NMDA Receptor-Mediated Na^+ Signals in Spines and Dendrites

Christine R. Rose and Arthur Konnerth

Institut für Physiologie, Ludwig-Maximilians-Universität München, D-80802 München, Germany

Spines and dendrites of central neurons represent an important site of synaptic signaling and integration. Here we identify a new, synaptically mediated spine signal with unique properties. Using two-photon Na^+ imaging, we show that suprathreshold synaptic stimulation leads to transient increases in Na^+ concentration in postsynaptic spines and their adjacent dendrites. This local signal is restricted to a dendritic domain near the site of synaptic input. In presumed active spines within this domain, the Na^+ level increases by 30–40 mM even during short bursts of synaptic stimulation. During a long-term potentiation induction protocol (100 Hz, 1 sec), the Na^+ level in the active spines

reaches peak amplitudes of ~ 100 mM. We find that the Na^+ transients are mainly mediated by Na^+ entry through NMDA receptor channels and are detected during the coincident occurrence of synaptic potentials and backpropagating action potentials. The large amplitudes of the Na^+ transients and their location on dendritic spines suggest that this signal is an important determinant of electrical and biochemical spine characteristics.

Key words: dendrite; spine; two-photon imaging; sodium; NMDA; coincidence detection; hippocampus

Dendritic spines represent the major postsynaptic input site for excitatory transmission in the brain (Harris and Kater, 1994). Despite this central feature, their role in information processing is still not completely understood (Koch and Zador, 1993; Harris, 1999; Segal et al., 2000). So far, two classes of spine signals have been observed during synaptic transmission. One class of synaptically induced spine signals is changes in the membrane potential. These can be recorded only at distant sites, because spines themselves are inaccessible to direct electrical measurements. In recent years, the development of high-resolution imaging techniques has enabled the detection of a second spine signal (Denk et al., 1996) that is composed of transient elevations in the intracellular Ca^{2+} concentration (Eilers and Konnerth, 1997; Yuste et al., 2000).

When the postsynaptic membrane is sufficiently depolarized, influx of Ca^{2+} into spines can be mediated through NMDA receptors and induce activity-dependent changes in synaptic properties such as long-term potentiation (LTP) or long-term depression (LTD) (Malenka, 1994). Close to the resting potential, another type of glutamate receptor, the so-called AMPA receptor, which in general is much less permeable to Ca^{2+} , is activated.

The major current carrier for both glutamate-gated channels, however, is Na^+ . Therefore, excitatory synaptic transmission is also expected to significantly alter the postsynaptic Na^+ concentration. Indeed, dendritic Na^+ accumulation induced by synaptic stimulation was observed in cerebellar Purkinje and hippocampal CA1 neurons (Lasser-Ross and Ross, 1992; Miyakawa et al., 1992; Callaway and Ross, 1997). In these studies, however, no spatial resolution of spines was obtained, and an analysis of the spatial distribution of the signals could not be performed. Moreover, no

estimate concerning the actual amplitude of the Na^+ changes was made.

A more detailed knowledge of postsynaptic Na^+ transients is desirable because changes in the electrochemical Na^+ gradient will alter the reversal potential of excitatory synaptic currents and Na^+ -dependent transporters such as $\text{Na}^+/\text{Ca}^{2+}$ -exchange (Blaustein and Lederer, 1999) and therefore could significantly influence synaptic function. Moreover, several studies indicated that Na^+ might play a role in activity-dependent synaptic plasticity. An earlier report showed that Na^+ influx through AMPA receptor channels is required for the induction of LTD in cerebellar Purkinje neurons (Linden et al., 1993). In hippocampal neurons, intracellular Na^+ increases have been proposed to increase the open probability of NMDA receptors via activation of the protein tyrosine kinase Src and thus might control the gain of excitatory transmission (Yu and Salter, 1998).

In the present study we used two-photon Na^+ imaging in combination with whole-cell patch-clamp recordings (Rose et al., 1999) to measure action potential (AP)-induced and synaptically induced Na^+ signals in spines and dendrites in a hippocampal slice preparation. This technique is particularly well suited for measurement of Na^+ transients in small cellular compartments because excitation is limited to the focal plane, allowing imaging in the intact, scattering tissue (Denk et al., 1990). Our results demonstrate the presence of local postsynaptic Na^+ transients in spines and dendrites. We provide evidence that Na^+ transients are mediated mainly by Na^+ entry through NMDA receptor-gated channels.

MATERIALS AND METHODS

Tissue preparation and patch-clamp recordings. CD-1 mice (Charles River; 3–5 weeks old) were anesthetized and decapitated. Transverse hippocampal slices (300 μm) were prepared as described previously, and standard techniques were used for somatic whole-cell patch-clamp recordings (Edwards et al., 1989). CA1 pyramidal cells were generally held at membrane potentials of -60 to -65 mV; granule cells of the dentate gyrus were held at -75 to -80 mV. Because of better space-clamp characteristics, voltage-clamp experiments were exclusively performed on granule cells. The intracellular solution for patch-clamp experiments

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Correspondence should be addressed to Christine R. Rose, Institut für Physiologie, Ludwig-Maximilians-Universität München, Biedersteiner Strasse 29, D-80802 München, Germany. E-mail: rose@lrz.uni-muenchen.de.

