We characterized the properties and functional roles of voltage-dependent potassium channels in the dendrites of Purkinje neurons studied in rat cerebellar slices. Using outside-out patches formed ≤250 μm away from the soma, we found that depolarization-activated potassium channels were present at high density throughout the dendritic tree. Currents required relatively large depolarizations for activation (midpoint, approximately −10 mV), had rapid activation and deactivation kinetics, and inactivated partially (20–70% over 200 msec) with both fast (time constant, 15–20 msec) and slow (300–400 msec) components. Inactivating and noninactivating components were both blocked potently by external tetraethylammonium (half-block by 150 μM) and 4-aminopyridine (half-block by 110 μM). The voltage dependence, kinetics, and pharmacology suggest a predominant contribution by Kv3 family subunits, and immunocytochemical experiments showed staining for both Kv3.3 and Kv3.4 subunits in the dendritic tree. In the proximal dendrite, potassium channels were activated by passively spread sodium spikes recorded at the same position, and experiments using dual recordings showed that the channels serve to actively dampen back-propagation of somatic sodium spikes. In more distal dendrites, potassium currents were activated by voltage waveforms taken from climbing fiber responses, suggesting that they help shape these responses as well. The requirement for large depolarizations allows dendritic Kv3 channels to shape large depolarizing events while not disrupting spatial and temporal summation of smaller excitatory postsynaptic potentials.

Key words: Kv3; Kv3.3; Kv3.4; tetraethylammonium; 4-aminopyridine; climbing fiber; Purkinje cell

Introduction

A growing body of experimental data has shown the presence of voltage-dependent ion channels in the dendrites of neurons (for review, see Johnston et al., 1996; Spruston et al., 1999; Häusser et al., 2000). In some neurons, notably hippocampal pyramidal neurons and layer 5 cortical pyramidal neurons, detailed studies have been made of dendritic sodium channels (Colbert et al., 1997; Jung et al., 1997), calcium channels (Christie et al., 1995; Oakley et al., 2001), and potassium channels (Hoffman et al., 1997; Golding et al., 1999; Bekkers, 2000; Johnston et al., 2000; Korngreen and Sakmann, 2000), and the properties of the channels have been linked with physiological functions, especially in controlling back-propagation of action potentials (Colbert et al., 1997; Jung et al., 1997; Williams and Stuart, 2000; Golding et al., 2001; Stuart and Häusser, 2001; Vetter et al., 2001; Watanabe et al., 2002).

The dendrites of cerebellar Purkinje neurons have active membrane properties (Llinás and Sugimori, 1980; Midtgaard et al., 1993; Callaway and Ross, 1997; Pouille et al., 2000; Womack and Khodakhah, 2002b). However, Purkinje neurons are strikingly different from other projection neurons studied so far in that their dendrites have a much lower density of sodium channels, and fast somatic action potentials fail to propagate effectively into the dendrites (Stuart and Häusser, 1994; Roth and Häusser, 2001; Vetter et al., 2001). Instead, the regenerative electrical activity in distal Purkinje cell dendrites takes the form of slow calcium-dependent action potentials and long-lasting plateaus (Llinás and Sugimori, 1980; Pouille et al., 2000). Correspondingly, patch-clamp recordings and immunocytochemistry have shown the presence of multiple types of calcium channels in Purkinje cell dendrites (Usowicz et al., 1992; Westenbroek et al., 1995; Yokoyama et al., 1995; Mouginot et al., 1997).

Very little is known about potassium channels in Purkinje neuron dendrites. Current-clamp recordings from dendrites show strong outward rectification, consistent with the presence of large depolarization-activated potassium conductances (Etzion and Grossman, 1998, 2001). Cell-attached patch recordings from the dendrites of cultured Purkinje neurons show the presence of voltage-dependent potassium channels with multiple single-channel conductance levels (Gruol et al., 1989, 1991), but little is known about their distribution, macroscopic kinetics, molecular basis, or functional roles. We used a combined electrophysiological and immunocytochemical approach to characterize the potassium channels in Purkinje cell dendrites, and we explored their functional roles by examining their activation by
different waveforms of dendritic depolarization arising from either synaptic input or somatic spikes.

Materials and Methods

Parasagittal slices of 300 μm thickness were cut from the cerebella of Long–Evans rats using a vibrating tissue slicer (Ted Pella, Redding, CA). Rats (14–21 d of age) were anesthetized by methoxyflurane before decapitation and removal of the cerebellum. All procedures involving animals were approved by the Harvard Medical Area Standing Committee on Animals. After cutting, slices were incubated at 35°C for 20 min and then stored at room temperature.

