Spatial Distribution of Synaptically Activated Sodium Concentration Changes in Cerebellar Purkinje Neurons

JOSEPH C. CALLAWAY AND WILLIAM N. ROSS
Department of Physiology, New York Medical College, Valhalla, New York 10595

Callaway, Joseph C. and William N. Ross. Spatial distribution of synaptically activated sodium concentration changes in cerebellar Purkinje neurons. J. Neurophysiol. 77: 145–152, 1997. The spatial distribution of Na⁺-dependent events in guinea pig Purkinje cells was studied with a combination of high-speed imaging and simultaneous intracellular recording. Individual Purkinje cells in sagittal cerebellar slices were loaded with either fura-2 or the Na⁺ indicator sodium binding benzofuran isophthalate (SBFI) with sharp electrodes or patch electrodes on the soma or dendrites. [Na⁺], changes were detected in response to climbing fiber and parallel fiber stimulation. These changes were located both at the anatomically expected sites of synaptic contact in the dendrites and in the somatic region. The variation in time course of these [Na⁺], changes in different locations implies that Na⁺ enters at the synapse and diffuses rapidly to locations of lower initial [Na⁺]. The synaptically activated somatic [Na⁺], changes probably reflect Na⁺ entry through voltage-sensitive Na⁺ channels because they were detected only when regenerative potentials were recorded in the soma. [Na⁺], changes in response to antidromically or intrasomatically evoked Na⁺ action potentials also were confined to the cell body. These observations are in agreement with other evidence that Na⁺ spikes are generated in the somatic region of the Purkinje neuron and spread passively into the dendrites. Plateau potentials, evoked by depolarizing pulses to the soma or dendrites, caused [Na⁺], changes only in the soma, indicating that the nonactivating Na⁺ channels contributing to this potential also were concentrated in this region. The climbing fiber-activated [Na⁺], changes were blocked by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione, indicating that these changes were not due to direct stimulation of the Purkinje neuron or activation of metabotropic receptors. Direct depolarization of the soma or dendrites never caused dendritic [Na⁺], increases, suggesting that the climbing fiber-activated [Na⁺], changes in the dendrites are due to Na⁺ entry through ligand-gated channels. A climbing fiber-like regenerative potential could be recorded in the soma after anode break stimulation, parallel fiber activation, or depolarizing pulses to the soma. The [Na⁺], changes evoked by all of these potentials were confined to the cell body region of the Purkinje cell. [Ca²⁺], changes in the dendrites evoked by the anode break potential were small relative to climbing fiber-activated changes, suggesting that a Ca²⁺ spike was not evoked by this response. The anode break and directly responses were blocked by tetrodotoxin. These results suggest that the somatically recorded climbing fiber response is predominantly a Na⁺-dependent event, consisting of a few fast action potentials and a slower regenerative response activating the same channels as the Na⁺ plateau potential.

INTRODUCTION

A detailed understanding of synaptic integration and plasticity requires a knowledge of how voltage and ligand-gated conductances are distributed throughout the neuronal arborization. The analysis of these mechanisms has become more complicated after the general recognition that different kinds of voltage-sensitive conductances can be found in the dendrites as well as the soma and axon (Llinás 1988). Most attention has been directed toward Ca²⁺-dependent mechanisms because of their critical role in the induction of synaptic plasticity and because they have been relatively easier to study. However, in some neurons, dendrites can fire Na⁺ action potentials (Wong et al. 1979) and possess subthreshold Na⁺ conductances that may amplify synaptic potentials (Stafstrom et al. 1985). Further, some reports have suggested that [Na⁺], changes might be important in the induction of long-term depression (LTD) (Linden et al. 1993). To assess the significance of these conductances and concentration changes, the location and properties of different Na⁺-dependent events need to be investigated in each cell type.

One part of this problem now appears to be answered—the site of Na⁺ spike generation in Purkinje cells. This issue is of special importance because this action potential determines the output message of the neuron. When this site is in the dendrites, a more elaborate model of cellular information processing must replace the simpler view of integration as an event occurring at a single locus at the axon hillock (Adams 1992; Sofiky and Koch 1993). Although there has been some controversy about the location of spike initiation (Regenh et al. 1992), experiments using dual patch recordings on the soma and dendrites found that propagation of Na⁺ spikes was completely passive in the dendrites under all conditions and that the density of Na⁺ channels declined rapidly with distance from the soma (Stuart and Hauser 1994). From these data Stuart and Hauser concluded that the spikes must be generated in the soma and/or axon of the Purkinje cell. This result supports previous experiments using extracellular (Hounsgaard and Yamamoto 1979) and microelectrode recordings (Llinás and Sugimori 1980a,b). It also explains why Na⁺ spikes produce elevations in [Ca²⁺], and [Na⁺], only in the somatic region (Lasser-Ross and Ross 1992; Lev-Ram et al. 1992).