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contained (in mM): 120 K⁺-gluconate, 10 HEPES, 32 KCl, 4 NaCl, 0.16 EGTA, 4 Mg-ATP, 0.4 NaGTP, 2 tetraammonium salt of sodium-binding benzofuran isophthalate (SBFI) (Molecular Probes, Eugene, OR) and was titrated with KOH to a pH of 7.3. During experiments, slices were perfused with physiological saline containing (in mM): 125 NaCl, 4 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2 CaCl₂, 1 MgCl₂, 20 glucose, continuously bubbled with 95% O₂/5% CO₂ resulting in a pH of 7.4. GABAergic transmission was blocked by using 10 μ M bicuculline methiodide. Experiments were performed at room temperature (22–24°C). To mimic burst activity of CA3 neurons, Schaffer collaterals were electrically stimulated by five 2 msec square pulses delivered at 50 Hz via a thin glass pipette filled with saline. To activate granule cells, the stimulation electrode was placed in the molecular layer of the dentate gyrus. The stimulation pipettes were placed at a distance of 10–15 μ m from the dendrites. Stimulation strength was set just below action potential firing threshold for subthreshold stimulation and just above firing threshold for suprathreshold stimulation.

Na⁺ imaging. Imaging was performed using a custom-built two-photon laser-scanning microscope based on a mode-locked Ti:sapphire laser system operated at 790 nm center wavelength, 80 MHz pulse repeat, <100 fsec pulse width (Tsunami and Millennia, Spectra Physics, Mountain View, CA), and a laser-scanning system (MRC 1024, Bio-Rad, Herts, UK) mounted on an upright microscope (BX50WI, Olympus, Tokyo, Japan) equipped with 20 $\times$ 0.5 NA and 60 $\times$ 0.9 NA water immersion objectives (Olympus). Neurons were filled with SBFI via the patch pipette for at least 45 min before the recordings were started. Images were acquired at 4–8 Hz. Background-corrected images were analyzed off-line with a custom-written routine in LabView (National Instruments, Austin, TX). *In situ* calibration of SBFI fluorescence with Na⁺ ionophores enabled the determination of the actual magnitude of the Na⁺ changes (Rose and Ransom, 1997; Rose et al., 1999). Unless specified otherwise, data are expressed as means $\pm$ SEM, and data traces shown are averages of one to three consecutive trials. Data were statistically analyzed by a Student's *t* test (significance level, *p* < 0.005).

RESULTS

By combining whole-cell patch-clamp recordings with two-photon laser-scanning microscopy (Denk et al., 1990), we analyzed synaptically and action potential-induced Na⁺ changes in apical dendrites and spines of neurons in hippocampal slice preparations of 3- to 5-week-old mice. Common patterns of activity in the hippocampus are bursts of two to seven action potentials (Otto et al., 1991; Lisman, 1997). To induce such burst activity in CA1 pyramidal neurons, Schaffer collaterals were electrically stimulated by trains of five pulses delivered at 50 Hz. Granule cells of the dentate gyrus were similarly activated by placing the stimulation electrode in the molecular layer of the dentate gyrus.

Spatial profile of Na⁺ transients

Suprathreshold synaptic activation leads to widespread elevations of Ca²⁺ throughout the dendritic tree because of backpropagation of action potentials and the resulting opening of voltage-gated Ca²⁺ channels (Jaffe et al., 1992; Markram et al., 1995; Spruston et al., 1995b; Yuste and Denk, 1995). Because apical dendrites of CA1 pyramidal neurons also express voltage-gated Na⁺ channels (Stuart et al., 1997), we first determined the spatial profile of synaptically induced dendritic Na⁺ accumulations. Figure 1 shows intracellular Na⁺ transients induced by suprathreshold synaptic stimulation (five stimuli resulting in four to five postsynaptic APs) (Fig. 1b) along different regions of a secondary apical dendrite of a CA1 pyramidal neuron.

When the stimulation electrode was placed in close proximity to the dendrite (10–15 μ m) (Fig. 1a), synaptically induced Na⁺ transients were maximal in the dendritic region, which was near (10–50 μ m) the pipette (Fig. 1c). Synaptic activation of the postsynaptic dendrite is expected from Ca²⁺ imaging measurements that were performed under similar conditions (Kovalchuk et al., 2000). Furthermore, to assure that these changes were not

