Functional roles of an ERG current isolated in cerebellar Purkinje neurons

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ABSTRACT

Transcripts encoding ERG potassium channels are expressed by most neurons of the central nervous system. By patch-clamp whole-cell recording from Purkinje neurons in slices of young (5-9 days old) mouse cerebellum we have been able to isolate a tail current \( I_{K(ERG)} \) with the same characteristics as previously described for ERG channels. In zero external \( Ca^{2+} \) and high \( K^+ \) (40 mM) the \( V_{1/2} \) of activation was \(-50.7\) mV, the \( V_{1/2} \) of inactivation was \(-70.6\) mV and the deactivation rate was double exponential and voltage dependent. \( I_{K(ERG)} \) was 93.0% blocked by WAY-123,398 (1 \( \mu \)M) and 78.2% by haloperidol (2 \( \mu \)M). The role of \( I_{K(ERG)} \) on evoked firing was studied in adult mice, where WAY-123,398 application decreased the first spike latency, increased the firing frequency and suppressed the frequency adaptation. However, the shape of individual action potentials was not affected. Stimulation of presynaptic climbing fibers evoked the Purkinje neuron “complex spike”, composed of an initial spike and several spikelets. \( I_{K(ERG)} \) block caused an increase of the number of spikelets of the “complex spike”. These data show, for the first time, an \( I_{K(ERG)} \) in a neuron of the central nervous system, the cerebellar Purkinje neuron, and indicate that such a current is involved in the control of membrane excitability, firing frequency adaptation and in determining the effects of the climbing fiber synapse.
INTRODUCTION

The “ether-a-go-go-related gene” (ERG) K⁺ channel was described for the first time in hippocampus (Warmke et al. 1994). In Drosophila, the eag gene family of K⁺ channels, which includes ERG, is involved in the control of neuromuscular excitability (Ganetzky and Wu 1983). However, in mammals the ERG current (I_{K(ERG)}) has been studied extensively only in non neuronal cells. In cardiac myocytes I_{K(ERG)} has been shown to correspond to the rapidly activating component of the delayed rectifier current (I_{Kr}; Sanguinetti et al., 1995) that contributes to the repolarization of the action potential (Shibasaki 1987; Sanguinetti and Jurkiewicz 1990). The relevance of this role is confirmed by the appearance of a cardiac arrhythmia (LQT2 syndrome) when the human ERG channel is mutated (Curran et al. 1995; Witchel and Hancox, 2000). Unusual kinetic properties, a fast inactivation and a slow activation gates, confer to I_{K(ERG)} a strong inward rectification and determine its contribution to the control of cardiac action potential shape, membrane excitability and discharge pattern. In the rat, in addition to the gene coding for the cardiac ERG channel (ERG1), other two genes have been identified (ERG2 and ERG3: Shi et al. 1997). In mammals, in spite of the abundant expression of the three ERG genes also in the central nervous system (Shi et al. 1997; Wymore et al. 1997; Maggio et al. 2000; Saganich et al. 2001), to the best of our knowledge I_{K(ERG)} has only been studied in non neuronal cells, in neuroblasts or in periferal autonomic neurons.

The result of the signal processing exerted by Purkinje neurons on synaptic inputs coming from parallel and climbing fibers is the only output signal which leaves the cerebellar cortex (Ito 1984). Therefore, a knowledge of the elements involved in such a signal processing is a crucial issue in cerebellar physiology. I_{K(ERG)} is involved in the control of excitability and in the specification of the firing pattern in neuron-neuroblastoma hybrid cells (Chiesa et al. 1997). However, nothing is known about the role of the I_{K(ERG)} in the modulation of synaptic signals. The climbing fiber afferent input to Purkinje neurons is strictly required for cerebellar control of motor performance (Welsh et al. 1995)
and for learning of novel motor tasks (Ito 2001). The postsynaptic signal evoked by climbing fiber activity, named “complex spike”, is associated with intradendritic Ca$^{2+}$ transients, with intriguing similarities with Ca$^{2+}$ spikes that in endocrine cells are involved in hormone secretion. ERG channels are involved in the control of excitability and hormone release in human β-cells (Rosati et al. 2000), in pituitary cells (Barros et al. 1997; Bauer et al. 1999, Lecchi et al., 2002), in glomus cells of the rabbit carotid body (Overholt et al., 2000) and in opossum esophageal circular smooth muscle (Akbarali et al., 1999). Therefore, it seems reasonable that, if Purkinje neurons express $I_{K(ERG)}$, this can have a modulatory action on the complex spike. In this paper we report for the first time the identification in a central neuron of an $I_{K(ERG)}$-like current and we show that in Purkinje neurons it is a determinant of the discharge pattern and that it exerts a control on the complex spike generated by climbing fiber activity. Part of the results has been published as abstracts (Sacco et al. 2001a,b).

MATERIALS AND METHODS

Whole-cell K$^+$ currents were recorded in voltage-clamp mode from Purkinje neurons in cerebellar slices prepared from immature (5-9 days old) and young adult (22-42 days old) CD-1 mice of either sex. Parasagittal cerebellar slices (200 µm thickness) were obtained following a previously described technique (Llinas and Sugimori 1980; Sacco and Tempia 2002), kept for one hour at 35˚C and then at room temperature in the extracellular saline solution containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 20 glucose, bubbled with 95 % O$_2$ / 5 % CO$_2$ (pH 7.4). The recording chamber was continuously perfused with the saline solution, bubbled with 95 % O$_2$ / 5 % CO$_2$. The Purkinje neuron upper surface was cleaned by a sodalime glass pipette filled with the extracellular saline solution. Patch pipettes (2.0-2.5 MΩ) contained one of the following solutions (in mM): (a) 140 potassium gluconate, 4 MgCl$_2$, 0.5 EGTA, 10 HEPES, 4 Na$_2$ATP, 0.4 Na$_3$GTP; pH: 7.3. (b) 138 KCl, 2 MgCl$_2$, 10 EGTA, 10 HEPES, 4 Na$_2$ATP, 0.4 Na$_3$GTP; pH: 7.3; Solution (a) was used
for all CC experiments and for the VC experiments shown in Fig. 1, while solution (b) was used for the VC experiments in which ZD7288 was applied to isolate $I_{K(\text{ERG})}$ (Fig. 2-5, 7). For recordings an EPC-9C patch-clamp amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany) was used. In VC, the uncompensated value of series resistance was 4-8 MΩ, and series resistance compensation was set at 80-90%. For the young Purkinje neurons used for VC recordings, in these conditions space clamp problems are minimal or absent even for the most distal cellular compartments (Sacco and Tempia 2002). The cell was kept at a holding potential ($V_H$) of −60 mV. All VC protocols started with a step of 5 seconds to voltages ranging from −80 to 0 mV followed by a shorter step (250, 500 or 1000 ms) to potentials between −120 and 0 mV. All VC data and CC data of long steps (5 or more sec) were filtered at 2.9 kHz and digitized at 10 kHz. CC data of 500 ms current steps were filtered at 8.5 kHz and digitized at 20 kHz. Digitized data were stored on a Macintosh computer (G3, Apple computer, Cupertino, CA, USA) using the Pulse software (HEKA Elektronik, Lambrecht/Pfalz, Germany) and analyzed by the Igor Pro software (Wavemetrics, Lake Oswego, OR, USA). The duration of action potentials was measured from the time at which the potential reached 10 % of the peak amplitude until the time at which it decayed of 90%.

