Functional Roles of an ERG Current Isolated in Cerebellar Purkinje Neurons

Tiziana Sacco, Alessandro Bruno, Enzo Wanke, and Filippo Tempia

1Department of Internal Medicine, Section of Human Physiology, University of Perugia, I-06126 Perugia; and 2Department of Biotechnology and Biosciences, University of Milano-Bicocca, I-20126 Milan, Italy

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Sacco, Tiziana, Alessandro Bruno, Enzo Wanke, and Filippo Tempia. Functional roles of an ERG current isolated in cerebellar Purkinje neurons. J Neurophysiol 90: 1817–1828, 2003. First published May 15, 2003; 10.1152/jn.00104.2003. Transcripts encoding ERG potassium channels are expressed by most neurons of the CNS. 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 [\(I_{\text{K(ERG)}}\)] with the same characteristics as previously described for ERG channels. In zero external Ca\(^{2+}\) and high K\(^+\) (40 mM) the \(V_{\text{1/2}}\) of activation was \(-50.7\) mV, the \(V_{\text{1/2}}\) of inactivation was \(-70.6\) mV, and the deactivation rate was double exponential and voltage dependent. \(I_{\text{K(ERG)}}\) was 93.0% blocked by WAY-123,398 (1 \(\mu\)M) and 78.2% by haloperidol (2 \(\mu\)M). The role of \(I_{\text{K(ERG)}}\) 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. \(I_{\text{K(ERG)}}\) block caused an increase of the number of spikelets of the “complex spike.” These data show, for the first time, an \(I_{\text{K(ERG)}}\) in a neuron of the CNS, 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 hippocampal cells (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 \([I_{\text{K(ERG)}}]\) has been studied extensively only in nonneuronal cells. In cardiac myocytes \(I_{\text{K(ERG)}}\) has been shown to correspond to the rapidly activating component of the delayed rectifier current \([I_{\text{Kd}}]\) (Sanguinetti et al. 1995) that contributes to the repolarization of the action potential (Sanguinetti and Jurkiewicz 1990; Shibasaki 1987). 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—fast inactivation and slow activation gates—confer to \(I_{\text{K(ERG)}}\) 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), another two genes have been identified (ERG2 and ERG3; Shi et al. 1997). In mammals, in spite of the abundant expression of the 3 ERG genes also in the CNS (Maggio et al. 2000; Saganich et al. 2001; Shi et al. 1997; Wymore et al. 1997), to the best of our knowledge \(I_{\text{K(ERG)}}\) has been studied only in nonneuronal cells, in neuroblasts, or in peripheral 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 that 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. \(I_{\text{K(ERG)}}\) 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 \(I_{\text{K(ERG)}}\) 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 (Akkarali et al. 1999). Therefore it seems reasonable that, if Purkinje neurons express \(I_{\text{K(ERG)}}\), this can have a modulatory action on the complex spike. In this study we report for the first time the identification in a central neuron of an \(I_{\text{K(ERG)}}\)-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).

METHODS

Whole cell K\(^+\) currents were recorded in voltage-clamp (VC) 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 (Linas and Sugimori 1980; Sacco and Tempia 2002), kept for 1 h at 35°C and then at room temp...
temperature in the extracellular saline solution containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 20 glucose, bubbled with 95% O₂-5% CO₂ (pH 7.4). The recording chamber was continuously perfused with the saline solution, bubbled with 95% O₂ and 5% CO₂. 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 MgCl₂, 0.5 EGTA, –80 mV, followed by a voltage pulse to –120 mV, followed by a voltage pulse to 120 mV. 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 90%.

The activation and inactivation curves were fitted with the Boltzmann equation

\[ I(V) = I_0 \left( \frac{1}{1 + \exp \left( \frac{V - V_1/2}{k} \right)} \right) + I_1 \]

where \( I(V) \) is the peak current as a function of \( V \); \( I_1 \) 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; \( V_{1/2} \) is the voltage at which the current is half of the maximal amplitude; and \( k \) represents the slope factor. The decay of \( I_{KERG} \), attributed to channel deactivation, was fitted either by a monoexponential equation

\[ I(t) = I(t_0) \left( \exp \left( -t/t_1 \right) \right) + I_0 \]

where \( I(t_0) \) 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.