Figure 1. Local dendritic Na⁺ transients induced by synaptic stimulation. *a*, Reconstruction of a stack of 27 optical sections taken at 1.5 μ m intervals through part of a CA1 pyramidal neuron filled with 2 mM SBFI (objective: 60 $\times$ 0.9 NA). The brackets numbered 1–6 indicate the dendritic regions from which fluorescence measurements in *c* were obtained. The position of the stimulation electrode is indicated schematically. Scale bar, 20 μ m. Distance of region 1 from the cell body is 120 μ m. *b*, Membrane potential change at the soma (*V_m*) induced by five afferent stimuli at 50 Hz as indicated in the bottom trace. *c*, Na⁺ transients in the dendritic regions 1–6 caused by suprathreshold afferent stimulation as in *b*. Na⁺ transients were maximal in region 4, which was closest to the stimulation pipette. Na⁺ changes in the different regions were measured successively, because the focal plane did not include the complete dendrite. *d*, Bar graph summarizing the normalized amplitude of activity-induced dendritic Na⁺ transients (mean $\pm$ SEM). The bars represent 4–16 observations taken from 16 cells. The bar at –40 μ m summarizes data from two experiments. The amplitude of Na⁺ transients decayed monoexponentially with increasing distance from the region of maximal response with a length constant (λ) of $\sim$ 30 μ m. The arrowhead in *c* indicates the time of synaptic stimulation.

caused by direct activation of voltage-sensitive channels by the stimulation current, we applied the postsynaptic blockers 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μ M) and DL-2-amino-5-phosphovaleric acid (APV; 100 μ M). During perfusion with these drugs, both membrane potential changes and Na⁺ transients were abolished, indicating that they were indeed caused by synaptic activity (*n* = 4; data not shown).

The amplitudes of Na⁺ transients reached $\sim$ 10 mM in the experiment depicted in Figure 1 [for calibration procedures, see Rose et al. (1999)]. This region of maximal response extended across a dendritic length of 20–30 μ m. With increasing distance from the region of maximal response, the site of synaptic input, the amplitude of the Na⁺ transients decayed in a monoexponential fashion with a length constant (λ_{Na^+}) of $\sim$ 30 μ m (*n* = 16 cells) (Fig. 1d). In other dendritic branches, small Na⁺ signals just above the detection threshold ($\sim$ 1–2 mM) were observed. This is in line with an earlier study in which we showed that short bursts of backpropagating action potentials cause only minor changes in

Figure 2. Na⁺ transients require suprathreshold stimulation. *a*, Left, Reconstruction of a stack of 24 optical sections taken at 5 μ m intervals through an SBFI-filled CA1 pyramidal cell. The white box indicates the area enlarged in the right panel. Right, Spiny dendrite from the same cell taken at higher resolution (15 stacks at 0.4 μ m). The position of the stimulation electrode is indicated schematically. The arrows indicate the dendritic region from which measurements in *b* were obtained. *b*, Suprathreshold synaptic stimulation (top traces) induced a prominent Na⁺ transient in the dendrite, whereas subthreshold stimulation (bottom traces) did not result in a measurable Na⁺ increase. *c*, Left, Stack of 20 optical sections (3.5 μ m intervals) through a granule cell of the dentate gyrus filled with SBFI. The white box indicates the area enlarged on the right. Right, Spiny dendrite from the same cell (15 stacks at 0.7 μ m); the stimulation electrode is indicated schematically. The arrows indicate the dendritic region from which measurements in *d* were obtained. *d*, Suprathreshold stimulation evoked a Na⁺ transient in the dendrite (top traces). Subthreshold stimulation, in contrast, did not result in a Na⁺ transient (bottom traces). Scale bars: *a*, 50 μ m, inset, 5 μ m; *c*, 20 μ m, inset, 5 μ m. Arrowheads in *b* and *d* indicate the time of synaptic stimulation. Calibration for *d* is shown in *b*.

Na⁺ concentration throughout the dendritic tree (Rose et al., 1999).

These results demonstrate that suprathreshold stimulation leads to local Na⁺ transients that occur in dendritic domains near the site of synaptic activation. In all subsequent experiments, the analysis of Na⁺ transients was confined to dendritic regions and their adjacent spines ~15–20 μ m proximal and distal to the position of the stimulation pipette.

Na⁺ transients require suprathreshold stimulation

Whereas suprathreshold activation has been reported to induce widespread dendritic Ca²⁺ transients (Markram et al., 1995; Yuste and Denk, 1995; Jaffe and Brown, 1997), subthreshold stimulation can elicit local dendritic Ca²⁺ signals that are confined to the site of activation (Markram and Sakmann, 1994; Eilers et al., 1995; Yuste and Denk, 1995; Kovalchuk et al., 2000). Synaptically induced dendritic Na⁺ signals, however, could be detected only with suprathreshold synaptic stimulation (Fig. 2). When the stimulation strength was set just below the threshold for action potential firing, no measurable change in the Na⁺ level was

observed. Increasing the stimulation strength just above firing threshold, in contrast, resulted in prominent Na⁺ transients in the apical dendrites of CA1 pyramidal cells ($n = 25$) (Fig. 2*a,b*). The amplitudes of these Na⁺ transients were dependent on the number of suprathreshold stimuli. Five stimuli (four to five action potentials) resulted in dendritic Na⁺ transients that amounted to 13.4 ± 1.1 mM ($n = 33$ cells) (Fig. 3*e*) and three stimuli (two to three action potentials) evoked Na⁺ transients of 8.6 ± 1.7 mM ($n = 6$ cells; data not shown), whereas a single stimulus resulting in one action potential evoked a Na⁺ rise close to the detection threshold (4.3 ± 1.0 ; $n = 7$ cells; data not shown).