The activation and inactivation curves were fitted with the Boltzmann equation:

\[(1) \quad I_{(V)} = (I_2 - I_1) / \{1 + \exp \left[-(V - V_{1/2}) / k\right]\} + I_1\]

where $I_{(V)}$ is the peak current as a function of $V_H$, $I_1$ and $I_2$ are the peak amplitudes of the currents at respectively the most negative and most positive potentials tested, $V$ is the holding voltage ($V_H$), $V_{1/2}$ is the voltage at which the current is half of the maximal amplitude and $k$ represents the slope factor.

The decay of $I_{K(\text{ERG})}$, due to channel deactivation, was fitted either by a mono-exponential equation:

\[(2) \quad I_0 = I_1 \left[\exp \left(-t / \tau\right)\right] + I_0\]
where $I(t)$ is the total current at time $t$, $I_1$ is the initial amplitude, $\tau$ is the onset time constant and $I_0$ is the non inactivating residual current; or by a double-exponential equation:

$$I(t) = \{I_s \exp(-t / \tau_s)\} + \{I_f \exp(-t / \tau_f)\} + I_0$$

where $I(t)$ is the total current at time $t$, $I_s$ and $I_f$ are the initial amplitudes of the two components related to $\tau_s$ and $\tau_f$ which are respectively the slow and the fast decay time constants and $I_0$ is the non inactivating residual current.

Since the ERG channel shows inward rectification and its conductance is directly related to the external $K^+$ concentration $[K^+]_o$. ERG $K^+$ currents ($I_{K(ERG)}$) were identified and studied in VC with an extracellular saline solution with high $[K^+]_o$ (calculated Nernst equilibrium potential for $K^+$: about $-30$ mV). Osmolarity was kept constant by lowering $Na^+$ concentration. The external saline solution used in VC recordings contained (in mM): 40 KCl, 87.5 NaCl, 2 CaCl$_2$, 1 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 20 glucose. This solution was additioned with tetrodotoxin citrate (TTX: 1 µM) to block Na$^+$ channels and with bicuculline methochloride (20 µM) to block spontaneous postsynaptic currents mediated by GABA$_A$ receptors. This solution, with physiological divalent cations concentrations ([Ca$^{2+}]_o$: 2 mM, [Mg$^{2+}]_o$: 1 mM), was used only for the experiments shown in Fig. 1. In all other VC experiments (Fig. 2-5 and 7) CaCl$_2$ was substituted with MgCl$_2$ (final MgCl$_2$ concentration: 3 mM) and ZD7288 (10 µM) was added. In CC, in order to avoid both spontaneous postsynaptic currents and those that could result from the application of $K^+$ channel blockers, the external saline solution was supplemented with bicuculline methochloride (20 µM) to block GABA$_A$ receptors, 50µM D(-)-2-amino-5-phosponopentanoic acid (D-AP5) to block NMDA type glutamate receptors and 10µM 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide disodium (NBQX) to block AMPA type glutamate receptors. The only exception is the recording of evoked complex spikes, where D-AP5 and NBQX were omitted. WAY-123,398 (1 µM) and haloperidol (2 µM) were applied by changing the perfusion line. All drugs were purchased from Tocris Cookson, Langford, UK, except for haloperidol
(Sigma) and WAY-123,398, which was kindly provided from Dr. W. Spinelli from Wyeth-Ayerst Research, Princeton, NJ, USA.
RESULTS

Immature cerebellar Purkinje neurons express an ERG-like current

ERG K⁺ currents ($I_{K(ERG)}$) in Purkinje neurons were identified and studied with an extracellular saline solution with high [K⁺]o. An initial set of experiments was performed with physiological [Ca²⁺]o concentration. The membrane potential was kept at –60 mV and every 30 seconds (or longer) it was stepped for 5 seconds to 0 mV and then it was stepped back to potentials ranging from –20 to –120 mV in increments of 20 mV. Immediately after the step from 0 to negative potentials a large inward tail current developed, which at the largest negative potentials had two components (Fig. 1A). An early component peaked within 50 ms while a later component was still slowly developing at the end of the 250 ms test pulse (Fig. 1A). When the ERG selective blocker WAY-123,398 (1 µM) was added to the slice perfusion solution, the early component of the inward K⁺ current virtually disappeared, while the later current was not affected (Fig. 1B). In fact, the later slowly activating component corresponds to $I_H$, a cationic inward rectifying current (see below). The WAY-123,398 sensitive component was obtained by subtraction of the current resistant to this compound from control traces (Fig. 1C). The WAY-123,398 sensitive tail current had a peak amplitude at –120 mV of 1.93 nA (± 0.17 s.e.m.; n = 4) and a fast onset. The rate of deactivation rapidly became faster moving towards more hyperpolarized potentials. These properties are very similar the $I_{K(ERG)}$ shown in other cell types (Shibasaki 1987; Faravelli et al. 1996).