This paper examines three other issues regarding the nature and location of different Na⁺ conductances in Purkinje cells. The first question is where do [Na⁺], changes occur when the cell is activated synaptically? If ligand-gated Na⁺ entry can be separated from voltage-gated entry, then concentration changes due to entry through the receptor channels will indicate the site of active synapses and receptors. If these sites can be determined with precision, then it may be possible to analyze how different synaptic contact points facilitate or depress with repeated activity. A further point
concerns the role of $[\text{Na}^+]$, changes in the induction of LTD. Based on experiments using cultured neurons, Linden et al. (1993) suggested that an increase in $[\text{Na}^+]$, is a required cofactor for this process. Determining the sites and sources of synaptically activated $[\text{Na}^+]$, increases may help determine how this particular form of synaptic plasticity occurs.

The second issue concerns the spatial distribution of Na$^+$ entry associated with the nonactivating Na$^+$ conductance (Crill 1996). Linás and Sugimori (1980a) showed that there was a strong Na$^+$-dependent plateau potential that supported burst firing in Purkinje cells. Because tetrodotoxin (TTX) blocks both the plateau channels and the fast spike channels, it has been difficult to analyze this nonactivating conductance directly. Indeed, it is not clear yet whether this conductance results from a channel distinct from the fast inactivating channel (Westenbroek et al. 1989), a chemically modulated form of the fast channel (e.g., Ma et al. 1994), the same channel with different gating properties (Alzheimer et al. 1993), or perhaps the same channel activated in a voltage "window" where some of the fast channels remain activated (DeSchutter and Bower 1994). Only in the first case is there a possibility for the spatial distribution of the Na$^+$ plateau conductance to extend beyond the region where the fast spikes are active. The determination of this distribution is important because of the possibility that nonactivating Na$^+$ channels in the dendrites could participate in boosting parallel fiber (PF) excitatory postsynaptic potentials (EPSPs) toward the soma (Stuart and Sakmann 1995).

The last issue concerns the conductances responsible for the classic climbing fiber (CF) response recorded in the soma. This response consists of a few fast Na$^+$ spikes followed by a rounded, 15–20 ms wide potential. At various times, this potential has been ascribed to the synaptic potential or Ca$^{2+}$ spikes. Our analysis suggests that this event in the soma is due almost entirely to regenerative Na$^+$ potentials, both fast spikes and a potential due to activation of the plateau channels. Some of this later work has been published in abstract form (Callaway et al. 1995b).

**METHODS**

The procedures used in these experiments followed those previously published (Callaway et al. 1995a; Lasser-Ross and Ross 1992; Lasser-Ross et al. 1991; Lev-Ram et al. 1992; Linás and Sugimori 1980a). Briefly, 150- to 200-mm sagittal slices from the vermis of 250–350 g guinea pigs were prepared after sodium pentobarbital anesthesia and decapitation with a small animal guillotine. Submerged slices were mounted in a recording chamber superfused with standard Krebs solution (in mM): 124 NaCl, 5 KCl, 1.2 NaH$_2$PO$_4$, 2.4 CaCl$_2$, 1.3 MgSO$_4$, 26 NaHCO$_3$, and 10 glucose, equilibrated with 95% O$_2$–5% CO$_2$, pH 7.4. Temperature was controlled at 30–32°C. TTX (1 mM, Sigma Chemical, St. Louis, MO) or 6-cyano-7-nitroquinolinine-2,3-dione (CNQX, 20 mM NaCl) was added in some experiments to block Na$^+$ channels or synaptic responses. For sharp microelectrode recordings and dye injections, the electrode tips were filled with 6 mM of the sodium indicator sodium binding benzofuran isothioplate (SBFI, impermeant salts, Molecular Probes, Eugene, OR) dissolved in 200 mM KAc. Patch electrode solutions contained (in mM) 4 NaCl, 10 Na-Gluconate, 130 K-Gluconate, 4 Mg-ATP, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES; pH 7.2). This solution was supplemented with 100–500 mM ethylene glycol-bis(β-aminoethylether)-$N,N’,N’’$-tetraacetic acid (EGTA) and 2 mM SBFI for $[\text{Na}^+]$, measurements, or 250 mM fura-2 and no EGTA for $[\text{Ca}^{2+}]$, measurements. Both sharp electrodes and patch electrodes were pulled from 1.5 mm OD, fiber-filled capillary tubing (No. 1511-M, Friedrich and Dimmock, Millville, NJ) using a model P-97 puller (Sutter Instrument, Novato, CA). Extracellular stimulating electrodes were bipolar sharpened tungsten wires exposed from insulation at the tips. They were placed on the white matter for CF activation or on the molecular layer for PF activation. Electrical recording and stimulating procedures were standard (Callaway et al. 1995a). Experiments were performed on the stage of an inverted microscope (Olympus, IMT-2F) (Callaway et al. 1995a). All electrodes were mounted on the stage and controlled with hydraulic micromanipulators. In this way, the implements and electrode positions could be preserved while changing the field of view. Electrodes were placed in position by viewing the preparation through a dissecting microscope mounted above the stage of the inverted microscope.