Activity-induced Na⁺ transients were not restricted to CA1 pyramidal cells but could also be evoked in granule cells of the dentate gyrus. As observed for CA1 pyramidal cells, measurable dendritic Na⁺ transients were elicited only with suprathreshold synaptic stimulation (Δ Na⁺ = 11.6 ± 1.5 mM; $n = 8$) (Fig. 2*c,d*).

Na⁺ transients in spines

We next attempted to identify “active” spines (Denk et al., 1995; Yuste and Denk, 1995), that is, the spines directly activated during synaptic stimulation. As reported above for dendritic Na⁺ transients, synaptically induced Na⁺ signals in spines were observed only with suprathreshold stimulation (the signal-to-noise ratio allowed reliable detection of Na⁺ transients in single spines of ~5 mM). Unambiguous identification of active spines was difficult because suprathreshold stimulation produced Na⁺ transients in all spines of the active domain. The plotting of a distribution histogram of peak Na⁺ transients of all spines, however, revealed a bimodal distribution of response amplitudes, clearly indicating the presence of two classes of spines (Fig. 3*d*).

In the first class of spines (75% of 152 spines; $n = 18$ cells), the Na⁺ level rose by 14.6 ± 1.8 mM (stimulation parameters set to five pulses at 50 Hz) (Fig. 3*b*), a level that was only slightly higher than that of the dendrites (~13 mM; see above and Fig. 3*c–e*). Moreover, the decay of the Na⁺ transients in these spines and in dendritic regions was monoexponential with time constants of 8.8 ± 1.6 sec (spines) and 9.0 ± 2.1 sec (dendrites; $n = 16$ cells) (Fig. 3*c,e*). In contrast, in the second class of spines (25% of 152; $n = 18$ cells), activity-induced Na⁺ transients were two to three times higher than those of the adjacent dendrite, reaching up to 35–40 mM (mean 35.7 ± 4 mM) (Fig. 3). Recovery followed a biexponential decay with a fast time constant (τ_f) of 1.6 ± 0.5 sec and a slow time constant (τ_s) that was very similar to the dendritic decay constant (8.5 ± 1.9 sec) (Fig. 3*e*). We assume, therefore, that these spines were the sites of synaptic input and thus the sites of synaptic Na⁺ influx (active spines). In the other class of spines, in which the amplitude and the time course of Na⁺ transients corresponded to that of the dendrites, the Na⁺ signals were probably caused by diffusion of free and SBFI-bound Na⁺ ions from their site of entry (“passive” spines).

Because of the relatively low image sampling frequency (4–8 Hz) and the required additional binning to improve the signal-to-noise ratio of the fluorescence signals from single spines, our measurements very likely underestimated the peak amplitude of the Na⁺ increase in active spines. Earlier calculations estimated that a single presynaptic action potential results in a rapid Na⁺ increase by ~30 mM in postsynaptic spines during excitatory transmission (Sejnowski and Qian, 1992). For the same reasons, τ_f , which most likely represents both active Na⁺ extrusion and diffusion of Na⁺ from spines to dendrites (Majewska et al., 2000), is probably overestimated. Furthermore, although SBFI concentration was low (2 mM) in comparison with the observed Na⁺

Figure 3. Synaptically induced Na⁺ transients in spines. *a*, Image of the spiny dendrite of a CA1 pyramidal cell chosen for the experiment depicted in *b* and *c*. Closed arrowheads indicate active spines; open arrowheads indicate passive spines from which measurements in *c* were taken. Scale bar: 5 μ m; distance from the cell body: 140 μ m. *b*, Suprathreshold stimulation (5 afferent stimuli at 50 Hz) induced five APs as measured at the soma. *c*, Left, Average activity-induced Na⁺ transient in spines (average of 19 spines) and in the dendrite (top traces). Middle traces, Na⁺ transients in three single passive spines. Bottom traces, Na⁺ transients in three single active spines. Data points of single spine traces were binned by a factor of 2 to improve the signal-to-noise ratio. Right, The recovery of dendritic Na⁺ transients and passive spines could be fitted by a monoexponential decay with similar time constants (τ). Recovery in active spines followed a biexponential time course (τ_f , fast decay constant; τ_s , slow decay time constant). The spine traces on the right represent averages of the single spine traces depicted on the left. *d*, Histogram of peak Na⁺ transients of all spines. Individual data were pooled in steps of 2.5 mM Na⁺. The data were fitted by two Gaussian functions revealing a bimodal distribution. *e*, Top, Mean amplitudes $\pm$ SEM of activity-induced Na⁺ increases in dendrites and spines. The amplitude in active spines was significantly higher than those in passive spines and dendrites. Bottom, Mean decay time constants (τ) $\pm$ SEM of activity-induced Na⁺ increases. The fast time constant (τ_f) of active spines was significantly different from the slow time constant (τ_s). Arrowheads in *c* indicate the time of synaptic stimulation.