During recording, slices were continuously superfused with physiological extracellular solution containing (in mM): 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, and 25 glucose, bubbled with 95% O2 and 5% CO2. Slices were visualized with an Olympus BX50WI (Olympus Optical, Tokyo, Japan) upright microscope using infrared differential interference contrast videomicroscopy (Stuart et al., 1993) with an immersion 60× objective.

Patch pipettes were pulled from borosilicate glass tubing (outer diameter, 1.65 mm; inner diameter, 0.75 mm; Dagan, Minneapolis, MN) and heat polished before use. Pipettes were filled with an internal solution consisting of (in mM): 140 KC1, 2 MgCl2, 10 EGTA, 2 Na2 ATP, 10 HEPES, pH adjusted to 7.3 with KOH. In some experiments, 30 mM KC1 was substituted with 30 mM KF, which enhanced seal formation and stabilized outside-out patches. Tip resistances in working solutions were 5–11 MΩ. The pipettes were brought close to the target while exerting positive pressure (30–60 millibar). This process helped in cleaning off glial cells that often wrap Purkinje cell dendrites. Purkinje cells were easily identified on the basis of their large size and distinctive morphology. Purkinje cells in the vermis were used for these experiments. Distance of the dendrite from which the patch was formed was measured from the center of the soma and off-line from pictures taken with a CCD camera and frame grabber (Scion, Frederick, MD).

We used outside-out patches for characterizing potassium currents because they allow recording of relatively large currents and also facilitate the application of different drugs to study the pharmacological profile of the channels. A potential disadvantage is that properties of channels might change because of changes in the phosphorylation state of the channels (although ATP was included in the internal solution to minimize such changes) or other consequences of dialysis. To evaluate this issue, in early exploratory experiments, we measured potassium currents from dendrites in the cell-attached configuration. It was not possible to use this configuration routinely, because most neurons were spontaneous and it was impossible to control the membrane voltage.

In a limited number of cells that did not show spontaneous firing, we determined activation curves in cell-attached patches using absolute voltages calculated after breaking through into the cell and measuring the resting potential. In seven cell-attached patches on dendrites, the voltage of half-maximal activation was −4.7 ± 2 mV and the slope factor was 21.6 mV/0.1 mV. The magnitude of the slope factor was determined from the peak current assuming ohmic behavior and a reversal potential of −95 mV. Activation and inactivation curves were fit with functions on the basis of the Boltzmann function, 1/(1 + exp[(V − V1)/k]), where V is the membrane potential, V1 is the potential at which the value of the Boltzmann function is 0.5, and k is the slope factor. Activation curves were fitted with a Boltzmann function raised to the fourth power. Inactivation curves were fitted with a Boltzmann function plus a constant. The curves shown in figures represent fits to the averaged data obtained from pooling all experiments together. The parameters reported in the text and Table 1 are the values obtained by averaging the results of independent fitting of individual experiments. In principle, this is a better procedure, because when data are averaged before plotting the curves, the apparent slope may be reduced if equally steep curves with slightly different midpoints are averaged. However, the differences between the two sets of parameters were minor.

Data are reported as mean ± SEM, and error bars in figures also represent SEM. SEMs of fit parameters were obtained by fitting data of individual experiments separately.

Immunocytochemistry. Postnatal 15–16-d-old Long–Evans rats were anesthetized with isoflurane and perfused through the left ventricle with 30 ml of ice-cold saline followed by 200 ml of 4% paraformaldehyde, 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. The brains were immersed in the same fixative for 4°C for 16 hr and then transferred to 30% sucrose in sodium phosphate buffer for 2–3 d. Twenty-five micro-meter thick sagittal sections were cut with a cryostat, mounted on glass slides, and then air-dried and rehydrated in 0.1 M phosphate buffer, pH 7.4, for at least 40 min. The sections were then treated for 10 min in 0.1 M PBS, pH 7.4, containing 0.3% hydrogen peroxide (Sigma, St. Louis, MO) and 0.3% normal goat serum (Sigma) at room temperature and then rinsed with TNT (TNT plus 1% goat serum; 20–22°C for 1 hr) to minimize unspecific binding and then incubated overnight at 4°C with primary diluted in TNT. Dilutions were 1:1,000 for mouse anti-calbindin (Sigma), 1:100 for rabbit anti-Kv3.4 (Alomone Labs, Jerusalem, Israel), and 1:300 for rabbit anti-Kv3.3 (kind gift from Lisa Taylor and Dr. Teresa Perney). Afterward, sections were rinsed in TNT (three times for 10 min each) and then incubated with the