To better isolate the early component of the tail current ($I_{K(ERG)}$) from the later component and from other currents, so that the biophysical properties of the $I_{K(ERG)}$ could be studied, we designed an external solution that allowed us to minimize other currents like sodium currents, calcium currents, calcium activated K⁺ currents and the current of the cationic inward rectifier ($I_H$). This was achieved by using a nominally calcium-free external solution (Ca²⁺ was substituted by Mg²⁺) additioned with tetrodotoxin (1 µM) to block Na⁺ currents, with bicuculline (20 µM) to block spontaneous postsynaptic
currents mediated by GABA_A receptors and the organic blocker ZD7288 (10 µM) to block \( I_H \). The effects of ZD7288 on \( I_{K(ERG)} \) were initially tested on the F-11 cell (Faravelli et al. 1996), where it produced a reduction of about 10% (not shown). The effects and side effects of ZD7288 were carefully tested also in cerebellar Purkinje neurons (Fig. 2). To this aim, in 4 Purkinje neurons tail currents at –120 mV were evoked from step potentials (duration: 5 s) ranging from –80 to 0 mV (Fig. 2A, blue traces). This protocol allowed us to estimate at the same time possible effects of ZD7288 on the currents evoked by the steps from –80 to 0 mV and on the tail currents at –120 mV, where the activation of \( I_{K(ERG)} \) and \( I_H \) is maximal (see Fig. 1A-B: red traces). ZD7288 (10 µM) almost completely blocked within 5 minutes the slowly developing inward current (Fig. 2A: arrows), as it is expected for \( I_H \). In contrast, the outward currents evoked by the steps from –80 to 0 mV were not significantly affected by ZD7288 (Fig. 2A). A detailed analysis of the early inward tail current (\( I_{K(ERG)} \)) that quickly develops at –120 mV (Fig. 2A-B) was performed on currents recorded after at least 5 minutes of ZD7288 application. Since \( I_{K(ERG)} \) at this potential peaked within 10 ms (see Fig. 2B) it was not necessary to correct control traces for the contribution of \( I_H \) to the peak amplitude of \( I_{K(ERG)} \). ZD7288 inhibited \( I_{K(ERG)} \) by 11.1% (± 1.1 s.e.m.; \( n = 4 \)). The plot of the peak amplitude of \( I_{K(ERG)} \) at –120 mV versus the prepulse potentials, reflecting the availability of ERG channels, revealed that the percentage of ZD7288 inhibition of \( I_{K(ERG)} \) was the same at all voltages (Fig. 2C). This was confirmed by the fact that the activation curves normalized to the value obtained by the prepulse to 0 mV were almost identical (Fig. 2D). Thus, in cerebellar Purkinje neurons, ZD7288 at the concentration of 10 µM completely blocks \( I_H \) and inhibits by about 11% \( I_{K(ERG)} \), but without affecting its activation curve. Furthermore, the deactivation kinetics of \( I_{K(ERG)} \) at –120 mV was also not affected (Fig. 2B). Finally, voltage gated outward currents evoked by depolarizing steps from the \( V_H \) of –60 mV to potentials from –80 to 0 mV were not significantly affected by ZD7288 (Fig. 2A). For these reasons, ZD7288 (10 µM) was systematically used in all the following experiments to characterize the biophysical properties of
In conditions in which it was the only time dependent inward current at the end of long depolarizing pulses.

Under these experimental conditions, tail currents at –120 mV, evoked from voltage steps ranging from –80 to 0 mV, displayed only one transient component, that appeared at –60 mV and gradually increased in amplitude with more depolarized step potentials (Fig. 3A). Such a transient component of tail currents was greatly inhibited by WAY-123,398 (1 µM; Fig. 3B). Since the sustained current remaining at the end of the transient component was not affected by the step potential (Fig. 3A) and since ERG is known to deactivate completely at very negative potentials like –120 mV (see also below: inactivation experiments), it was possible to precisely measure the amplitude of the transient current both before and during block of WAY-123,398. The application of WAY-123,398 caused a reduction of 93.0% (± 1.9% s.e.m.; n = 5) of the transient tail current evoked by steps to 0 and then back to –120 mV, in line with the sensitivity of ERG channels for this drug (Fig. 3B). Therefore, we used WAY-123,398 to further isolate \( I_{K(ERG)} \) by subtraction of the WAY-123,398 resistant current from control traces (Fig. 3C). The peak amplitude of the WAY-123,398 sensitive tail current obtained by stepping back from 0 to –120 mV was 0.98 nA (± 0.10 s.e.m.; n = 6). This value is significantly smaller that the peak amplitude of the WAY-123,398 sensitive current (1.93 ± 0.17 nA) obtained with the same stimulation protocol, but in the different ionic conditions of the experiments shown in Fig. 1. Such a difference can be attributed to the different divalent cations content of the extracellular solutions. In fact, in the experiments with ZD7288, the external Ca\(^{2+}\) was substituted by Mg\(^{2+}\), which is known to decrease the amplitude of \( I_{K(ERG)} \) (Faravelli et al., 1996).

The activation curves of \( I_{K(ERG)} \), reflecting channel availability, were constructed by plotting the normalized peak amplitude of tail currents against the pulse potential (Fig. 3D; n = 8). The activation curves of individual cells were fitted by Boltzmann functions with a \( V_{1/2} \) of –50.7 mV (± 2.0 mV s.e.m.;
n = 8) and a slope factor of 5.6 mV (± 0.4 s.e.m.; n = 8). The dashed line in Fig. 3D is a Boltzmann curve constructed using these average values.

In order to study the voltage dependence of the quasi steady-state inactivation of $I_{K(ERG)}$, the voltage was stepped for 5 seconds to 0 mV to obtain a full activation of ERG channels. Then, it was stepped back to different potentials (–20 to –120 mV) to allow the quick recovery from inactivation (Fig. 4A-C). Since ERG channels at potentials where they recover from inactivation also deactivate, the decay of tail currents, due to channel deactivation, was fitted by exponential functions and the amplitude was extrapolated at +1 ms after the voltage step. This allowed us to estimate the amplitude of the current just after removal of inactivation without the decline due to the deactivation that already occurs when the tail current reaches a peak (Fig. 4D). The chord conductance was calculated by dividing the extrapolated current amplitudes by the driving force. The chord conductance normalized to the value obtained at –120 mV was plotted as a function of the membrane potential (Fig. 4D). The data points of individual cells were fitted by Boltzmann functions with a $V_{1/2}$ of –70.6 mV (± 4.8 s.e.m.; n = 6) and a slope factor of 23.9 mV (± 3.4 mV s.e.m; n = 6). The dashed line in Fig. 4D is a Boltzmann curve constructed using these average values.

The same protocol used to study the inactivation also allowed us to measure the time constants of deactivation (Fig. 4E). At the more negative potentials, the deactivation followed a double exponential function, with a clear voltage dependence of both time constants (Fig. 4E; n = 6). At more depolarized potentials the deactivation was fitted by single exponential functions. In a semi-logarithmic plot, the voltage dependence of deactivation time constants was linear, with the single exponential time constants lying in the same line as $\tau_f$ values (Fig. 4E; n = 6).