changes (~ 35 mM in spines), the dye will act as a buffer for Na⁺ and might alter Na⁺ dynamics in spines and dendrites, as has

Figure 4. NMDA dependence of synaptically induced Na⁺ transients. *a*, Blocking glutamate receptors of the NMDA type with APV strongly reduced the stimulus-induced Na⁺ transient in the spines and the dendrite. *b*, CNQX, a blocker of non-NMDA receptors, blocked activity-induced Na⁺ transients. Increasing the stimulus intensity restored the Na⁺ transients. *a*, CA1 pyramidal neuron; *b*, dentate gyrus granule cell. Arrowheads in *a* and *b* indicate the time of synaptic stimulation. Calibration for *b* is shown in *a*; distance from the cell body in *a* = 120 μ m; in *b* = 80 μ m.

been reported for Ca²⁺ signals measured with fluorometric Ca²⁺ indicators (Helmchen et al., 1996). Finally, decay times of Na⁺ transients are dependent on temperature. In an earlier study, we showed that increasing the bath temperature from 23 to 33°C decreased the dendritic decay constant of action potential-induced Na⁺ transients by a factor of 1.7, indicating that Na⁺/K⁺-ATPase activity plays a dominant role in restoring the electrochemical Na⁺ gradient (Rose et al., 1999).

NMDA receptor dependence

To further analyze the mechanism of activity-induced Na⁺ transients, we studied the contribution of ionotropic glutamate receptors. In these and all subsequent experiments (except for those illustrated in Fig. 7*a,b*), fluorescence signals from all spines of a given dendrite were averaged. This resulted in average spine Na⁺ transients that were slightly larger than those of the parent dendrites (compare Figs. 3*c*, 4–6, 7*c*).

Blocking of glutamate receptors of the NMDA type with APV resulted in a reversible reduction of the stimulus-induced Na⁺ transients by $\sim 80\%$ in spines and dendrites, although the stimulation still elicited action potentials (Figs. 4*a*, 5*c*) ($n = 6$). During perfusion with CNQX (10 μ M), a blocker of non-NMDA receptors, the stimulation failed to elicit action potentials, and the activity-induced Na⁺ transient was reduced by $\sim 93\%$ (Figs. 4*b*, 5*c*) ($n = 4$). Increasing the stimulus intensity caused a pronounced depolarization and restored the Na⁺ transient (Fig. 4*b*) ($n = 2$). This was most likely caused by an efficient removal of the Mg²⁺ block of NMDA receptor channels at the site of synaptic input.

Na⁺ transients induced by suprathreshold stimulation were suppressed by $>77\%$ during voltage clamp. Removing extracellular Mg²⁺ relieved this voltage dependence and resulted in the expected augmentation of the amplitude of the Na⁺ increase to

Figure 5. Membrane potential dependence of Na⁺ transients. *a*, Na⁺ transients induced by suprathreshold stimulation were suppressed when cells were held in the voltage-clamp mode. Removing extracellular Mg²⁺ relieved this voltage dependence. *b*, In the absence of extracellular Mg²⁺, the amplitudes of the Na⁺ transients were similar when the cells were held in the current-clamp and voltage-clamp mode. *c*, Normalized mean amplitudes of dendritic activity-induced Na⁺ transients ± SEM in control conditions (1), during perfusion with APV (2), CNQX (3), during voltage clamp (4), and during voltage clamp in 0 Mg²⁺ (5). *d*, Normalized mean amplitudes of activity-induced Na⁺ transients ± SEM during current clamp and voltage clamp. *a*, *b*, Dentate gyrus granule cells; distance from the cell body in *a* = 60 μm; in *b* = 90 μm. Arrowheads in *a* and *b* indicate the time of synaptic stimulation.

~172% of control levels (Fig. 5*a,c*) (*n* = 7). In the absence of extracellular Mg²⁺, the amplitudes of the Na⁺ transients were undistinguishable between stimulations performed when cells were held in the current-clamp mode (allowing the generation of action potentials) compared with those in the voltage-clamp mode (Fig. 5*b,d*) (*n* = 9). This demonstrates that the influx of Na⁺ through voltage-gated Na⁺ channels during backpropagating action potentials does not significantly contribute to the observed Na⁺ transients, even during concomitant synaptic activation of NMDA receptors.

These results indicate that the observed postsynaptic Na⁺ transients are largely mediated by Na⁺ entry through NMDA receptor channels, whereas Na⁺ entry through AMPA receptor channels or voltage-gated Na⁺ channels plays only a minor role. Indeed, because of the considerably longer time course of synaptic NMDA receptor currents (Spruston et al., 1995a), Na⁺ influx through these NMDA channels will be larger by far than that through AMPA-gated channels.