### Table 1. Comparison of potassium currents in outside-out patches from dendrites and soma of cerebellar Purkinje neurons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dendrite</th>
<th>Soma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midpoint</td>
<td>−10 ± 2 mV (n = 16)</td>
<td>−12 ± 2 mV (n = 18)</td>
</tr>
<tr>
<td>k/4</td>
<td>5.8 ± 0.3 mV (n = 16)</td>
<td>4.5 ± 0.3 mV (n = 18)</td>
</tr>
<tr>
<td>τ (0 mV)</td>
<td>3.1 ± 0.4 msec (n = 16)</td>
<td>4.3 ± 0.5 msec (n = 18)</td>
</tr>
<tr>
<td>τ (20 mV)</td>
<td>2 ± 0.1 msec (n = 16)</td>
<td>1.9 ± 0.2 msec (n = 18)</td>
</tr>
<tr>
<td>Deactivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>τ (−70 mV)</td>
<td>1.1 ± 0.2 msec (n = 4)</td>
<td>0.8 ± 0.2 msec (n = 7)</td>
</tr>
<tr>
<td>τ (−40 mV)</td>
<td>2.8 ± 0.9 msec (n = 4)</td>
<td>2.8 ± 0.4 msec (n = 7)</td>
</tr>
<tr>
<td>Inactivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V0</td>
<td>−37.6 ± 4.5 mV (n = 6)</td>
<td>−38.8 ± 4.3 mV (n = 7)</td>
</tr>
<tr>
<td>k</td>
<td>12 ± 0.6 mV (n = 16)</td>
<td>15.6 ± 1.2 mV (n = 17)</td>
</tr>
<tr>
<td>τ fast</td>
<td>18.7 ± 3.3 msec (n = 26)</td>
<td>14 ± 2.5 msec (n = 15)</td>
</tr>
<tr>
<td>Noninactivating (100 msec)</td>
<td>55 ± 2% (n = 28)</td>
<td>75 ± 2% (n = 28)</td>
</tr>
<tr>
<td>Block by 1 mM TEA</td>
<td>76 ± 4% (n = 10)</td>
<td>79 ± 3% (n = 9)</td>
</tr>
</tbody>
</table>

All data are from outside-out patches except the data for inactivation of somatic currents, which are from outside-out nucleated patches. The voltage dependence of the inactivation curve was determined using a 5 sec prepulse. For dendrites, data were confined to patches at a distance from soma of at least 100 μm for activation and inactivation and at least 70 μm for TEA block. Parameters reported in this table were obtained by averaging values from fitting of individual experiments.
Results

Voltage dependence and kinetics of dendritic potassium current

We characterized the voltage dependence and kinetics of voltage-activated potassium current in outside-out patch recordings taken from Purkinje cell dendrites ≤250 μm from the soma. All patches had detectable voltage-activated potassium current, and most had currents of several hundred picoamperes. Figure 1A shows a typical family of currents recorded from a patch taken from a Purkinje cell dendrite 140 μm from the soma. Figure 1B shows peak conductance as a function of voltage for collected data from 16 patches formed at dendritic locations >100 μm from the soma. Currents required relatively strong depolarizations to activate, with half-maximal activation at \(-10 \pm 2\) mV \((n = 16)\). The conductance versus voltage curve was well fit by a Boltzmann function raised to the fourth power, with a slope factor of 23 ± 1 mV.

Activation was rapid (Fig. 1C), with a 10–90% rise-time of 3.1 ± 0.4 msec at 0 mV \((n = 16)\) (Fig. 1D). Activation kinetics were strongly voltage dependent, and rise-times decreased to near 1 msec for depolarizations positive to 40 mV. Deactivation of the current was also very fast (Fig. 2). Deactivation could generally be fit well by a single exponential, with average time constants of 2.8 ± 0.9 msec at −40 mV and 1.1 ± 0.2 msec at −70 mV \((n = 4)\).

Dendritic potassium currents showed partial inactivation, typically decaying by 30–50% over 200 msec for voltage steps positive to 0 mV. The time course of decay could generally be fit well by two exponential functions (Fig. 3A). Measured for maximal activation by steps to 70 mV, the faster time constant had an average value of 19 ± 3 msec \((n = 26)\) and the slower time constant was 377 ± 131 msec. On average, the fast inactivating component contributed 24 ± 4% of the total peak current, the slow inactivating component contributed 31 ± 6%, and 45 ± 5% remained after 200 msec. Figure 3, B and C, shows the steady-state voltage dependence of inactivation, determined by changing the holding potential for 5 sec before a 100 msec test pulse to 70 mV. A notable feature is that inactivation becomes substantial only when depolarizations reach voltages at which significant current is activated (positive to −40 mV). This property differentiates these currents from A-type potassium currents mediated either by Kv4 or Kv1.4 (Pak et al., 1991; Retting et al., 1992) channels, which inactivate strongly at subthreshold voltages.