Recent data indicate that the widely used antipsychotic drug haloperidol is a blocker of human ERG (HERG) channels (Suessbrich et al. 1997). Since no evidence is available of effects of haloperidol on $I_{K(ERG)}$ in any central nervous system neuron, we tested this compound on the $I_{K(ERG)}$ recorded in
cerebellar Purkinje neurons. At a concentration (2 µM) corresponding to that used for therapeutic
effects, haloperidol exerted a maximal block of 78.2% of $I_{K(ERG)}$ after 10 minutes of application ($\pm$
4.5% s.e.m.; $n = 4$; Fig 5A-B). The peak amplitude of the haloperidol sensitive current obtained by
voltage steps from 0 to −120 mV was 0.77 nA ($\pm$ 0.09 s.e.m.; $n = 4$).

Tail currents obtained by subtraction of the haloperidol resistant component (Fig. 5C) were
studied to obtain the voltage dependence of the *quasi* steady-state inactivation of $I_{K(ERG)}$ (Fig. 5D), as
described above for the WAY-123,398 sensitive current. The curves representing the chord
conductance after removal of inactivation were fitted by Boltzmann functions with a $V_{1/2}$ of −68.6 mV
($\pm$ 3.3 s.e.m.; $n = 4$) and a slope factor of 21.6 mV ($\pm$ 4.9 s.e.m.; $n = 4$). The dotted line in Fig. 5D is a
Boltzmann curve constructed using these average values. The curve for the haloperidol sensitive
current (Fig. 5D: dotted line) was very similar to the curve for the WAY-123,398 sensitive current (Fig.
5D: dashed line). Also the time constants of deactivation of the haloperidol sensitive current (Fig. 5E:
data points) displayed similar values and a similar voltage dependence as the WAY-123,398 sensitive
current (Fig. 5: dashed lines).

**Contribution of $I_{K(ERG)}$ to intrinsic properties of immature Purkinje neurons**

In order to study the physiological roles of $I_{K(ERG)}$ in Purkinje neurons, whole-cell current clamp
recordings were performed in cerebellar slices from mice of the same age as those used for voltage-
clamp recordings (P5-P9) and from adult mice. In the more physiological conditions in which current
clamp recordings were performed, including the presence of extracellular Ca$^{2+}$, a low concentration of
intracellular Ca$^{2+}$ buffer and absence of Na$^+$ channel blockers, it was necessary to carefully isolate the
effects of ERG blockers on Purkinje neurons. To this aim, possible effects due to block of $I_{K(ERG)}$ in the
 glutamatergic synaptic terminal of parallel fibers and climbing fibers with a consequent release of
 glutamate, or in the GABAergic terminals of inhibitory interneurons with a consequent release of
GABA, were avoided by blocking the main postsynaptic receptors to these neurotransmitters expressed by cerebellar cortical neurons (see Methods).

In such immature Purkinje neurons, the resting membrane potential was \(-56.2\) mV (± 2.4 s.e.m.; n = 10). The application of WAY-123,398 tended to slightly depolarize the cell, but this trend was not statistically significant (mean resting potential: \(-55.8\) mV ± 2.5 s.e.m.; n = 10; Student’s paired t-test: P > 0.05). The input resistance \(R_N\), measured by the voltage deflection evoked by the injection of a low intensity hyperpolarizing current (-50 or -100 pA, 200 ms), was 224.1 MΩ (± 19.8 s.e.m.; n = 10) in the control solution containing the synaptic receptor blockers. Application of WAY-123,398 slightly increased \(R_N\) to 232.5 MΩ (± 24.6 s.e.m.; n = 10; Student’s paired t-test: P > 0.05).

Five immature Purkinje neurons were depolarized by current injections (1 nA; n = 5), which evoked one or more action potentials. However, 4 of the 5 Purkinje neurons fired only one full action potential, that in some cases was followed by inactivating spikelets of by calcium spikes. Only one cell displayed a continuous firing during the whole duration of the depolarizing step. The mean duration of the first action potential of evoked discharge in these immature cells was 2.45 ± 0.55 ms in the standard solution and 2.47 ± 0.55 ms during application of WAY-123,398 (Fig. 6; n = 5; Student’s paired t-test: P > 0.05). However, since \(I_{K(ERG)}\) activation is slow and could require several spikes to reach a significant accumulation of open channels (Schönherr et al. 1999), in the cell with repetitive firing the tenth action potential of each series was also analyzed. Also in this cell WAY-123,398 application did not affect the duration of the first action potential (control and WAY-123,398: 3.0 ms; Fig. 6), but it produced a broadening of the tenth action potential with an increase of duration from 5.1 to 6.3 ms (not shown).

\(I_{K(ERG)}\) has been shown to affect the firing pattern of a neuron-neuroblastoma cell line (Chiesa et al. 1997). However, an analysis of the firing pattern requires a stable and reproducible firing that in immature Purkinje neurons was not present (see also Woodward et al. 1969; Crepel 1972). Therefore,
we extended to adult Purkinje neurons the experiments on intrinsic properties, including the firing pattern.

**Contribution of $I_{K(ERG)}$ to intrinsic properties of adult Purkinje neurons**

A prerequisite to study the physiological roles of $I_{K(ERG)}$ in adult Purkinje neurons is to provide some clue of the presence of this type of current at the same age. Adult Purkinje neurons have an extensive dendritic tree that does not allow a reliable voltage clamp, so that a quantitative study of the biophysical properties of membrane currents is not possible. In spite of this problem, we tried to obtain some qualitative information on the presence of $I_{K(ERG)}$ using the best experimental protocol employed in immature animals (high $[K^+]_o$, nominally calcium free extracellular solution and $I_h$ block by ZD7288). In these conditions we searched for an inward current evoked by voltage steps from 0 mV to negative potentials (-80, -100 and -120 mV) that decayed progressively faster at more negative potentials and that was blocked by WAY-123,398 (1 µM). These requisites were met in 3/3 Purkinje neurons (an example is shown in Fig. 7), indicating that in Purkinje neurons the expression of ERG into adulthood (Maggio et al. 2000; Saganich et al. 2001) is paralleled by the functional presence of $I_{K(ERG)}$.