To further quantify the Na⁺ influx through NMDA receptors, we performed experiments in which cells were held in the

Figure 6. Correlation between charge and Na⁺ increase. *a*, Membrane currents (*I*_{NMDA}) and corresponding Na⁺ transients at four different increasing stimulation intensities. Experiments were performed in the voltage-clamp mode in the absence of extracellular Mg²⁺ and in the presence of CNQX. *b*, Plot of the mean values ± SEM of six experiments. Individual data points were pooled in steps of 5 mM Na⁺. The mean values follow a linear regression line (*r* = 0.994) with a slope of 5.3 pC/mM Na⁺. *c*, Histogram of mean values ± SEM for measured charge per millimolar Na⁺ for experiments in which no CNQX was present (1; compare Fig. 5*a*) and for experiments in which CNQX was added to the saline (2). Experiments were performed in dentate gyrus granule cells; distance from the cell body in *a* = 100 μm.

voltage-clamp mode in the absence of extracellular Mg²⁺ and in the presence of CNQX, and the stimulation intensity was increased stepwise (Fig. 6*a*) (*n* = 6). We then plotted the measured charge against the corresponding Na⁺ increase and found a linear correlation with a slope of 5.3 pC/mM Na⁺ (Fig. 6*b,c*). This result corresponds closely to the prediction because the influx of 1 mM Na⁺ into a given dendrite with a length of 20 μm and a diameter of 2 μm will result theoretically in a loss of 6 pC negative charge.

In the voltage-clamp experiments performed in the presence of CNQX (see above), the correlation between charge and [Na⁺]_i increase was not significantly different from the experiments performed with no CNQX added (Figs. 5*a*, third row, 6*c*). This control experiment clearly confirms that the influx of Na⁺ through AMPA-gated channels does not contribute significantly to the measured, synaptically induced Na⁺ transients.

Na⁺ transients during an LTP induction protocol

Repetitive activation of excitatory synapses in the hippocampus causes a long-lasting increase in synaptic strength called LTP that is considered to be a cellular model for learning and memory (Bliss and Collingridge, 1993; Malenka, 1994). Usually, LTP is induced by a tetanic synaptic stimulation using stimulation frequencies of 100 Hz for 1 sec. The critical trigger for LTP is a rise in intracellular Ca²⁺ after NMDA receptor channel opening (Malenka, 1994).

To analyze Na⁺ changes during a typical LTP induction protocol, we performed a synaptic stimulation at 100 Hz for 1 sec while measuring Na⁺ transients in dendrites and spines of CA1 pyramidal neurons. In these experiments, the calcium chelator BAPTA (10 mM) was added to the pipette solution to prevent postsynaptic Ca²⁺ accumulation and LTP induction. As illustrated in Figure 7, *a* and *b*, the Na⁺ concentration increased by 44.6 ± 4.2 mM ($n = 7$) in dendrites during the tetanus. Again (compare Fig. 3), two classes of spines could be distinguished. Although Na⁺ transients in passive spines were similar to those measured in the dendrite, Na⁺ increased to values of >100 mM in active spines. These results demonstrate that tetanic stimulation causes enormous postsynaptic Na⁺ transients, which may play a role in the induction process of LTP. Furthermore, during a short period (1–2 sec), the driving force for Na⁺ will break down and synaptic transmission will collapse. It is important to note that Na⁺ entry through cation channels activated by metabotropic glutamate receptors may partially contribute to the Na⁺ transients during this prolonged stimulation (Congar et al., 1997).

Na⁺ transients during coincident presynaptic and postsynaptic activity

Na⁺ spikes actively invade dendrites of hippocampal pyramidal cells (Jaffe et al., 1992; Spruston et al., 1995b), and the coincidence of presynaptic and postsynaptic activity can lead to supralinear increases in postsynaptic Ca²⁺ (Magee and Johnston, 1997; Markram et al., 1997; Koester and Sakmann, 1998; Schiller et al., 1998). To analyze whether such a supralinearity also exists for postsynaptic Na⁺ signals in dendrites and spines, we investigated the effect of coincident presynaptic and postsynaptic activity on the amplitude of synaptically induced Na⁺ transients. Figure 7*c* shows such an experiment. As illustrated above (compare Fig. 2), suprathreshold synaptic activation caused a prominent Na⁺ transient, whereas subthreshold stimulation did not result in measurable Na⁺ increases. Backpropagating APs, evoked by 2 msec square pulse current injection at the soma at 50 Hz, caused no or only minor Na⁺ changes (on average 17% of control) (Fig. 7*d*) (cf. Rose et al., 1999). Combining backpropagating APs with subthreshold synaptic stimulation, however, resulted in a supralinear summation of Na⁺ influx into both spines and dendrites. The pairing of presynaptic and postsynaptic activity induced Na⁺ transients that reached ~75% of those with suprathreshold stimulation (Fig. 7*d*) ($n = 7/8$ cells). These results demonstrate that the occurrence of postsynaptic APs in coincidence with presynaptic activity was the critical parameter for the induction of NMDA-receptor-mediated postsynaptic Na⁺ transients.

