Potassium currents modulation of calcium spike firing in dendrites of cerebellar Purkinje cells

Abstract The pattern of sustained Ca\(^{2+}\) spike firing was investigated, using macropatch clamp and intracellular recordings, in guinea pig cerebellar Purkinje cells. Under our standard experimental conditions (30°C, 5 mM [K\(^+\)]_o, 2 mM [Ca\(^{2+}\)]_o, 1 μM tetrodotoxin), each firing period started with uniform firing and gradually turned into a doublet pattern with a large spike afterhyperpolarization (AHP) between the doublets. Macropatch clamp recordings from localized dendritic regions revealed that each doublet is composed of two similar inward current deflections. This result indicated, for both peaks, an active process in the recording site and contradicted the possibility that they reflect firing in two completely separated dendritic regions. When [K\(^+\)]_o was increased the transition to a doublet pattern occurred earlier and the doublets became more pronounced. A similar but more prominent effect occurred following application of 1–10 μM 4-amino pyridine, which also reduced the threshold, increased the spike amplitude, and shortened the initial delay of evoked Ca\(^{2+}\) spike firing. In contrast, membrane depolarization, increased [Ca\(^{2+}\)]_o, and application of quinidine (but not apamine) markedly suppressed the generation of doublet pattern. During uniform initial firing, a short hyperpolarizing pulse that mimicked a large AHP induced a subsequent doublet. A short depolarizing pulse following a single spike induced an artificial doublet followed by a large AHP. These results indicate that the pattern of Ca\(^{2+}\) spike firing in the dendrites of Purkinje cells is dynamically modulated by a highly aminopyridine-sensitive K\(^+\) current, and probably also by a Ca\(^{2+}\) -activated potassium current.

Introduction

In recent years it has become apparent that the dendrites of central nervous system (CNS) neurons cannot be considered as passive summators of spatially distributed synaptic inputs (Llinas 1988). Purkinje cells provided the first firm demonstration of dendritic Ca\(^{2+}\) spikes (Llinas and Nicholson 1971; Llinas and Hess 1976), and since then their active dendritic membrane properties were the subject of detailed investigation. Voltage-gated channels can sustain Ca\(^{2+}\) influx in highly restricted dendritic compartments of the Purkinje cell (Denk et al. 1995; Eilers et al. 1995). This Ca\(^{2+}\) response is suggested to have several roles such as prolongation and amplification of excitatory synaptic inputs (Jaeger and Bower 1994) and involvement in the induction of long-term depression (Konnerth and Eilers 1994). Thorough understanding of Purkinje cell dendritic properties should take into account the activity of different outward currents (Gruol et al. 1989, 1991; Midtgaard et al. 1993), which may influence dendritic processing by direct modulation of membrane potential as well as by regulation of Ca\(^{2+}\) influx through the voltage-gated Ca\(^{2+}\) channels. However, the characterization of dendritic outward currents in Purkinje cell has been limited by space-clamp problems that confound voltage-clamp studies and by lack of selective ion channel blockers (Gruol et al. 1991).

In vitro, Purkinje cells display spontaneous or evoked full-blown dendritic Ca\(^{2+}\) spikes, as well as Ca\(^{2+}\) plateaus (Llinas and Sugimori 1980a; Hounsgaard and Midggaard 1988). Some of the cells alternate autorhythmically between prolonged firing and electrical quiescent periods (Yamamoto 1974; Llinas and Sugimori 1980b; Chang et al. 1993) and demonstrate sustained Ca\(^{2+}\) spike firing, often with appearance of doublets (see Llinas and Sugimori 1980b, their Fig. 4). Although sustained Ca\(^{2+}\) spike firing is not normally seen in vivo unless inhibition is blocked (Jaeger and Bower 1994), this behavior may be utilized for exploring fundamental properties of dendritic nonlinear activity (Llinas and Sugimori 1980b; Hounsgaard and Midggaard 1988; Tank et al. 1988; Lev Ram et al. 1992; Midggaard et al. 1993).
In the present study, we analyze in detail the pattern of spontaneous and evoked dendritic Ca\(^{2+}\) spike firing. We demonstrate that the characteristic doublets, previously interpreted as peaks originating in different dendritic branches, result from a dynamic regulation of Ca\(^{2+}\) spike generation by potassium currents.

**Materials and methods**

Cerebellar slices were prepared from adult male albino guinea pigs (200–650 g) using procedures previously described by Linas and Sugimori (1980a). Briefly, the brain was exposed and removed under pentobarbital sodium anesthesia (Nembutal 40 mg kg\(^{-1}\)). The vermis was isolated from the cerebellum by removing the hemispheres with two parasagittal cuts. While maintained in cold (6°C) Ringer’s solution, sagittal slices of 300 μm thickness were cut using a vibrotome (Campden Instruments). The slices were incubated for at least 1 h at room temperature in a standard Ringer’s solution saturated with a mixture of 95% \(\text{O}_2\) and 5% \(\text{CO}_2\). The standard Ringer’s solution contained: 124 mM NaCl, 5 mM KCl, 1.25 mM NaH\(_2\)PO\(_4\), 2 mM MgSO\(_4\), 26 mM NaHCO\(_3\), 2 mM CaCl\(_2\) and 10 mM glucose. Slices were placed in superfused-type and interface-type chambers, where macropatch clamp and intracellular recordings, respectively, were performed at 30°C. In some experiments, temperature was varied over a range of 28–33°C (see Results). Tetrodotoxin (TTX; Sigma), 4-aminopyridine (4-AP; Sigma), 3,4 diaminopyridine (3,4 DAP; Sigma), and amanite (Sigma) were added to the Ringer’s solution immediately before use. Quinidine (Sigma) was dissolved in ethanol and added to the Ringer’s solution. The concentration of ethanol in these experiments reached 0.05%.

