\text{NaP}}$ activation only at the soma), we calculate that the peak $[\text{Na}^+]$, 200 $\mu$m from the soma would be reached 8.7 times later and have an amplitude 3.8 times smaller than peak $[\text{Na}^+]$, at 50 $\mu$m from the soma. In contrast to this prediction, we observed $\Delta F/F$ changes in soma and all dendritic RIs that were nearly identical in amplitude and time of onset (see Fig. 2C). These observations (particularly the time course) are expected if $[\text{Na}^+]$, rose because of simultaneous Na$^+$ influx at all RIs.

A second possibility is that the somatic PD might evoke only the transient Na$^+$ current ($I_{\text{Na}}$) in the dendrite in association with repetitive dendritic action potentials. According to this idea, the PD is large enough to inactivate $I_{\text{Na}}$ only in the soma. In the dendrite the PD-associated depolarization may become smaller because of electrotonic decay until, at some point, the dendritic depolarization would be small enough to avoid full inactivation of $I_{\text{Na}}$, but large enough to evoke repetitive, Na$^+$-dependent, dendritic action potentials. If this is true, we may have recorded the rise of $[\text{Na}^+]$, caused by repetitive dendritic action potentials, not by dendritic $I_{\text{NaP}}$ activation. Several of our observations make this scenario unlikely. First, we obtained two intradendritic recordings 60–80 $\mu$m away from the soma, and we observed an electrical response (PD) similar to that recorded with intrasomatic recordings (data not shown). Second, repetitive action potentials evoked in ACSF by 1-s current pulses resulted in much smaller $\Delta F/F$ changes in both soma and dendrites than a 1-s-duration PD (our unpublished observations). Finally, if the dendrite could not generate $I_{\text{NaP}}$ there would be some proximal dendritic region where the somatically evoked, electrotonically decaying PD was still large enough to inactivate $I_{\text{Na}}$. No rise of $[\text{Na}^+]$, would be observed in this region because there was no Na$^+$ influx (i.e., $I_{\text{Na}}$ is inactivated and there is no $I_{\text{NaP}}$), but such a region was never observed.

In light of the above considerations, the simplest and best explanation for the rise of dendritic $[\text{Na}^+]$, that we observed is Na$^+$ influx due to dendritic $I_{\text{NaP}}$. Thus our imaging results support previous indirect electrical evidence (Schwindt and Crill 1995) that the apical dendrite can generate $I_{\text{NaP}}$. Our experimental protocol also provides a new way of measuring possible changes in $I_{\text{NaP}}$ due to neuromodulation. Measurement of $I_{\text{NaP}}$-induced changes in $[\text{Na}^+]$, avoids the problem of determining whether $I_{\text{NaP}}$ was in fact altered when both $I_{\text{NaP}}$ and K$^+$ currents were activated together and perhaps altered together.

Recently, Calloway and Ross (1997) used similar methods to monitor the rise of $[\text{Na}^+]$, associated with PDs evoked in cerebellar Purkinje cells. They found the rise of $[\text{Na}^+]$, to be confined to the soma, and concluded that the generation of $I_{\text{NaP}}$ (which also underlies the PD in Purkinje cells) was confined to that region. Evoked action potentials (Stuart and Hausser 1994) and the associated rise of $[\text{Na}^+]$ (Calloway and Ross 1997; Lasser-Ross et al. 1992) also were confined to the soma of Purkinje cells. In contrast, electrical recording has revealed that action potentials actively propagate into the dendrites of layer 5 pyramidal neurons (see introduction), which can generate $I_{\text{NaP}}$ (Schwindt and Crill 1995; this study). These contrasting results indicate that the spatial distribution of Na$^+$ channels differs among central mammalian neurons; they also are consistent with the idea proposed by Alzheimer et al. (1993) that the same Na$^+$ channels generate both the transient and the persistent Na$^+$ current.

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