DISCUSSION

Local Na⁺ transients in apical dendrites and spines

Our results demonstrate a new and unique type of synaptically induced signal in dendrites and spines that consists of postsynaptic Na⁺ transients. Although a direct comparison is problematic

Figure 7. Na⁺ transients during tetanic stimulation and during coincident presynaptic and postsynaptic activity in CA1 pyramidal cells. *a*, Image of the spiny dendrite chosen for the experiment depicted in *b*. Closed arrowheads indicate active spines; open arrowheads indicate passive spines from which measurements in *b* were taken. Scale bar, 5 μ m. *b*, Top, Membrane potential change induced by synaptic stimulation at 100 Hz/1 sec. Bottom, Average Na⁺ transients in 16 spines and in the dendrite induced by the tetanic stimulation; Na⁺ transients in two single passive spines; and Na⁺ transients in two single active spines. Data points of single spine traces were binned by a factor of 2 to improve the signal-to-noise ratio. *c*, Effect of coincident presynaptic and postsynaptic activity on the amplitude of synaptically induced Na⁺ transients. Suprathreshold synaptic activation caused Na⁺ transients in both dendrite and adjacent spines, whereas subthreshold stimulation did not result in measurable Na⁺ increases. Backpropagating APs evoked only minor Na⁺ changes. Combining backpropagating APs with subthreshold synaptic stimulation, however, resulted in a supralinear summation of Na⁺ transients of both spines and dendrites. *d*, Histogram comparing the normalized mean amplitudes $\pm$ SEM of activity-induced Na⁺ transients during suprathreshold stimulation (1), subthreshold stimulation (2), backpropagating APs (3), and pairing of backpropagating APs with subthreshold stimulation (4). The arrowheads in *b* and *c* indicate the time of synaptic stimulation; distance from the cell body in *a* = 150 μ m; in *c* = 250 μ m.

because spatial and temporal characteristics of measured ion changes depend on the binding characteristics of the indicator used (Helmchen et al., 1996), the Na⁺ signals appear to differ in several ways from postsynaptic Ca²⁺ signals reported earlier. First, suprathreshold stimulation protocols evoke widespread elevations in Ca²⁺ because of influx of Ca²⁺ through voltage-gated Ca²⁺ channels activated by backpropagating action potentials (Jaffe et al., 1992; Markram et al., 1995; Spruston et al., 1995b;

Yuste and Denk, 1995). Na⁺ transients elicited by suprathreshold stimulation seem to be more restricted to defined dendritic regions directly at and adjacent to the stimulation site. As reported earlier (Rose et al., 1999), influx of Na⁺ via voltage-gated Na⁺ channels during short trains of backpropagating action potentials causes only minor Na⁺ increases throughout the dendritic tree.

Second, postsynaptic Na⁺ signals in spines as well as in dendrites were observed only with suprathreshold stimulation, whereas already single, subthreshold stimuli induce Ca²⁺ signals in spines (Denk et al., 1995; Eilers et al., 1995; Yuste and Denk, 1995; Koester and Sakmann, 1998; Schiller et al., 1998). The absence of subthreshold Na⁺ signals in spines might be related to the fact that these signals were too small to be detected with our technique. In any case, this result indicates that Na⁺ accumulations in active spines during subthreshold stimulation are probably negligible (<5 mM) in comparison with those evoked by suprathreshold stimulation (30–40 mM).

Third, our results strongly suggest that the dominating pathways for Na⁺ entry during suprathreshold synaptic activation are NMDA receptor channels. Postsynaptic Ca²⁺ signals, in contrast, can be mediated by several mechanisms: influx through NMDA receptor channels, influx through voltage-gated Ca²⁺ channels, and release from intracellular stores (Markram and Sakmann, 1994; Denk et al., 1995; Nakamura et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Schiller et al., 2000).

Our ability to detect large Na⁺ gradients between active spines and the adjacent dendrite with our relatively low image-sampling frequency (4–8 Hz) was surprising. The existence of such gradients that were maintained over hundreds of milliseconds suggests the existence of a diffusion barrier or a buffer system, or both, for Na⁺ in the spines. The apparent diffusion coefficient for free Na⁺ is $\sim 13 \times 10^{-6}$ cm²/sec (Push and Neher, 1988) and about twice that value for SBFI-bound Na⁺ [assuming that the diffusion coefficient is similar to that of fura-2 (Push and Neher, 1988)]. Unrestricted equilibration of Na⁺ and SBFI-bound Na⁺ between distances of ~ 1 μ m will be accomplished, therefore, within milliseconds. Because diffusion from spines to the adjacent dendrite is slowed by a factor of 10–100 by the spine neck (Svoboda et al., 1996), Na⁺ gradients between spine and adjacent dendrites should be largely equilibrated within ~ 100 msec.

Significant gradients between spines and the adjacent dendritic shaft have also been reported for Ca²⁺ signals (Jaffe and Brown, 1997; Yuste et al., 1999; Kovalchuk et al., 2000; Majewska et al., 2000), suggesting that spines may be chemically isolated from dendrites. In addition to the diffusion barrier caused by spine necks, calcium pumps or other Ca²⁺ uptake systems and Ca²⁺ buffers might be responsible for the observed biochemical isolation of spines (Koch and Zador, 1993; Maeda et al., 1999). Similarly one could speculate about a high presence of Na⁺ pumps (Na⁺–K⁺–ATPase) in spine necks that might account for the compartmentalization in Na⁺. Alternatively, the spine head may contain molecules that bind and buffer Na⁺.