High-speed fura-2 and SBFI fluorescence measurements using a cooled CCD camera were as described in Lasser-Ross et al. (1991) and Callaway et al. (1995a). Fluorescence was detected using a 20×0.75 NA dry objective (Nikon, Garden City, NY). To increase the accuracy of the measurements, the excitation intensity was improved by using an Opti-Quip (Highland Mills, NY) model 1600 power supply (ripple <0.02%) and a Hamamatsu L2481-01 75-W Mercury-Xenon arc lamp, which is more stable than ordinary bulbs. Excitation for either indicator dye was at 380 nm selected with a 15-nm-wide interference filter (Omega Optical, Brattleboro, VT). Typical intensity fluctuations were <0.3%. Bleaching and baseline drift were corrected by subtracting an identical measurement without stimulation. Optical changes were expressed as $\Delta F/F$ (change in fluorescence from resting levels divided by the resting fluorescence corrected for autofluorescence). The autofluorescence correction was made by making comparable measurements at locations in the slice away from the dyed cell and subtracting this measurement from the experimental ones. Changes in $[\text{Ca}^{2+}]$, or $[\text{Na}^+]$, are expected to be proportional to $\Delta F/F$ for small values of $\Delta F/F$ if the resting concentration of these ions is uniform. Homogeneity for resting $[\text{Ca}^{2+}]$, has been established (Tank et al. 1988); we assume uniformity for resting $[\text{Na}^+]$. No attempt was made to convert $\Delta F/F$ into true ion concentrations. Cell images in the figures were taken at resting potential using 380-nm excitation for both indicator dyes. The gray scale images of $\Delta F/F$ in the figures show the spatial distribution of the change in fluorescence between a time before stimulation (usually at resting potential) and the end of the stimulation period. $[\text{Ca}^{2+}]$, measurements are from a single trial. $[\text{Na}^+]$, measurements usually required signal averaging to produce clean records. The number of trials in each experiment are indicated in the figure legends. Recordings were made by tracking the dye-filled microelectrode through the slice. Seals and patch recordings were made with the blind technique of Blanton et al. (1989) using a Neurodata IR-283 amplifier (New York, NY) in the current-clamp mode. Cells obtained in this way usually were not on the surface, making it difficult to resolve the finest structures. In particular, we could not see the axon or the details of the fine dendrites.