The physiological role of $I_{K(ERG)}$ in the action potential discharge properties of Purkinje neurons could be studied in cerebellar slices from adult mice, since action potentials can be reliably current-clamp recorded also in cells with an extensive dendritic tree without the limitations present in voltage clamp mode. During all recordings, AMPA, NMDA and GABA$_A$ receptors were blocked. In contrast to immature mice, all adult Purkinje neurons spontaneously fired action potentials. The application of WAY-123,398 at −60 mV had no effect on the cell input resistance (control: 161.6 MΩ ± 21.4 s.e.m.; WAY-123,398: 162.0 MΩ ± 17.9; n = 3). In order to obtain stable and reproducible firing in response to depolarizing current steps, for the following experiments the membrane potential was manually kept as close as possible to −70 mV by continuous injection of constant hyperpolarizing current, thereby
abolishing the spontaneous firing that was present in all cells (n = 6). Every 10 seconds the membrane potential was recorded, the input resistance was measured by the voltage deflection evoked by the injection of a low intensity hyperpolarizing current (-50 pA, 200 ms) and the cell excitability was tested by the injection of a depolarizing current sufficient to elicit low frequency firing of simple spikes (+100 to +250 pA, 500 ms). In these conditions, WAY-123,398 significantly affected neither the membrane potential (potential difference in WAY-123,398: +0.9 mV ± 0.3 s.e.m., n = 4; Student’s paired t-test: p > 0.05) nor $R_N$ (control: 90.8 M$\Omega$ ± 7.8 s.e.m., n = 4; WAY-123,398: 95.4 M$\Omega$ ± 9.3 s.e.m., n = 4; Student’s paired t-test: p > 0.05). The application of WAY-123,398 affected the duration neither of the first (control: 0.65 ± 0.04 ms; WAY-123,398: 0.69 ± 0.07 ms n = 5; Student’s paired t-test: p > 0.05; Fig. 8A) nor of the tenth (control: 0.69 ± 0.08 ms; WAY-123,398: 0.73 ± 0.10 ms; n=5; Student’s paired t-test: p > 0.05; Fig. 8B) action potential evoked by depolarizing steps. It should be noted that the mean action potential duration was considerably shorter in these adult Purkinje neurons (about 0.7 ms) relative to immature ones (about 2.5 ms).

In order to study the role of $I_{K(ERG)}$ on the firing properties of adult Purkinje neurons, initially, long depolarizing current injections were delivered (Fig. 9A). All adult Purkinje neurons (n = 6) responded to such stimulation with a repetitive firing that usually showed a frequency adaptation in the first 10 or 20 spikes (Fig.9A-B). Therefore, we performed a detailed analysis of Purkinje neuron evoked firing using shorter depolarizing current injections lasting 500 ms, that were sufficient to obtain at least 10 spikes in most of the cells (Fig.9C-D). This analysis (Table I) revealed that, despite the variability of the responses within each cell, in 3 out of the 6 analyzed cells the application of WAY-123,398 caused significant changes (one-way ANOVA). Thus, in these 3 cells, following WAY-123,398 application the latency of the first evoked spike was shortened, the number of spikes fired in 500 ms was decreased, the instantaneous frequency measured at the first and at the ninth interspike interval was enhanced and the frequency adaptation was reduced (Table I). The responses in control
conditions of the 3 cells sensitive to WAY-123,398 had several differences relative to the 3 cells that were not affected, like a shorter first spike latency, a higher firing rate at the first interspike interval and a stronger frequency adaptation (Table I). The last of these parameters, measured as the slope factor of the linear function describing the decrease of the instantaneous frequency, had a value larger than 3 Hz/spike in the WAY-123,398 responsive cells, while in the other 3 cells the slope was smaller than 2 Hz/spike and in one cell the firing presented with a slight acceleration rather than adaptation (Table I). Thus, these results show that Purkinje neurons that display a frequency adaptation larger than 3 Hz/spike are significantly affected by WAY-123,398, indicating that in such cells $I_{K(ERG)}$ is involved in the control of excitability, firing frequency and frequency adaptation.

**The climbing fiber postsynaptic potential is controlled by $I_{K(ERG)}$**

The activation of a climbing fiber by a second electrode placed in the granule cell layer of adult mice slices evoked an all-or-none excitatory postsynaptic potential containing an initial spike followed by several spikelets (Fig. 10A,C). The application of WAY-123,398 (1 µM) prolonged the duration of the complex spike, so that Purkinje neurons often fired an additional spikelet (Fig. 10B,C). On average, the number of spikelets in control complex spikes was 4.1 (± 0.6 s.e.m.; n = 5), while during WAY-123,398 application it increased to 4.7 (± 0.4 s.e.m.; n = 5). The difference was statistically significant (paired Student’s t-test: p < 0.05). The same effect could be observed also on the second complex spikes evoked by paired pulse stimulation (Fig. 10D).

**DISCUSSION**

**ERG current**

In this study we describe in a central neuron, the Purkinje neuron of the cerebellar cortex, a K⁺ current with biophysical and pharmacological properties corresponding to ERG channels ($I_{K(ERG)}$). The ERG
current is characterized by a very fast recovery from inactivation and a slow deactivation with voltage-dependent time constants (Bauer et al. 1990; Faravelli et al. 1996; Bianchi et al 1998). Since neurons express a huge repertoire of ion channels, in order to detect and characterize $I_{K(ERG)}$, it was necessary to isolate it from the other currents displayed by Purkinje neurons. The ERG channel shows inward rectification and its conductance is directly related to the external K$^+$ concentration $[K^+]_o$ (Shibasaki 1987). Moreover, the activation of ERG channels is very sensitive to the extracellular Ca$^{2+}$ concentration $[Ca^{2+}]_o$, which causes allosteric changes in channel gating that shift the activation curve to the right (Johnson et al. 2001). Both properties were exploited to optimize the detection of $I_{K(ERG)}$ by using an extracellular solution with a high $[K^+]_o$ and with nominally zero $[Ca^{2+}]_o$. The absence of external Ca$^{2+}$ allowed us to eliminate Ca$^{2+}$ currents and Ca$^{2+}$ dependent currents.