When the voltage dependence of the inactivation of dendritic currents by a 5 sec prepulse was fit with a Boltzmann function plus a constant, the average midpoint was −37.6 ± 4.5 mV, and the slope factor was 12 ± 0.6 mV \((n = 6)\). Inactivation was nearly complete with depolarizations up to 10 mV (Fig. 3G, open circles). The midpoint of inactivation determined using 100 msec prepulses was −33.3 ± 3 mV, and the slope factor was 11.3 ± 3.4 mV, with an average fraction of 38 ± 6% \((n = 10)\) remaining noninacti-
Parameters are for fits to the averaged data. Table 1 shows the values for $V_h$ distal dendrites. Overall, currents in somatic patches (Fig. 4) were taken at positions varying from the soma itself to the cell body. Solid curves are Boltzmann functions raised to the fourth power, with a midpoint of $95 \text{ mV}$ and a linear current–voltage curve for $90 \text{ mV}$ to $70 \text{ mV}$ (10 mV intervals). B, Peak conductance–voltage relationship for somatic patches (mean ± SEM; 18 cells). Conductance was calculated assuming a reversal potential of $−95 \text{ mV}$ and a linear current–voltage curve for open channels. Solid curve is a Boltzmann function raised to the fourth power, with a midpoint potential of $−11.5 \text{ mV}$ and slope factor 18 mV. C, Magnitude of peak potassium current (step from $−90$ to $70 \text{ mV}$) in outside-out patches, plotted as a function of distance from the soma from which the patch was formed (closed circles). To facilitate the comparison, only data obtained with 7–11 MΩ pipettes are plotted. The white symbol indicates mean ± SEM for patches from the cell body with the same range of electrode resistances. The solid line is least-squares to the data and has an x-intercept of 652 pA and slope of $−2.3 \text{ pA/μm}$. D, The ratio of the current remaining after 100 msec to the peak current is plotted versus distance from soma (closed circles). Steps were to 70 mV. The white symbol indicates mean ± SEM for patches from the cell body. Solid line is least-squares to the data and has an x-intercept of 0.77 and slope of $−0.0016 \text{ μm}^{-1}$.

There was also a trend evident in the degree of inactivation with distance from the soma (Fig. 4D), with inactivation being more pronounced with increasing distance from the soma. For depolarizations to 70 mV, the ratio of noninactivating current (measured after 100 msec) to peak current decreased from 0.75 ± 0.02 ($n = 28$) for somatic patches to $0.67 ± 0.02$ ($n = 71$) for dendrites $≤100 \text{ μm}$ from the soma and to $0.55 ± 0.02$ ($n = 28$) for distal dendrites ($>100 \text{ μm}$ from the soma). Interestingly, although somatic currents on average inactivated less completely during 100 msec or 200 msec pulses, the value of the fast inactivation time constant was similar to that of dendritic patches. Moreover, the parameters of the steady-state inactivation curve obtained using a 5 sec prepulse were very similar for somatic and dendritic currents (Table 1).

**Sensitivity to TEA and 4-aminopyridine**

The potassium currents in dendritic membranes were highly sensitive to block by external TEA (Fig. 5A). At 0.1 mM, TEA blocked the current by $46 ± 4\%$, and 1 mM TEA blocked $76 ± 4\%$ of the current. The dose–response curve (Fig. 5B) suggested that $≈80\%$ of the total potassium current is highly sensitive to TEA with a half-blocking concentration of $≈100 \text{ μM}$ for this component. There also appears to be a component of current ($≈20\%$ of the whole) with much lower sensitivity to TEA, being only partly blocked by 10 mM.

It has been found previously that somatic potassium currents in mouse Purkinje neurons are effectively blocked by external TEA (Raman and Bean, 1999; Southan and Robertson, 2000).
The high sensitivity to 4-AP of the potassium current in the dendritic membrane of Purkinje cells requires relatively large depolarizations to be activated and has rapid activation and deactivation kinetics as well as high sensitivity to both external TEA and 4-AP. These properties are all characteristic of members of the Kv3 family (Martina et al., 1998; Rudy and McBain, 2001; Lien et al., 2002). We therefore used immunocytochemistry to test whether particular members of the Kv3 family of subunits are present in the dendrites of Purkinje neurons. We found clear staining for both Kv3.3 and Kv3.4 subunits (Fig. 6). With antibodies to both subunits, staining was present both in cell bodies and throughout the dendritic tree. With Kv3.3 antibodies, there was prominent staining of both cell bodies and dendrites (Fig. 6A–C), suggesting that Kv3.3 subunits are highly expressed in both compartments. Staining of cell bodies and proximal dendrites was especially strong. The same results were obtained in four different slices from three different animals. Antibodies to Kv3.4 subunits also stained both cell bodies and dendrites (Fig. 6D–G); however, staining generally appeared stronger in the dendrites than in the cell bodies, and there were often segments of distal dendrites that showed very strong staining (Fig. 6H, arrow). The same results were obtained in six different slices from three different animals.