**RESULTS**

Climbing fiber induced $[\text{Na}^+]$, changes

Previously (Lasser-Ross and Ross 1992) we reported that the CF response caused a small $[\text{Na}^+]$, change in the dendrites of Purkinje cells. However, the small size of the fluorescence signals prevented a detailed analysis of the spatial distribution or origin of these signals. In these new experiments, we usually used patch electrodes to load the cell with
higher concentrations of SBFI, enabling us to achieve larger fluorescence signals. Figure 1 shows the spatial distribution of the $[\text{Na}^+]$ changes and their time courses in selected dendritic locations in response to a train of CF stimuli. At the end of the train of 10 responses, the changes ($\Delta F/F$) were predominantly along the main dendrites, with comparatively smaller changes along the segment closest to the soma ($n = 10$). However, the time course of the $[\text{Na}^+]$ change was not the same at all locations. Along the main distal dendrites, $[\text{Na}^+]$ began rising from the first response and peaked at the time of the last response. In the fine dendrites, to the side of the main distal dendrites and in the proximal dendrite, the changes rose later and peaked later. This pattern is consistent with $\text{Na}^+$ entry along the main shaft and diffusion outward towards the finer dendrites. However, the resolution of our data cannot rule out some entry at locations away from the primary dendrites. Note that this pattern is completely different from the distribution of $[\text{Ca}^{2+}]$, changes evoked by the CF (e.g., Miyakawa et al. 1992, Fig. 3). These CF-induced $[\text{Ca}^{2+}]$ changes were highest in the fine dendrites, lower on the thicker dendrites, and lowest in the soma—a pattern consistent with a spatially uniform $\text{Ca}^{2+}$ entry in the dendrites, with concentration changes determined largely by the relative surface to volume ratio in the different regions.

The pattern of dendritic $[\text{Na}^+]$ changes is consistent with $\text{Na}^+$ entry through ligand-gated channels opened by the CF neurotransmitter because anatomic investigations have placed the CF contacts on the large dendritic branches (Larramendi and Victor 1967; Palay and Chan-Palay 1974). CNQX, a known blocker of the CF synaptic potential (Konnerth et al. 1990; Perkel et al. 1990), completely blocked the CF-induced $[\text{Na}^+]$ changes (data not shown). This experiment rules out $[\text{Na}^+]$ increases caused by direct stimulation of the Purkinje cell or increases as a consequence of metabotropic receptor activation. By itself, it does not rule out a contribution from voltage-dependent $\text{Na}^+$ channels in the dendrites because the CF EPSP could open these channels if they were present. However, the possibility of CF induced voltage-dependent $[\text{Na}^+]$ entry in the dendrites is unlikely because direct depolarization of the somas (Lasser-Ross and Ross 1992) or the dendrites (Fig. 5) caused no dendritic $[\text{Na}^+]$ increase.

The CF response also caused a large $[\text{Na}^+]$, increase in the somatic region where there are no synaptic contacts. Some of this increase may have come from the $\text{Na}^+$ spikes activated by the CF response. To test this possibility, we measured the $[\text{Na}^+]$ change resulting from five CF responses and a train of 40 antidromic action potentials on the same sweep (Fig. 2). Consistent with previous results (Lasser-Ross and Ross 1992), the $[\text{Na}^+]$ changes from the antidromic action potentials were entirely confined to the soma. The antidromically activated $[\text{Na}^+]$ changes were entirely due to the $\text{Na}^+$ spikes because there was no change in this signal amplitude when 10 $\mu$M CNQX was added to the bath (data not shown). Although each CF response evoked 2 fast action potentials, making a total of 10 from all the CF responses, the amplitude of the $[\text{Na}^+]$ change from the CF responses was almost the same as from the train of antidromic spikes. Therefore the CF-induced $[\text{Na}^+]$ increase was more than three times the expected amplitude from the spikes alone. The remainder of the somatic $[\text{Na}^+]$ increase probably came from the slow part of the CF response, through the noninactivating $\text{Na}^+$ channels described below.

Parallel fiber induced $[\text{Na}^+]$, changes

Parallel fiber stimulation directly over a dendritic branch caused a small, very localized increase in $[\text{Na}^+]$, at the point of stimulation when the resulting EPSP was subthreshold (Fig. 3, left). With stronger stimulation (Fig. 3, right), the dendritic $[\text{Na}^+]$ increase was larger and extended over a
wider area (n = 4). The increase rose faster and peaked earlier at the center of the spot of elevated [Na\(^+\)]. The slower time course and lower amplitude at the edges of the spot is consistent with diffusion to the sides from a central point of Na\(^+\) entry. However, as with the CF response, we cannot rule out some contribution resulting from direct entry at the periphery.

The focal distribution of [Na\(^+\)], increases in these experiments is consistent with entry through ligand-gated channels. To further test this conclusion, we repeated this experiment in the presence of 20 mM CNQX to block the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that underlie the PF EPSP (Konnerth et al. 1990; Perkel et al. 1990). With the same stimulus intensity, the PF-evoked [Na\(^+\)], changes were eliminated completely (data not shown). As with the CF-evoked [Na\(^+\)], changes, this experiment rules out a contribution from direct stimulation of the dendrites or as a result of metabotropic receptor activation.