Recordings of single-cell unit discharges were performed using the loose macropatch clamp technique. This technique provided high-quality recordings and therefore was suitable for experiments in which several ionic or pharmacological manipulations were performed. Somatic recordings were done with microforge-polished electrodes of tip diameter 20 μm (resistance of 0.4–0.6 MΩ). Dendritic recordings were done with electrodes of tip diameter 5 μm (resistance of 1–1.5 MΩ). A loose seal resistance of 100–200 kΩ and 0.5–1 MΩ, respectively, was noted for the somatic and dendritic recordings. In each experiment, an electrode filled with Ringer’s solution was mounted on a micromanipulator and connected through a Ag-AgCl wire to an Axopatch-1D headstage (CV-4; Axon Instruments, USA). The electrode was placed on the desired layer under direct visual control (Wild M5A binocular, ×60 magnification). Although single cells were not visualized routinely, the cerebellar layers were clearly discernible under these conditions. Somatic recordings were performed by placing the electrodes in the somatic layer, while for middendritic recordings the electrodes were placed in the central part of the molecular layer approximately 250–300 μm distal to the somatic region. The cells that were selected exhibited stable, spontaneous firing of both rapid somatic Na\(^+\) action potentials, and slow Ca\(^{2+}\)-dependent (blocked by 0.2 mM Cd\(^{2+}\); \(n=4\)) dendritic spikes (recorded in the soma as inward and outward currents, respectively; Housegaard and Yamamoto 1979; Linas and Sugimori 1992), alternating periodically between firing and quiescent periods (Fig. 1A). After stable recording was established, 0.5–1 μM TTX was added to block sodium currents. However, about half of the macropatch recordings were started in the presence of TTX. Most of the cells that were detected fired sharp calcium spikes (Fig. 1A); nevertheless, some cells exhibited multicomponent spike bursts (see Linas and Sugimori 1980a; their Fig. 11) and were excluded from this study. Unless otherwise noted in the text, recordings were done from the somatic layer by using the macropatch clamp technique as described above.

Intracellular recordings were obtained by using glass microelectrodes filled with 4 M potassium acetate (resistance 50–120 MΩ), with a conventional bridge-balance amplifier. The recordings were done in a TTX-containing solution from somatic or mid-dendritic regions as noted in the text. Evoked Ca\(^{2+}\) spike firing was studied either in cells with stable resting potential (below –55 mV) or in spontaneously active cells whose oscillations were suppressed by a 0.2–0.7-nA hyperpolarizing d.c. injection.

**Data acquisition and analysis**

Macropatch current recordings were filtered (DC –10 kHz bandpass), digitized (National Instruments board; AT-MIO-16F-5), and stored on a PC disk. Acquisition and analysis were made using self-programmed software (Labview 3.0.1; National Instruments). For firing pattern analysis, 8–15 cycles of autorhythmic spike firing were digitized for each condition at 2.5–5 kHz. The program measured the duration of the active and the quiescent periods on-line and analyzed interspike intervals (ISIs) off-line. For analyzing Ca\(^{2+}\) spike parameters, spikes from one to three active periods for each condition were digitized at 20 kHz. Using a peak detector, each Ca\(^{2+}\) spike was presented in a separate trace with pre- and postperiods adequate for baseline determination. Intracellular recordings were filtered (cutoff frequency 10 kHz) and stored on a PC disk. Statistical significance was determined by application of Student’s paired t-test except for doublet index, which was evaluated using Wilcoxon signed-ranks test.

**Doublet index**

In order to quantify the appearance of doublet firing, a doublet index (DI) was defined. This index was designed to recognize variation in the magnitude of adjacent ISIs and to increase specifically for patterns including doublets. The index gets higher values as the doublets became more widespread in the pattern or more pronounced (i.e., as the ISIs between doublets become larger relative to the ISIs within them).

Mathematically DI is defined for a given active period sequence of \(n\) ISIs \((I_1, I_2, \ldots, I_n)\) as follows: First a sequence of consecutive ratios \(R_1^*, R_2^*, \ldots, R_{n-1}^*\) is defined:

\[
R_j^* = \frac{\max\{I_j, I_{j+1}\}}{\min\{I_j, I_{j+1}\}}  \quad 1 \leq j \leq n - 1
\]

The sequence is calculated between pairs of consecutive ISIs, with the larger ISI in each couple as the numerator.

Next the skipping ratios sequence \(R_1^\#, R_2^\#, \ldots, R_{n-2}^\#\) is defined as follows:

\[
R_k^\# = \frac{\max\{I_k, I_{k+2}\}}{\min\{I_k, I_{k+2}\}}  \quad 1 \leq k \leq n - 2
\]

This sequence is calculated in the same way as the consecutive ratio sequence but for pairs with one ISI between them.

DI is defined by the expression:

\[
DI = \sum_{k=1}^{n-1} \frac{R_k^\#}{n-1} - \sum_{k=1}^{n-2} \frac{R_k^\#}{n-2}
\]

The final index is the ratio between the mean of the two sequences defined above. Since the doublet pattern is characterized by intermittent ISI alternations, as doublets become more widespread or pronounced, the consecutive ratios increase relative to the skipping ratios and DI value increases. In contrast, when ISI alternations are not intermittent, they increase both ratio sequences in a similar manner and consequently DI gets a value approaching 1.

**Results**

Different cells recorded using the macropatch clamp technique exhibited variation in the duration of the active and