**Activation by action potentials and synaptic potentials**

Under what conditions are dendritic potassium channels activated, and what is their functional role? To explore these questions, we made current-clamp recordings of the voltage trajectory of dendritic membrane under various circumstances and then performed voltage-clamp experiments to examine whether those voltage patterns activated dendritic potassium currents in outside-out patches, often formed using the same pipette used to make the current-clamp recordings. Purkinje neurons often fire spontaneous action potentials in a highly regular manner (Häusser and Clark, 1997), and in cerebellar slices, spontaneous firing becomes faster and more regular when ongoing basal synaptic activity is blocked (Häusser and Clark, 1997). Figure 7A shows how the action potential height of spontaneous action potentials changed with distance from the soma in our experiments. The inset in Figure 7A shows a typical example in which simultaneous current-clamp recordings were made in the cell body and dendrite (in this case, at a distance of 60 μm). The spontaneous spikes in the cell body had amplitude of 56 mV, whereas those measured in the dendrite 60 μm away had an amplitude of 37 mV. Collected results are shown in Figure 7A; the decline with a distance of spontaneous action potentials is very similar to that observed previously by Stuart and Häusser (1994) and Pouille et al. (2000) for action potentials elicited by current injection. The data show that within ~70–80 μm of the soma, spontaneous spikes commonly have peaks positive to ~40 mV, the voltage at which activation of dendritic potassium current becomes substantial. Thus, at least for the proximal dendrite, there is the potential for dendritic potassium channels to be activated during spontaneous action potentials. However, it is also necessary to establish that activation of the dendritic channels is sufficiently rapid to be activated during the action potential in the dendrite, especially because action potentials in Purkinje neurons are very narrow (0.53 ± 0.02 msec; half-height width; measured in the soma; 23°C; n = 14 cells). The experiment in Figure 7B shows directly that dendritic potassium channels are activated by the action potentials they experience. In this experiment, the recording pipette was first used to make a

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**Figure 5.** Pharmacology of dendritic potassium currents. A, Effects of increasing concentrations of external TEA on currents evoked by 200 msec pulses from −90 to 70 mV in a dendritic patch (excised 50 microns from soma). B, Dose–response curve for block of dendritic current by external TEA. The solid curve represents fit by the logistic equation to the data up to 3 mM TEA, 0.8/[1 + ([TEA]/K)_d] + 0.2, with K_d = 85 μM. C, Effect of 4-AP on currents from a dendritic outside-out patch (excised 75 microns from soma). D, Dose–response curve for block of dendritic current by 4-AP. The solid curve represents fit by the logistic equation with variable slope (Hill coefficient) given by 0.81/[1 + ([4-AP]/K)_d] + 0.19, with K_d = 86 μM and n = 1.7.
current-clamp recording from the dendrite (at 35 μm from the soma), and spontaneous spikes with amplitude of ~35 mV (occurring at a frequency of ~50 Hz) were recorded (top trace). The dendritic pipette was then detached to form an outside-out patch, and we tested whether the previously recorded segment of spontaneous activity used as command voltage could activate the potassium channels in the patch. There was substantial activation during the spikes. In this patch, peak current activated by a step to 70 mV was 170 pA; thus, expressed in terms of conductance and assuming a reversal potential of 95 mV, the current of 5 pA elicited at the peaks of the dendritic action potentials (10 mV) represents ~6% of the maximal conductance when the channels are fully activated. In three experiments with patches taken from distances between 35 and 60 μm from the soma, dendritic spikes activated 8 ± 3% of the maximal potassium conductance in the patch.