![Diagram](image-url)
\[ I_{o} = [I_{[\exp(-\Delta\tau_{2})]} + [I_{[\exp(-\Delta\tau_{2})]}] + I_{o} \] 

where \( I_{o} \) is the total current at time \( t \); \( I_{1} \) and \( I_{2} \) are the initial amplitudes of the two components related to \( \tau_{1} \) and \( \tau_{2} \), which are, respectively, the slow and the fast decay time constants; and \( I_{o} \) is the non-inactivating residual current.

Because the ERG channel shows inward rectification and its conductance is directly related to the external K\(^+\) concentration [K\(^+\)], 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 −50 mV). Osomolarity was kept constant by lowering the 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. To this solution was added tetrodotoxin citrate (TTX: 1 \( \mu \)M) to block Na\(^+\) channels and bicuculline methochloride (20 \( \mu \)M) to block spontaneous postsynaptic currents mediated by GABA\(_{A}\) receptors. This solution, with physiological divalent cation 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 (Figs. 2–5 and 7) CaCl\(_{2}\) was substituted with MgCl\(_{2}\) (final MgCl\(_{2}\) concentration: 3 mM) and ZD7288 (10 \( \mu \)M) was added. In VC, 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 \( \mu \)M) to block GABA\(_{A}\) receptors. 50 \( \mu \)M D-(−)-2-amino-5-phosphonoantoc acid (D-AP5) to block NMDA-type glutamate receptors and 10 \( \mu \)M 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzof[1]quinoxaline-7-sulfonamide 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 \( \mu \)M) and haloperidol (2 \( \mu \)M) 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).

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 [Ca\(^{2+}\)]\(_{o}\) concentration. The membrane potential was kept at −60 mV and every 30 s (or longer) it was stepped for 5 s 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, whereas 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 \( \mu \)M) was added to the slice perfusion solution, the early component of the inward K\(^+\) current virtually disappeared, whereas 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 following text). 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 SE; \( n = 4 \)) and a fast onset. The rate of deactivation rapidly became faster moving toward more hyperpolarized potentials. These properties are very similar to the \( I_{K(ERG)} \) shown in other cell types (Faravelli et al. 1996; Shibasaki 1987).

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 (Ca\(^{2+}\) was substituted by Mg\(^{2+}\)) to which was added tetrodotoxin (1 \( \mu \)M) to block Na\(^+\) currents and bicuculline (20 \( \mu \)M) to block spontaneous postsynaptic currents mediated by GABA\(_{A}\) receptors and the organic blocker ZD7288 (10 \( \mu \)M) 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. 1, A and B). ZD7288 (10 \( \mu \)M) almost completely blocked within 5 min 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. 2, A and B) was performed on currents recorded after ≥5 min of ZD7288 application. Because \( I_{K(ERG)} \) 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 SE; \( n = 4 \)). 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 \( \mu \)M completely blocks \( I_{H} \) and inhibits \( I_{K(ERG)} \) by about 11%, 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 \( \mu \)M) was systematically used in all the following experiments to characterize the biophysical properties of \( I_{K(ERG)} \) 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, which 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
Because the sustained current remaining at the end of the transient component was not affected by the step potential (Fig. 3A) and because 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% SE; 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 $I_{\text{K(ERG)}}$, 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 SE; n = 6). This value is significantly smaller than 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 cation contents 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 $I_{\text{K(ERG)}}$ (Faravelli et al. 1996).

The activation curves of $I_{\text{K(ERG)}}$, 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 SE; n = 8) and a slope factor of 5.6 mV (±0.4 SE; n = 8). The dashed line in Fig. 3D is a Boltzmann curve constructed using these average values.