Functional implications of postsynaptic Na⁺ transients

The NMDA receptor channels are widely accepted as coincident detectors of presynaptic and postsynaptic activity (Malenka, 1994). They were shown to mediate a supralinear increase in postsynaptic Ca²⁺ signals during pairing of an action potential with synaptic stimulation (Yuste and Denk, 1995; Magee and Johnston, 1997; Markram et al., 1997; Koester and Sakmann, 1998; Schiller et al., 1998). Our results indicate that NMDA receptor activity might be involved in yet another signal for

coincidence detection, which consists of Na⁺ transients. Postsynaptic Na⁺ transients seem to be ideally suited for coincidence detection because they are restricted to the site of activation and exhibit all-or-none characteristics in that they require the occurrence of postsynaptic APs in coincidence with presynaptic activity.

Although the direct physiological role of Na⁺ accumulations in spines is unclear, it is firmly established that the inwardly directed Na⁺ gradient regulates many cellular processes, among them intracellular Ca²⁺ homeostasis via Na⁺/Ca²⁺ exchange (Blaustein and Lederer, 1999). In presynaptic terminals of cultured hippocampal cells, an elevation of Na⁺ was accompanied by an increase in Ca²⁺, causing enhanced transmitter release (Bouron and Reuter, 1996). A reduction in the Na⁺ gradient slowed Ca²⁺ extrusion in cerebellar neurons (Kiedrowski et al., 1994; Fierro et al., 1998). In granule cell presynaptic terminals, Ca²⁺ extrusion via the Na⁺/Ca²⁺ exchange leads to Na⁺ accumulation (Regehr, 1997). These studies indicate that Na⁺ accumulations in spines might shape the time course of postsynaptic Ca²⁺ transients and vice versa. Therefore, activation of Na⁺/Ca²⁺ exchange also might contribute partly to the synaptically induced Na⁺ transients observed in our study. The Na⁺ changes measured during tetanic stimulation in the presence of 10 mM BAPTA, however, indicate that considerable Na⁺ increases also occur without a contribution of Na⁺/Ca²⁺ exchange. Furthermore, a rise in postsynaptic Na⁺ will slow the Na⁺-dependent uptake of glutamate or may even lead to its reversal and therefore could influence excitatory transmission (Attwell et al., 1993).

Another emerging possibility is that increases in Na⁺ concentration might induce synaptic plasticity by selectively increasing the open probability of NMDA receptors, a process that is controlled by a channel-associated Src kinase (Yu and Salter, 1998). This mechanism might be especially powerful during more intensive stimulation protocols and could be important in the Src-dependent induction of LTP in CA1 hippocampal neurons (Lu et al., 1998). Thus, synaptically induced NMDA-dependent Na⁺ accumulations in spines might provide an efficient mechanism of autoregulation of NMDA conductance and input-specific strengthening of active synapses. It is important to note that in isolated patch recordings, [Na⁺] elevations by 30–40 mM were sufficient to upregulate the NMDA receptor function (Yu and Salter, 1998). Under our conditions of stimulation with an LTP-inducing protocol, we saw Na⁺ peak levels of >100 mM in active spines.

Finally, an important possibility that must be considered is that increases in intracellular Na⁺ will reduce the driving force for glutamatergic currents. At rest, the reversal potential of AMPA and NMDA receptor-mediated currents is close to 0 mV. Short synaptic bursts will briefly reduce their reversal potential to approximately –35 mV [calculation based on assumed peak intracellular concentrations of 35 mM Na⁺ (see Results) and 130 mM K⁺, and extracellular concentrations of 145 mM Na⁺ and 8 mM K⁺; see Materials and Methods and Dietzel and Heinemann (1985)]. At this potential, NMDA receptor currents are already significantly reduced because of voltage-dependent block by Mg²⁺ (Spruston et al., 1995a). With more intense synaptic activity and larger ion changes, the reversal potential for glutamatergic currents will be even more negative, which would eventually result in dendritic saturation (Sejnowski and Qian, 1992; Bush and Sejnowski, 1994). Glutamatergic currents will be largely diminished, thereby protecting the postsynaptic input sites from excessive accumulations of Na⁺ and Ca²⁺, which have been

implicated in inducing cellular excitotoxicity (Lee et al., 1999). This intriguing prediction, however, will probably be difficult to test experimentally at present, because peak ionic changes persist for only very short periods (Fig. 7). Nevertheless, it seems reasonable to speculate that dendritic saturation caused by increases in postsynaptic Na^+ levels during excitatory synaptic transmission could play an important role in the input-specific protection of overexcitation of synapses. The development of specific Na^+ chelators that are effective in the range of tens of millimolar will help to test this hypothesis.

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