Activation of potassium current by the spikes in the proximal dendrite would be predicted to actively dampen the dendritic spike beyond the truncation from the passive properties of the dendrite. The high sensitivity of the dendritic potassium channels to external TEA provides a way of testing this prediction. Figure 8 shows such an experiment in which a prerecorded waveform taken from current-clamp stimulation of a Purkinje neuron cell body was applied as a voltage-clamp command at the cell body. A second electrode recorded the voltage response at a dendritic location 35 μm away with 300 nM TTX present in the external solution to prevent spontaneous firing of the neuron and avoid eliciting uncontrolled sodium currents in the cell body. As expected, the voltage recorded at the dendrite (height, 35 mV) was much smaller than that at the cell body (94 mV). When 1 mM TEA was added to the external solution, the spikes measured at the dendrite became larger and also broader. This suggests that dendritic potassium channels have the effect of both truncating and narrowing the dendritic spike, at least in the proximal dendrite. In collected data from three cells, the half-duration of dendritic

Figure 6. Immunostaining for Kv3.3 and Kv3.4 subunits in somata and dendrites of Purkinje neurons. A, Confocal image of Purkinje cell layer after staining with antibodies to Kv3.3 (red). B, Same slice stained using primary antibodies to calbindin (green). C, Merged image. D, Higher magnification merged image of staining by Kv3.3 (red) and calbindin (green). E–G, Staining with antibodies to Kv3.4 (E, red), calbindin (F, green), and merged image (G). H, Higher magnification merged image of staining by Kv3.3 (red) and calbindin (green). Note the colocalization of the antibodies in many regions of distal dendrites (including that marked by an arrow). For both Kv3.3 and Kv3.4 staining, controls were run by staining adjacent sections and processing them in parallel except that the antibodies were preincubated with 100-fold excess of antigenic peptide; there was only very faint nonspecific staining of cell body cytoplasm present in the controls.

Figure 7. Activation of dendritic potassium current by truncated dendritic spikes. A, Peak amplitude of dendritic action potentials (from spontaneous firing of neurons with no injected current) plotted against distance from soma. The dashed line represents the average voltage at which dendritic K⁺ currents activate by ~10%. Inset, voltage traces of spontaneous activity obtained recording simultaneously from soma and dendrite of a Purkinje neuron. B, Voltage traces recorded from another dendrite (top); the recordings used as voltage command activated potassium currents in the outside-out patch obtained from the same dendrite.
spikes increased from 0.66 ± 0.03 msec in control to 0.87 ± 0.03 msec after block of potassium currents by 1 mM TEA. Purkinje neurons receive excitatory synaptic input from the inferior olive by means of climbing fibers, which form powerful 1:1 synaptic connections with Purkinje neurons. Stimulation of a single climbing fiber evokes a large, all-or-none EPSP on which multiple action potentials are superimposed, the "complex spike" (Eccles et al., 1966). Complex spikes probably represent complicated interaction of synaptic currents with intrinsic membrane properties of both dendrites and cell body (Llinás and Sugimori, 1980; Callaway and Ross, 1997; Pouille et al., 2000; Cavelier et al., 2002). The superimposed fast spikes originate in the soma (Stuart and Häusser, 1994) and are not evident in dendrites more distal than 70 μm or so. However, the underlying maintained depolarization can have a magnitude of 40–50 mV even in distal dendrites (Stuart and Häusser, 1994; Callaway and Ross, 1997). Figure 9 shows the response to climbing fiber stimulation measured in a dendrite 75 μm from the soma with the depolarization reaching nearly −30 mV. When the recording electrode was pulled away to form an outside-out patch and the patch was stimulated by the recorded waveform, there was a substantial potassium current (33 pA) elicited, which reached a peak a few milliseconds later than the peak of the voltage response. In this patch, the peak current activated by a step to 70 mV was 670 pA, and the current during the climbing fiber response corresponded to ~15% of the maximal available potassium conductance. This experiment makes it clear that substantial dendritic potassium currents are activated during climbing fiber responses and shows that they must influence the duration and shape of the response. This experiment was performed in the dendrites of five different neurons, at distances ranging from 45 to 85 μm times (four using the climbing fiber response of each cell as voltage command and one using the responses from another cell). The peak of the climbing fiber EPSP varied from −35 to −24 mV, and the average potassium conductance activated by the EPSP as waveform was 8 ± 4% of the conductance activated by a step from −90 to 70 mV.