In the experimental conditions used in these experiments, the inward current of Purkinje neurons consists of three components. A first component activates slowly and is almost completely blocked by ZD7288 (Fig. 2), so that it can be attributed to the hyperpolarization-activated cationic current called $I_H$ (Crepel and Penit-Soria 1986; Harris and Constanti 1995). After elimination of $I_H$, a second component, which develops quickly and decays with a voltage-dependent rate, remains as the only current with time-dependent kinetics. This second component was identified in this paper as $I_{K(ERG)}$ and was largely suppressed by the ERG channel specific blocker WAY-123,398 and by haloperidol. The third component is a steady current that remains after block of $I_H$ and $I_{K(ERG)}$. Such a residual current does not show any voltage dependent gating and was not further analyzed in this study. The selective block of the second component by WAY-123,398 allowed us to further isolate $I_{K(ERG)}$ from the passive currents of the third component. However, the activation, inactivation and deactivation properties of $I_{K(ERG)}$ were essentially the same even without subtraction of such residual currents (see Fig. 2-5), providing us with the possibility to characterize $I_{K(ERG)}$ independently from its pharmacological profile.
The activation curve, with a $V_{1/2}$ of $-50.7$ mV, accurately corresponds to the value of $-51$ mV reported in mouse smooth muscle cells, which express the channel ERG1b (Ohya et al., 2002). This study, like ours, was conducted with a high $[K^+]_o$ and almost $[Ca^{2+}]_o$ free conditions. However, the recently cloned smooth muscle ERG1 channel (ERG1-sm), expressed in a heterologous system and recorded in low $[K^+]_o$ and physiological $[Ca^{2+}]_o$, has a more depolarized $V_{1/2}$ of activation of $-36$ mV (Shoeb et al., 2003). Also cloned rat ERG2 and ERG3 channels studied in a low $[K^+]_o$ and physiological $[Ca^{2+}]_o$, display less negative $V_{1/2}$ of activation values respectively of $-3.5$ and $-44.0$ mV (Shi et al., 1997). In the same study, the human ERG channel (HERG) had a $V_{1/2}$ of activation of $-21$ mV. Cloned mouse ERG1a (mERG1a) also has a $V_{1/2}$ of activation at a rather depolarized voltage of $-5.9$ mV (Selyanko et al., 1999). However, the $[Ca^{2+}]_o$ is known to strongly shift to the right the activation curve, so that, in zero $[Ca^{2+}]_o$, the $V_{1/2}$ of activation of HERG becomes $-44 \pm 6$ mV (Johnson et al. 2001). The slope of the activation curve of 5.6 is smaller than the values reported in other preparations, like 11.0 in smooth muscle cells (Ohya et al., 2002), 10 in ERG1-sm (Shoeb et al., 2003), 7.6, 8.3 and 7.3 respectively in HERG, rat ERG2 and ERG3 (Shi et al., 1997), 12.2 in mERG1a (Selyanko et al., 1999).

The kinetics of deactivation is relatively in line with mouse smooth muscle $I_{K(ERG)}$, where the time constant was 30 ms at $-120$ mV (Ohya et al., 2002), while in our study at this voltage the fast time constant was 17 ms and the slow one was 63 ms. At $-80$ mV Ohya et al. (2002) report a deactivation time constant of 108 ms, while in cerebellar Purkinje neurons we obtained 128 ms. These values are also in line with cloned ERG1-sm (Shoeb et al., 2003). Deactivation at $-120$ mV of mERG1a and of NG108-15 cells followed a double exponential time course like in our study, with fast time constants in these two cell types of 33 and 34 ms and slow time constants of 197 and 189 ms. Taken together, activation and deactivation data support the identification of the current described in this paper with $I_{K(ERG)}$, with properties similar to those reported in a study performed in native cells and with ionic
conditions close to those used in our experiments (Ohya et al., 2002). Notable differences of $I_{K(ERG)}$ in mouse Purkinje neurons relative to mouse smooth muscle cells are a smaller slope factor of the activation curve and a double exponential deactivation with a fast time constant shorter than the only time constant of the latter study (Ohya et al., 2002). Both differences could be due to the expression in Purkinje neurons of ERG3 in addition to ERG1 (Saganich et al., 2001), since rat ERG3 activation has a relatively steep slope of 7.3 mV and faster kinetics than ERG1 (Shi et al., 1997). Other possible reasons of the differences in biophysical properties in different tissues are the different expression of beta subunits (Abbott et al., 1999) or the predominance of a certain splice variant.

The quasi steady-state inactivation of $I_{K(ERG)}$, with a $V_{1/2}$ of about –70 mV, was similar to the value obtained in mouse smooth muscle cells with short pulses followed by a step to –120 mV (Ohya et al., 2002). In contrast, in heterologous expression studies, the $V_{1/2}$ of inactivation was either more depolarized (Shoeb et al., 2003) or shifted towards more negative values (Smith et al., 1996; Shi et al., 1997), but with slope factors around 20 in agreement with our data.

The sensitivity of $I_{K(ERG)}$ to WAY-123,398 and to haloperidol further confirms the identification of $I_{K(ERG)}$ with ERG currents reported in other systems. In fact, WAY-123,398 is selective for ERG currents (Spinelli et al. 1993; Faravelli et al. 1996), being ineffective on other types of K$^+$ current including $K_{(ATP)}$, $SK_{(Ca)}$, $BK_{(Ca)}$ and other members of the eag family such as ELK2 (Rosati et al. 2000; Becchetti et al. 2002; unpublished data). Recent data indicate that the widely used antipsychotic drug haloperidol is also a blocker of human ERG (HERG) channels (Suessbrich et al. 1997). Such a block explains the cardiac side effects of haloperidol (Suessbrich et al. 1997). The high affinity effect of haloperidol has been shown to be selective for HERG channels over a wide range of other K$^+$ channels like Kv1.1, Kv1.2, Kv1.4, Kv1.5, IsK, Kir2.1 (Suessbrich et al. 1997) and ELK2 (Becchetti et al. 2002). Our finding of the inhibition of $I_{K(ERG)}$ in a neuron of the central nervous system suggests that
some of the neurotropic effects of haloperidol could be explained by a direct block of ERG channels in brain regions where $I_{\text{K(ERG)}}$ is involved in the control of cell firing.

The presence of functional ERG channels in cerebellar Purkinje neurons is in agreement with \textit{in-situ} hybridazation studies, which reported that these neurons express the ERG-1 and ERG-3 genes (Maggio et al. 2000; Saganich et al. 2001). In conclusion, our results show that Purkinje neurons express ERG channels that are functional and have similar properties as ERG currents described in other cellular preparations.