To study the voltage dependency of the quasi steady-state inactivation of $I_{\text{K(ERG)}}$, the voltage was stepped for 5 s 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. 4, A–C). Because ERG channels at potentials where they recover from inactivation also deactivate, the decay of tail currents—attributed 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 attributed 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 $V_{1/2}$ value of −70.6 mV (±4.8 SE; n = 6) and a slope factor of 23.9 mV (±3.4 mV SE; 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 dependency of both time constants (Fig. 4E; n = 6). At more depolarized potentials the deactivation was fitted by single-exponential functions. In a semilogarithmic plot, the voltage dependency of deactivation time constants was linear, with the single-exponential time constants lying in the same line as $\tau_d$ 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). Because no evidence is available on the effects of haloperidol on $I_{\text{K(ERG)}}$, in any CNS neuron, we tested this compound on the $I_{\text{K(ERG)}}$ 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 $I_{\text{K(ERG)}}$ after 10 min of application (±4.5% SE; n = 4; Fig. 5, A and B). The peak amplitude of the

**FIG. 3.** Activation curve of $I_{\text{K(ERG)}}$ isolated by application of $I_h$ 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 $I_h$ blocker ZD7288 (10 μM). B: same protocol as in A, after addition of ERG channel blocker WAY-123,398 (1 μM). Note great attenuation of tail currents. C: further isolation of WAY-123,398-sensitive component by subtraction of $B$ from $A$. D: $I_{\text{K(ERG)}}$, activation curve, obtained from 8 Purkinje neurons, constructed by dividing peak amplitude of WAY-123,398-sensitive component of tail currents at −120 mV by peak amplitude after pulse to 0 mV. Fitting curve is Boltzmann function with $V_{1/2}$ value of −50.7 mV and slope factor of 5.6 mV. Error bars are SE.
haloperidol-sensitive current obtained by voltage steps from 0 to −120 mV was 0.77 nA (±0.09 SE; \( n = 4 \)).

Tail currents obtained by subtraction of the haloperidol-resistant component (Fig. 5C) were studied to obtain the voltage dependency of the quasi steady-state inactivation of \( I_{\text{K(ERG)}} \) (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 \( V_{1/2} \) value of −68.6 mV (±3.3 SE; \( n = 4 \)) and a slope factor of 21.6 mV (±4.9 SE; \( n = 4 \)). 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). Moreover, the time constants of deactivation of the haloperidol-sensitive current (Fig. 5E, data points) displayed similar values and a similar voltage dependency as that of the WAY-123,398–sensitive current (Fig. 5, dashed lines).

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

To study the physiological roles of \( I_{\text{K(ERG)}} \) in Purkinje neurons, whole cell CC 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 CC 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 attributed to blocking of \( I_{\text{K(ERG)}} \) 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 SE; \( n = 10 \)). 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 SE; \( n = 10 \)); Student’s paired t-test: \( P > 0.05 \)]. The input resistance (\( R_{\text{in}} \)), 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 SE; \( n = 10 \)) in the control solution containing the synaptic receptor blockers. Application of WAY-123,398 slightly increased \( R_{\text{in}} \) to 232.5 MΩ (±24.6 SE; \( n = 10 \); 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, which in some cases was followed by inactivating spikelets of 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, given that \( I_{K(ERG)} \) 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), although 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 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 Crepel 1972; Woodward et al. 1969). 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 \([K^+]_r\), 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 potentials between −30 and −120 mV in 10-mV increments, during application of \( I_{Na} \) blocker ZD7288 (10 \( \mu \)M). \( B \); same protocol as in \( A \), after addition of haloperidol (2 \( \mu \)M). Note great attenuation of currents. \( C \); isolation of haloperidol-sensitive component by subtraction of \( B \) from \( A \). \( D \); quasi steady-state inactivation curve of haloperidol-sensitive current, constructed as in Fig. 4D (\( n = 4 \)). Dotted line is best-fitting Boltzmann function, with \( V_{1/2} \) value of −68.6 mV and slope factor of 21.6. For comparison, best-fitting Boltzmann function of WAY-123,398-sensitive current shown in Fig. 4D is also drawn (dashed line). \( E \); semilogarithmic plot of time constants of deactivation of haloperidol-sensitive current vs. membrane potential. Fast time constants are measures from 4 cells, whereas slow time constants are from one cell because deactivation of other 3 cells was fitted by single-exponential function. For comparison, best-fitting curves of WAY-123,398-sensitive current shown in Fig. 4E are drawn as dashed lines. Error bars in \( D \) and \( E \) are SE.
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 [(means ± SE) control: 161.6 ± 21.4 MΩ; WAY-123,398: 162.0 ± 17.9 MΩ; n = 3]. 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 s 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 did not significantly affect either the membrane potential (potential difference in WAY-123,398: +0.9 ± 0.3 mV SE, n = 4; Student’s paired t-test: P > 0.05) or R_{in} (control: 90.8 ± 7.8 MΩ, n = 4; WAY-123,398: 95.4 ± 9.3 MΩ, n = 4; Student’s paired t-test: P > 0.05). The application of WAY-123,398 did not affect the duration either 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) or 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 that of immature ones (about 2.5 ms).