**Discussion**

**Molecular composition of dendritic potassium channels**

The predominant potassium currents in outside-out patches from Purkinje cell dendrites had rapid activation and deactivation kinetics and high sensitivity to external TEA and 4-AP, consistent with Kv3 family potassium channels (Grissmer et al., 1994; Martina et al., 1998; Rudy and McBain, 2001; Lien et al., 2002). In concordance, the immunocytochemical experiments show the presence of Kv3.4 and Kv3.3 protein in both dendrites and somata of Purkinje neurons. Previously, Kv3 family channels were most commonly found localized in somata, axons, and presynaptic terminals of neurons (Rudy and McBain, 2001), but dendritic Kv3.3 subunits have been described in Purkinje cells of an apteronid fish (Rashid et al., 2001), consistent with our results. Our results showing prominent staining for Kv3.4 protein in Purkinje neurons differs from a previous study (Veh et al., 1995; Laube et al., 1996). The antibodies used for this previous work were raised against a region in the C terminus of the Kv3.4a protein that is ~25% different in the Kv3.4c splice variant (Veh et al., 1995; Vullhorst et al., 2001), whereas the antibodies that we used were raised to a region (residues 177–195 of the N-terminal region) that is conserved between Kv3.4a and Kv3.4c proteins. It is possible that Kv3.4c subunit expression is higher than that of Kv3.4a subunits or is more easily detected by antibodies.

Kv3.1 RNA is present at low levels of expression in Purkinje neurons (Weiser et al., 1994), and expression declines dramatically between P10 and P17 (M. K. Jarvinen, M. Fry, D. M. Porter,
and R. A. Maue, personal communication). Consistent with this, we found that antibodies to Kv3.1b protein produced no detectable staining of either Purkinje cell dendrites or somata in P15–P16 rats while robustly staining hippocampal neurons (G. L. Yao, unpublished data), consistent with previous results (Weiser et al., 1995).

Different Kv3 family subunits have different kinetic properties. Homomers of Kv3.3 subunits inactivate slowly (hundreds of milliseconds) (Rudy and McBain, 2001). Kv3.4a homomeric channels inactivate rapidly (tens of milliseconds) and completely (Rettig et al., 1992; DiChot et al., 1998; Rudy and McBain, 2001), and Kv3.4c channels inactivate more slowly and less completely than Kv3.4a channels (G. Baranauskas, T. Tkatch, and D. J. Surmeier, personal communication). Kv3 family heteromers have been shown to form in heterologous expression systems (Weiser et al., 1994; Rudy and McBain, 2001) and mediate currents with kinetic properties intermediate between the component subunits. Thus, the components of dendritic current with different inactivation kinetics might represent channels formed by different mixtures of Kv3.3, Kv3.4a, and Kv3.4c subunits, perhaps with other Kv3 family subunits as well.

Approximately 20% of the current in dendritic patches was left unblocked by 3 mM TEA or 3 mM 4-AP. Additional analysis will be required to identify the channels underlying this component. Sacco and Tempia (2002) have described a TEA-resistant A-type potassium current in whole-cell recording from mouse Purkinje neurons much younger (3–9 d old) than the rat Purkinje neurons we studied (14–21 d old); the TEA-resistant current comprised approximately one-third of the total in their experiments, suggesting developmental changes in contributions from different potassium channels (cf. Yool et al., 1988).

Comparison with somatic potassium channels

Channels in somatic patches had very similar voltage dependence, kinetics, and pharmacology as dendritic channels (Table 1). Our results for somatic potassium channels are very similar to those obtained previously in the somata of mouse Purkinje neurons (Raman and Bean, 1999; Southan and Robertson, 2000). Together, these results suggest that the predominant voltage-activated potassium channels in the cell bodies of Purkinje neurons are also of the Kv3 family, and our immunocytochemical results show staining of both Kv3.3 and Kv3.4 subunits in the somata as well as dendrites of Purkinje neurons.

Purkinje neurons are known to possess both SK-type and BK-type calcium-activated potassium channels (Yool et al., 1988; Gähwiler and Llano, 1989; Gruol et al., 1991; Jacquin and Gruol, 1999; Cingolani et al., 2002; Womack and Khodakhah, 2002a, 2003; Edgerton and Reinhart, 2003), and there is some evidence that these channels are expressed to at least some extent in dendrites (Gruol et al., 1991; Cingolani et al., 2002; Womack and Khodakhah, 2003). There was usually no sign of calcium-activated current in our recordings; calcium channel activity was apparently not maintained in the patches, because we never saw inward calcium currents. Only very seldom, and for strong depolarizations (V_m, >20 mV), did we observe individual openings of large conductance channels resembling the BK type of calcium-dependent potassium channels. The characterization of calcium-activated potassium channels in Purkinje cell dendrites may require different experimental approaches (Womack and Khodakhah, 2002a).