\textbf{Membrane excitability and firing pattern}

The role of $I_{\text{K(ERG)}}$ in immature Purkinje neurons is clearly different from the role in adult ones. In immature Purkinje neurons, $I_{\text{K(ERG)}}$ gives a negligible contribution to the resting potential. The repolarization of the first evoked action potential was also not affected by $I_{\text{K(ERG)}}$ block, as expected from the slow activation kinetics of ERG channels, that necessitates repetitive firing to accumulate enough open-state ERG channels to participate to spike repolarization (Schönherr et al. 1999). Immature Purkinje neurons often fired a single full action potential even in response to long depolarizing steps. Our results show that, in cells with this type of firing, $I_{\text{K(ERG)}}$ does not contribute to the repolarization of the only action potential fired. However, one Purkinje neuron was capable to produce a sustained discharge, and in this cell an analysis of the tenth action potential of evoked firing suggested a contribution of $I_{\text{K(ERG)}}$ in repolarization. In contrast, in adult Purkinje neurons action potential repolarization was not affected by block of $I_{\text{K(ERG)}}$, even when the tenth spike was analyzed. This difference can be explained by the shorter duration of action potentials in adult Purkinje neurons (0.7 ms) relative to immature ones (2.5 ms). Previous studies, which found a contribution of $I_{\text{K(ERG)}}$ in spike repolarization, were conducted on cells that fired action potentials with durations markedly longer than typical central nervous system neurons (Chiesa et al. 1997; Schönherr et al. 1999).
The roles of $I_{K(ERG)}$ in the control of the properties of action potential firing were investigated only in adult Purkinje neurons, because at the immature age at which currents were recorded most cells had not yet developed a sustained discharge. In adult Purkinje neurons with a pronounced frequency adaptation, the responses to depolarizing steps starting from $-70$ mV were clearly affected by the $I_{K(ERG)}$ blocker. The reduction of the first spike latency and the enhancement of the firing frequency caused by WAY-123,398 application, indicate a significant role of $I_{K(ERG)}$ in the control of membrane excitability. These results suggests that in adult Purkinje neurons, when they are depolarized to fire action potentials, the membrane excitability is inhibited by the accumulation of open-state ERG channels (Schönherr et al. 1999). This action of $I_{K(ERG)}$ on membrane excitability has also been observed in other cell types like neuron-neuroblastoma hybrid cells (Chiesa et al. 1997). In vivo, during active movements, the mossy and the climbing fiber afferences to Purkinje neurons evoke brief bursts of action potential discharge which are the signal that, via deep cerebellar nuclei, impinge on motor centers as a cerebellar contribution to motor commands (Ito 1984). Therefore, the control exerted by $I_{K(ERG)}$ on the repetitive action potential firing elicited by depolarizing current injections is likely present also in physiological conditions.

The information contained in neuronal discharge also depends on the type of firing pattern, like spike frequency adaptation or acceleration. In neuron-neuroblastoma hybrid cells the addition of $I_{K(ERG)}$ has been shown to produce spike frequency adaptation (Chiesa et al. 1997). Such a role in a central neuron represents a mechanism of the production of a firing pattern, which in the case of Purkinje neurons is involved in movement control. Our finding that the block of $I_{K(ERG)}$ by WAY-123,398 can largely suppress spike frequency adaptation in Purkinje neurons indicates that this current is one mechanism that is physiologically active in shaping the firing pattern in this cell type, in competition with others like the spike acceleration due to an $I_{K(A)}$ current (Hounsgaard and Midtgaard 1988). It is interesting to note that, in Purkinje neurons, similarly to $I_{K(ERG)}$, also the subthreshold activated $I_{K(A)}$. 

which might be responsible for spike acceleration, seems not to be involved in action potential repolarization (Sacco and Tempia 2002). Several mechanisms of modulation of ion channels involved in these two contrasting effects would easily shift the firing pattern from regular spiking to an accelerating or adapting one. Several examples of $I_{K(ERG)}$ modulation have recently been described in lactotrophs (Schledermann et al. 2001), in cardiac myocytes (Heath and Terrar 2000) and in a heterologous system (Cui et al. 2000). Therefore, the identification of the modulation mechanisms of $I_{K(ERG)}$ acting in cerebellar Purkinje neurons will help to understand how the firing pattern in these neurons can be shifted from one mode of discharge to another.

**Complex spike modulation**

The complex spike is composed by several spikelets and has a duration longer than simple spikes, similarly to Ca$^{2+}$ action potentials in endocrine cells. These properties could allow a sufficient activation of ERG channels (Schönherr et al. 1999). Our results show for the first time that also a single postsynaptic event, the Purkinje neuron complex spike, is large and long enough to produce an accumulation of open ERG channels sufficient to exert an effect on membrane repolarization. Since complex spikes fired by Pukinje neurons are involved in the dynamic control of movements (Welsh et al. 1995), our finding that an ERG blocker can add a spikelet to the complex spike suggests that $I_{K(ERG)}$ contributes to the signal processing involved in cerebellar motor control. Furthermore, since the complex spike-elicited intradendritic [Ca$^{2+}$], increase is strictly required for the induction of long-term synaptic plasticity at the parallel fiber-Purkinje neuron synapse (Ito 2001), the modulation exerted by the ERG current on the complex spike is likely to influence also the induction of such a change in synaptic efficacy. Taken together, the effects of $I_{K(ERG)}$ on excitability, firing pattern and complex spike modulation suggest that in Purkinje neurons this current is one important determinant of signal
processing with possible consequences on dynamic and plastic properties. These results open the way to studies on the physiological roles of $I_{K(ERG)}$ in other types of central nervous system neurons.

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FIGURE LEGENDS

Fig. 1. Identification of an ERG-like component in tail currents of cerebellar Purkinje neurons
A: Currents evoked by voltage pulses (250 ms) to potentials between –20 and –120 mV, in 20 mV increments, following steps (5 s) to 0 mV. External K+ concentration was 40 mM, [Ca²⁺]o was 2 mM and [Mg²⁺]o was 1 mM.
B: Same currents as in A, but during application of the ERG channel blocker WAY-123,398 (1µM).
C: WAY-123,398 sensitive current obtained by subtraction of B from A

Fig. 2. Effects of ZD7288 on membrane currents of cerebellar Purkinje neurons.
A: Currents evoked by steps from a V_H of –60 mV to voltages from –80 to 0 mV, followed by a voltage pulse to –120 mV. Traces obtained before (blue) and during (red) application of ZD7288 (10 µM) are superimposed. Note the large outward currents evoked by steps from –20 to 0 mV and the block by ZD7288 of the slow component of tail currents. External K⁺ concentration was 40 mM, but external Ca²⁺ was substituted by Mg²⁺.
B: Tail currents of panel A are shown on an expanded time scale to visualize the fast and slow components (control: blue traces) and the block of the slow component by ZD7288 (red traces).
C: Peak amplitudes of the fast component of tail currents plotted versus the pulse potential. Blue circles and solid line: control. Red squares and dashed line: ZD7288.
D: Normalized peak currents of the fast component of tail currents plotted versus the pulse potential. Symbols are the same of panel C.