When the PF EPSPs were suprathreshold, an additional [Na\(^+\)], increase was detected in the soma. This signal, while present in the data of Fig. 3, is difficult to see in the grey scale image. However, in other cells (e.g., Fig. 4) this component was much clearer. The somatic signal reasonably is assigned to the Na\(^+\) spikes and possibly to the Na\(^+\) plateau potential (see below). There was no signal in the region between the stimulation zone in the distal dendrites and the soma, indicating that dendritic Na\(^+\) channels play no role in boosting the EPSP towards the soma (n = 3). A signal from regenerative sodium spikes should be detectable because the same apparatus measured sodium spike transients in thinner hippocampal pyramidal cell dendrites (Jaffe et al. 1992). With stronger stimulation, the EPSP often evoked a larger regenerative potential in the soma that resembled the CF response. The same pattern of two spots of Na\(^+\) entry was observed. This response was not due to activation of the climbing fiber by the stimulating electrode because the pattern of dendritic [Na\(^+\)], changes was completely different from that observed when the CF was activated from an electrode over the white matter (Fig. 1). Rather, this experiment shows that this intrinsic regenerative response also can be evoked by PF input if the EPSP is large enough.

**Sodium plateau potential**

It was not possible to study the Na\(^+\)-dependent plateau potential in isolation because any depolarization sufficient to induce this potential always caused a burst of fast Na\(^+\) spikes. Also, they were both equally sensitive to TTX. However, strong depolarizing pulses usually evoked a short burst of action potentials that rapidly inactivated, leaving just the plateau potential for the remainder of the pulse. Figure 5 shows a cell filled with SBFI from a patch electrode on a dendrite \(\sim 60 \text{ mm} \) from the cell body (arrow). When the cell was stimulated with a depolarizing pulse, [Na\(^+\)], rose throughout the duration of the pulse in the soma and did not change in the dendrites. This plateau signal was eliminated completely in TTX (not shown). Similar results were found when SBFI was loaded from patch or sharp electrodes on the soma (n = 11). We conclude that the channels causing the plateau associated [Na\(^+\)], change, like the channels causing the spike related [Na\(^+\)], increase, are not found in significant density in the dendrites. Whether the same channels are responsible for both [Na\(^+\)], changes is still to be determined.

**Ionic basis of the climbing fiber response**

These experiments provide a framework for reevaluating the nature of the somatically recorded climbing fiber electrical response. Because of the complex interaction of Na\(^+\) and Ca\(^{2+}\) conductances in the Purkinje cell, dissection of this response has been challenging. Direct pharmacological experiments are difficult because blockade of either of these conductances will prevent the response completely by blocking presynaptic action potentials or transmitter release. However, indirect approaches have been more rewarding.

Analysis of the effects of inhibition on CF-evoked [Ca\(^{2+}\)], changes (Callaway et al. 1995a) showed that most of the [Ca\(^{2+}\)], change was caused by a fast Ca\(^{2+}\) spike in the dendrites; blocking this spike caused little change in the somatically recorded electrical CF response. This indicates that fast Ca\(^{2+}\) spiking is not important in generating this somatic potential.

Further evidence concerning this response was provided when we noticed that an almost identical electrical potential could be recorded in the soma after anode break stimulation. Figure 6 shows fluorescence and electrical responses to CF and anode break stimulation in a cell filled with fura-2. The *insets* show the close resemblance between the two potentials. The main difference is that the CF response rose faster, probably because it is driven by a large, fast EPSP. The CF-induced [Ca\(^{2+}\)], changes were large and widespread in the dendrites. The anode break induced [Ca\(^{2+}\)], changes were much smaller in the dendrites and more evenly distributed (n = 3). The spatial distribution of this response was
variable. In this cell, the \([\text{Ca}^{2+}]\) change was detected out to the tip of the dendrites. In other cells, the \([\text{Ca}^{2+}]\) changes were predominantly in the somatic region with little or no detectable changes in the distal dendrites. We also found that the anode break electrical response was smaller in dendritic recordings even though \(\text{Ca}^{2+}\) spikes recorded from these locations were larger than at other locations in the dendrites or soma (data not shown). This result suggests that the anode break response does not evoke a dendritic \(\text{Ca}^{2+}\) spike. However, we do not have sufficient numbers of these recordings or any dual patch recordings to make a rigorous statement about the decline in amplitude of the anode break response with distance from the cell body.