To study the role of $I_{KERG}$ 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. 9, A and 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 ≥10 spikes in most of the cells (Fig. 9, C and D). This analysis (Table 1) 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, after 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 1). 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 1). 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, whereas 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 1). Thus these results show that Purkinje neurons that display a frequency adaptation greater than 3 Hz/spike are significantly affected by WAY-123,398, indicating that in such cells $I_{KERG}$ is involved in the control of excitability, firing frequency, and frequency adaptation.

The climbing fiber postsynaptic potential is controlled by $I_{KERG}$

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. 10, A and 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. 10, B and C). On average the number of spikelets in control complex spikes was 4.1 (±0.6 SE; n =

FIG. 7. Evidence of $I_{KERG}$ presence in adult Purkinje neurons. A and 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 early current 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. Traces have been aligned for better comparison. B: aligned tenth action potentials of evoked discharge.
5), whereas during WAY-123,398 application it increased to 4.7 (±0.4 SE; n = 5). The difference was statistically significant (paired Student’s t-test: P < 0.05). The same effect could also be observed 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\(^{\text{ERG}}\)]. 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; Bianchi et al. 1998; Faravelli et al. 1996). Because neurons express a huge repertoire of ion channels, to detect and characterize I\(^{\text{ERG}}\) 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 Ca²⁺ 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\(^{\text{ERG}}\) by using an extracellular solution with a high \([K^+]_o\) and with nominally zero \([Ca^{2+}]_o\). The absence of external Ca²⁺ allowed us to eliminate Ca²⁺ currents and Ca²⁺-dependent currents.

In the conditions used in these experiments, the inward current of Purkinje neurons consists of 3 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 study as I\(^{\text{ERG}}\) 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 $I_K$ 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 Figs. 2–5), providing us with the possibility of characterizing $I_{K(ERG)}$ independently from its pharmacological profile.

The activation curve, with a $V_{1/2}$ value of $-50.7 \pm 1.2$ 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). In addition cloned rat ERG2 and ERG3 channels studied in a low $[K^+]_o$ and physiological $[Ca^{2+}]_o$ display less negative $V_{1/2}$ activation values, respectively, of $-3.5$ and $-44.0$ mV (Shi et al. 1997).