Fig. 3. Activation curve of I_{K(ERG)} isolated by application of the I_H blocker ZD7288
A: Tail currents evoked by voltage steps to –120 mV from potentials between 0 and –80 mV in 10 mV increments, during application of the $I_h$ blocker ZD7288 (10 µM).

B: Same protocol as in A, after addition of the ERG channel blocker WAY-123,398 (1 µM). Note the great attenuation of tail currents.

C: Further isolation of the WAY-123,398 sensitive component by subtraction of B from A.

D: $I_{K(ERG)}$ activation curve, obtained from 8 Purkinje neurons, constructed by dividing the peak amplitude of the WAY-123,398 sensitive component of tail currents at –120 mV by the peak amplitude after the pulse to 0 mV. The fitting curve is a Boltzmann function with a $V_{1/2}$ of –50.7 mV and a slope factor of 5.6 mV. Error bars are s.e.m.

Fig. 4. Inactivation curve and deactivation kinetics of $I_{K(ERG)}$ isolated by application of the $I_h$ blocker ZD7288

A: Tail currents evoked by voltage steps from 0 mV to potentials between 0 and –120 mV in 10 mV increments, during application of the $I_h$ blocker ZD7288 (10 µM).

B: Same protocol as in A, after addition of the ERG channel blocker WAY-123,398 (1 µM). Note the almost complete elimination of the fast developing, decaying component. During block of $I_h$ and $I_{K(ERG)}$ a third component of inward currents is isolated. Note that these currents are dominated by a sustained component, indicating that they are mainly due to channels with essentially passive properties.

C: Isolation of the WAY-123,398 sensitive component by subtraction of B from A.

D: $I_{K(ERG)}$ quasi steady-state inactivation curve, obtained from 6 Purkinje neurons, constructed by dividing the initial amplitude, extrapolated at +1 ms, of the WAY-123,398 sensitive component of tail currents by the driving force (see text for details). The fitting curve is a Boltzmann function with a $V_{1/2}$ of –70.9 mV and a slope factor of 23.9 mV.
E: Semi-logarithmic plot of time constants of deactivation of $I_{K(ERG)}$ versus membrane potential. Between $-120$ and $-100$ mV the decay of $I_{K(ERG)}$ was fitted by double exponential functions, while between $-90$ and $-60$ mV the time course followed single exponential functions. Dashed lines are linear best fitting curves. Error bars in D and E are s.e.m.

Fig. 5. Sensitivity of $I_{K(ERG)}$ to haloperidol

A: Tail currents evoked, as in Fig. 4A, by voltage steps from 0 mV to potentials between -30 and $-120$ mV in 10 mV increments, during application of the $I_h$ blocker ZD7288 (10 µM).

B: Same protocol as in A, after addition of haloperidol (2 µM). Note the great attenuation of the currents.

C: Isolation of the haloperidol sensitive component by subtraction of B from A.

D: Quasi steady-state inactivation curve of the haloperidol sensitive current, constructed as in Fig. 4D ($n = 4$). The dotted line is best fitting Boltzmann function, with a $V_{1/2}$ of $-68.6$ mV and a slope factor of 21.6. For comparison, the best fitting Boltzmann function of the WAY-123,398 sensitive current shown in Fig. 4D is also drawn (dashed line).

E: Semi-logarithmic plot of time constants of deactivation of the haloperidol sensitive current versus membrane potential. Fast time constants are measures from 4 cells, while slow time constants are from one cell since the deactivation of the other 3 cells was fitted by a single exponential function. For comparison, the best fitting curves of the WAY-123,398 sensitive current shown in Fig. 4E are drawn as dashed lines. Error bars in D and E are s.e.m.

Fig. 6: Current clamp recordings from immature Purkinje neurons

First spike of evoked discharge before (solid trace) and during (dashed trace) WAY-123,398 application.
Fig. 7. Evidence of $I_{K(ERG)}$ presence in adult Purkinje neurons

A-B: Tail currents evoked by voltage steps from 0 to –80, -100 and –120 mV in control conditions (A) and during application of WAY-123,398 (B). Arrows point to an early current that is inhibited by WAY-123,398.

C: WAY-123,398 sensitive current obtained by subtraction of B from A.

Fig. 8. Lack of effect of WAY-123,398 on action potential repolarization in adult Purkinje neurons

A: First action potential of evoked discharge before (solid trace) and during (dashed trace) WAY-123,398 application. The traces have been aligned for better comparison.

B: Aligned tenth action potentials of evoked discharge.

Fig. 9. Effects of WAY-123,398 on action potential discharge of adult Purkinje neurons

A: Repetitive firing evoked by long depolarizing pulses (200 pA, 5 s). Upper trace: control; lower trace: WAY-123,398. Spike sizes are not uniform because the low sampling frequency caused undersampling.

B: Plot of the instantaneous frequency versus the interspike intervals in which it has been measured (interspike #), derived from the record shown in panel A. Note that the instantaneous frequency of the first 10 to 15 spikes decays until reaching a sustained level. Empty circles: control; filled squares: WAY-123,398.

C: Firing evoked by depolarizing pulses (200 pA, 500 ms). Note the shorter first spike latency in WAY-123,398 and the stronger frequency adaptation in the control.
D: Analysis of the instantaneous frequency of one Purkinje neuron, performed on 5 pairs of traces obtained as in C. Symbols are as in B. Note the higher initial frequency and the less steep decay in WAY-123,398. Error bars are s.e.m.

Fig. 10. Complex spikes evoked by paired-pulse stimulation of presynaptic climbing fibers.

A. In control external solution.

B. During WAY-123,398 application (1 µM).

C. First complex spikes of paired pulse stimulations shown in A (solid trace) and B (dashed trace) shown on an expanded time scale. The complex spikes are preceded by antidromic spikes (arrow). Symbols (open circles for the control and filled squares for WAY-123,398) are drawn on each spikelet of which the complex spikes are composed.

D. Second complex spikes of paired pulse stimulations displayed as in C. The complex spikes are preceded by antidromic spikes (arrow). Note the presence, during WAY-123,398 application (dashed traces), of an additional spikelet.
<table>
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<th>NOT ADAPTING CELLS</th>
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* = P < 0.05

** = P < 0.01
Figure 1
Figure 2
Figure 5
Figure 7
A  
1st action potential

B  
10th action potential

Figure 8
Figure 10

A

control

B

WAY-123,398