The anode break response was also detected in neurons containing SBFI (Fig. 7). Again, the anode break and CF electrical responses recorded in the soma were almost identical. However, the anode break induced \([\text{Na}^+]\) change was detected only in the soma \((n = 5)\). In this and all other cells tested, the anode break response was completely blocked by TTX. Together these experiments suggest that the CF response and the anode break response are predominantly \(\text{Na}^+\)-dependent events, consisting of a combination of fast spikes and a slower regenerative potential—all occurring in the soma. Whether \(\text{Ca}^{2+}\) conductances contribute in a significant way is not clear. Some \([\text{Ca}^{2+}]\) changes remained when the fast CF-induced \(\text{Ca}^{2+}\) spike was blocked by inhibition (Callaway et al. 1995a), and a similar small \([\text{Ca}^{2+}]\) change was detected after the anode break response (Fig. 6). However, the \(\text{Ca}^{2+}\) conductance responsible for these \([\text{Ca}^{2+}]\) changes may make only a small contribution to the regenerative potential.

**DISCUSSION**

**Na\(^+\) plateau potentials**

Our data contribute useful information to the classification of the channels underlying the plateau potential in Purkinje cells, but they do not settle the issue. We found that: neither the \([\text{Na}^+]\) increases associated with this plateau nor the \([\text{Na}^+]\) changes caused by \(\text{Na}^+\) spikes were detected in the dendrites and a large \([\text{Na}^+]\) increase was detected even when the cell was depolarized far enough to completely inactivate the fast spikes. The large signal at depolarized
potentials appears to rule out a significant contribution from a "window" current (active only in a small range of potentials where channels are partially activated but not completely inactivated). All the results are consistent with spike channels and plateau channels being different forms of the same Na⁺ channel but differing in their inactivation properties. These characteristics are close to those of Na⁺ channels in neurons from rat and cat sensorimotor cortex described by Alzheimer et al. (1993) and Crill (1996). They found that the fast and persistent forms of the Na⁺ current could be explained by a uniform population of Na⁺ channels that can switch individually between different gating modes. They also found that the two currents in the cortical neurons appeared to have different voltage activation curves, even

FIG. 5. [Na⁺]ᵢ changes evoked by a 3-s-long depolarizing pulse that evoked a few spikes and a long plateau response. Image shows cell filled with SBFI and stimulated with a patch electrode on dendrites (arrow; same cell as Fig. 1). Both time-dependent traces and gray scale image show that changes were detected only in somatic region. Because electrical recording was in dendrites, amplitude of Na⁺ spikes was small. Inset: depolarization evoked a short, ~20-ms-wide, regenerative response before settling into steady plateau potential. Optical data are an average of 10 sweeps.

FIG. 6. [Ca²⁺]ᵢ changes evoked by anode break stimulation are much smaller than changes caused by climbing fiber response. Optical records are from soma (gray trace) and 2 dendritic locations indicated by boxes in image. Insets: aligned to same absolute voltage, show that both stimuli evoked similar electrical responses as recorded with a patch electrode on soma. Both electrical responses reach approximately same peak potential.

FIG. 7. Climbing fiber responses cause [Na⁺]ᵢ increases all over Purkinje cell while anode break response caused an increase only in soma. Climbing fiber responses were evoked during hyperpolarizing pulse. Some of this [Na⁺]ᵢ increase was caused by hyperpolarizing pulse itself, probably by Na⁺ entry through an anomalous rectifier channel (Crepel and Penit-Soria 1986). Anode break-induced [Na⁺]ᵢ increase was larger than typical climbing fiber induced changes, probably because of long-lasting 2nd phase to plateau. Insets: both electrical responses, recorded with a sharp electrode in the soma, reached approximately same peak potential. Same cell and box locations as in Fig. 2. Optical data are an average of 20 sweeps.
though they resulted from the same channels (Brown et al. 1994). The characteristics of $[\text{Na}^+]$ changes we observed in the soma are compatible with $\text{Na}^+$ entry through similar kinds of channels.