Functional significance

Unlike many other types of neurons, sodium-dependent action potentials are not effectively propagated in the dendrites of Purkinje neurons because dendritic sodium-channel density is low (Stuart and Häusser, 1994). It is therefore somewhat unexpected to find strong dendritic expression of Kv3 family potassium channels whose high threshold and rapid activation are most obviously suited to give rapid repolarization of large, narrow action potentials in fast-spiking cells. Despite the absence of backpropagating sodium spikes, the density of voltage-activated potassium current in Purkinje cell dendrites is similar or higher than in several cell types in which sodium action potentials do actively propagate into dendrites (Hoffman et al., 1997; Bekkers, 2000; Korngreen and Sakmann, 2000; Martina et al., 2000). Our results suggest that one functional role of these channels is to help quickly damp even the passive propagation of action potentials into the dendritic tree (cf. Roth and Häusser, 2001). Crucial for this function is the rapid activation kinetics of dendritic currents, even at voltages at the foot of the activation curve.

The inactivation properties of Kv3 channels of Purkinje cell dendrites differ considerably from the inactivation of potassium current in dendrites of pyramidal neurons, which is primarily attributable to Kv4 family channels (Maletic-Savatic et al., 1995; Hoffman et al., 1997; Hoffman and Johnston, 1998). The midpoint of inactivation for the inactivating component of dendritic channels in Purkinje neurons (~38 mV) is much more positive than that in dendrites of pyramidal neurons (~56 mV) (Hoffman and Johnston, 1998). The channels in pyramidal neuron dendrites would be almost completely inactivated at the more depolarized voltages typical of spontaneously firing Purkinje neurons, whereas the Kv3 channels are still available.

Our results with climbing fiber stimulation show that climbing fiber EPSPs are large enough to activate potassium channels, even in fairly distal dendrites, so that the presence of the channels will help determine the magnitude and duration of such EPSPs. Distal dendrites are also known to support slow calcium-dependent spikes and plateau potentials that can reach potentials near 0 mV (Llinás and Sugimori, 1980; Pouille et al., 2000). Unlike sodium spikes, calcium spikes are, if anything, larger in more distal dendrites (Pouille et al., 2000). It seems likely that the Kv3 channels that we found in distal as well as proximal dendrites help terminate such calcium spikes. In addition, the threshold for calcium spikes is fairly high, so that the rapidly activating potassium currents may influence the threshold. Consistent with a role of Kv3 channels in regulating calcium spikes, low concentrations of 4-AP have been found to decrease threshold and increase amplitude for calcium spikes recorded in the dendrites of guinea pig Purkinje neurons (Etzion and Grossman, 1998, 2001). The partial inactivation of the dendritic potassium currents would be expected to influence the timing of calcium spikes and plateau potentials; indeed, a role for an inactivating potassium conductance in controlling the timing and amplitude of dendritic calcium spikes has been suggested previously in turtle Purkinje cells (Midtgaard et al., 1993). Inactivation properties may also be important for climbing fiber responses that occur in rapid succession. Kv3.4 channels can dramatically change their inactivation properties after phosphorylation (Covarrubias et al., 1994), raising the possibility that modulatory transmitters could affect the integrative properties of the dendrites by this mechanism.

In many cases, Kv3 family channels have activation curves with midpoints near 10 mV (Rudy and McBain, 2001), so that 5–10% of activation requires depolarization to approximately ~20 mV or beyond. In contrast, the current in Purkinje neuron
dendrites has a midpoint near −10 mV and significant current begins to activate at −40 mV. In general, Kv3 family channels are found in soma, axons, and presynaptic terminals of fast-spiking cells, and their requirement for strong depolarization means that they are only activated during full-blown action potentials (Rudy and McBain, 2001). However, there are no full-blown sodium spikes in the dendrites of Purkinje neurons. The voltage dependence of the channels in Purkinje cell dendrites ensures that the channels can be activated even by truncated sodium spikes as well as by calcium spikes and climbing fiber responses. Yet the requirement for depolarization beyond −40 mV means that they will not be effectively activated by moderate-sized EPSPs from parallel fiber synapses or interfere with temporal or spatial summation of such EPSPs at subthreshold voltages.

References


Callaway JC, Ross WN (1997) Spatial distribution of synaptically activated sodium channels in soma, axons, and presynaptic terminals of fast-spiking cells and their requirement for strong depolarization means that they are only activated during full-blown action potentials (Rudy and McBain, 2001). However, there are no full-blown sodium spikes in the dendrites of Purkinje neurons. The voltage dependence of the channels in Purkinje cell dendrites ensures that the channels can be activated even by truncated sodium spikes as well as by calcium spikes and climbing fiber responses. Yet the requirement for depolarization beyond −40 mV means that they will not be effectively activated by moderate-sized EPSPs from parallel fiber synapses or interfere with temporal or spatial summation of such EPSPs at subthreshold voltages.