It is important to note that the lack of plateau or spike related $[\text{Na}^+]$ changes in the dendrites does not prove that both potentials are caused by the same channels or even that they are colocalized. It is possible that spike-related $\text{Na}^+$ entry could come through noninactivating channels even if a plateau is not generated, as when the spikes were evoked antidromically. With this possibility the data are also consistent with inactivating channels predominantly in the axon hillock and noninactivating channels predominantly in the soma.

**Ligand-gated signals**

These experiments clearly demonstrate that measurable $[\text{Na}^+]$ changes can be produced by $\text{Na}^+$ entry through ligand-gated channels. This possibility was suggested previously (Lasser-Ross and Ross 1992). In those experiments, we did not have the spatial resolution or pharmacology to clearly support this hypothesis. In these new experiments PF-related signals were localized in the dendrites directly under the stimulating electrode. For CF activation, the $[\text{Na}^+]$ changes were predominantly along the main dendrites. Both locations correspond to the known sites of synaptic contact in Purkinje cells (Larramendi and Victor 1967; Palay and Chan-Palay 1974). Although these signals were small, they were detected with sufficient signal to noise ratio and spatial resolution to suggest that they might be used in experiments comparing aspects of the potentiation and depression of synaptic currents in different regions of the dendrites. Also, if local $[\text{Na}^+]$ increases are required for the induction of LTD (Linden et al. 1993), then these ligand gated changes are the only likely source.

For both PF- and CF-activated ligand-gated signals, there was evidence of diffusion from the putative site of $\text{Na}^+$ entry to nearby locations where the $[\text{Na}^+]$ concentration was lower. It is possible that some of this diffusion could be mediated by the indicator dye “shuttling” $\text{Na}^+$ from regions of high concentration to nearby regions of lower concentration, as has been suggested for $\text{Ca}^{2+}$ ions (e.g., Connor and Nikolakopoulou 1982). However, the diffusion rate for $\text{Na}^+$ in these cells (measured in the presence of the indicator SBFI) is much higher than the rate for $\text{Ca}^{2+}$ (measured in the presence of fura-2) in other cells (e.g., Callaway et al. 1993). This higher diffusion rate is consistent with diffusion rates measured with other techniques without added buffers (e.g., Hodgkin and Keynes 1956). Therefore it is likely that diffusion of $\text{Na}^+$ is significant when there is no indicator in the cell. Rapid diffusion should help to maintain $[\text{Na}^+]$, close to resting levels at the synapse and other sites of localized $\text{Na}^+$ entry.

**Climbing fiber response**

These experiments showed that the CF-like wide regenerative electrical response, as recorded in the soma, could be evoked via CF activation, by anode break stimulation, and by PF stimulation. A similar potential also could be evoked with intrasomatic depolarization (inset, Fig. 5). The fact that this response could be evoked through many pathways establishes that it is an intrinsic regenerative potential in the Purkinje cell and not dependent on the unique EPSP of the CF synapse. These and previous experiments (Callaway et al. 1995a) indicate that this response is not due to a $\text{Ca}^{2+}$ spike, is blocked by TTX, and is largest in the cell body. Because both the inactivating and noninactivating $\text{Na}^+$ channels are located in the cell body, it is likely that this potential is a somatically localized regenerative event. However, the small and widespread $[\text{Ca}^{2+}]$, increase associated with this response suggests that $\text{Ca}^{2+}$ channels also contribute to some extent.

The all-or-none character of the somatic CF response helps insulate the Purkinje cell output from variations in the strength of the CF synaptic current due to repetitive activation (Konnerth et al. 1990; Perkel et al. 1990) or other factors. In particular, as we have shown previously, stellate cell inhibition (Callaway et al. 1995a) or activation of an A-like $K^+$ current (Midtgaard et al. 1993) have little affect on the somatically recorded CF response. In contrast, both the A-current and dendritic inhibition can dramatically affect potentials and $[\text{Ca}^{2+}]$, changes in the dendrites.

We thank N. Lasser-Ross for discussions and help in some of the experiments.

This work was supported in part by grants from the Human Frontier Science Program and the National Science Foundation (IBN-9209784) and a National Research Service Award postdoctoral fellowship (NS09257) to J. C. Callaway.

Present address and address for reprint requests: J. C. Callaway, Dept. of Anatomy and Neurobiology, University of Tennessee at Memphis, 665 Monroe Ave., Memphis, TN 38163.

Received 22 May 1996; accepted in final form 6 September 1996.

REFERENCES


DeSchutter, E. and Bower, J. M. An active membrane model of the