In the same study, the HERG channel had a $V_{1/2}$ activation value of $-21$ mV. Cloned mouse ERG1a (mERG1a) also has a $V_{1/2}$ activation value 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); and 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), whereas 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, whereas 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 as 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

### Table 1. Effects of WAY-123,398 on firing properties of adult Purkinje neurons

<table>
<thead>
<tr>
<th></th>
<th>Cell 81</th>
<th>Cell 87</th>
<th>Cell 90</th>
<th>Cell 86</th>
<th>Cell 88</th>
<th>Cell 85</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First spike latency</strong></td>
<td>41.3 ± 0.5</td>
<td>73.9 ± 1.1</td>
<td>41.5 ± 2.1</td>
<td>153.0 ± 11.8</td>
<td>170.7 ± 6.7</td>
<td>159.9 ± 8.2</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>*</td>
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<tr>
<td><strong>Number of spikes</strong></td>
<td>14.0 ± 0.4</td>
<td>9.6 ± 0.4</td>
<td>13.5 ± 3.3</td>
<td>5.5 ± 1.0</td>
<td>10.1 ± 0.5</td>
<td>11.2 ± 0.6</td>
</tr>
<tr>
<td>in 500 ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>WAY</strong></td>
<td>25.1 ± 2.6</td>
<td>21.4 ± 1.1</td>
<td>26.5 ± 2.9</td>
<td>7.4 ± 0.5</td>
<td>11.0 ± 0.6</td>
<td>11.2 ± 0.5</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>First interspike inst. freq. (Hz)</strong></td>
<td>61.6 ± 1.5</td>
<td>42.1 ± 4.1</td>
<td>71.8 ± 3.6</td>
<td>18.8 ± 4.4</td>
<td>32.8 ± 1.6</td>
<td>28.4 ± 1.3</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>*</td>
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<td>*</td>
<td>n.s.</td>
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<td>n.s.</td>
</tr>
<tr>
<td><strong>Ninth interspike inst. freq. (Hz)</strong></td>
<td>33.4 ± 3.5</td>
<td>13.1 ± 0.7</td>
<td>43.8 ± 10.2</td>
<td>5.9 ± 1.0</td>
<td>36.5 ± 2.2</td>
<td>23.4 ± 2.6</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
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<td>n.s.</td>
</tr>
<tr>
<td><strong>Adapt. Slope (Hz/ intersp.)</strong></td>
<td>-3.69 ± 0.30</td>
<td>-3.20 ± 0.14</td>
<td>-4.28 ± 0.35</td>
<td>-1.82 ± 0.20</td>
<td>-0.62 ± 0.10</td>
<td>+0.50 ± 0.04</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
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<tr>
<td><strong>N</strong></td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>6</td>
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</table>

Values are means ± SE; n = number of cells. Contr; control; WAY, WAY-123,398. *P < 0.05; **P < 0.01; n.s., not significant.
deactivation data support the identification of the current described in this study 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 attributable to the expression in Purkinje neurons of ERG3 in addition to ERG1 (Saganich et al. 2001), given that rat ERG3 activation has a relatively steep slope of 7.3 mV and faster kinetics than that of ERG1 (Shi et al. 1997). Other possible reasons of the differences in biophysical properties in different tissues are the different expressions 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 \( V_{1/2} \) value 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 toward more negative values (Shi et al. 1997; Smith et al. 1996), 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 (Faravelli et al. 1996; Spinelli et al. 1993), being ineffective on other types of \( K^+ \) current including \( I_{K(A)} \), \( I_{K(Ca)} \), and \( I_{K(Ca)} \), and other members of the eag family such as ELK2 (Becchetti et al. 2002; Rosati et al. 2000; unpublished data). Recent data indicate that the widely used antipsychotic drug haloperidol is also a blocker of 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 CNS suggests that some of the neurotropic effects of haloperidol could be explained by a direct block of ERG channels in brain regions where \( I_{K(ERG)} \) is involved in the control of cell firing.

The presence of functional ERG channels in cerebellar Purkinje neurons is in agreement with in situ hybridization 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.

Membrane excitability and firing pattern

The role of \( I_{K(ERG)} \) in immature Purkinje neurons is clearly different from the role in adult neurons. In immature Purkinje neurons, \( I_{K(ERG)} \) gives a negligible contribution to the resting potential. The repolarization of the first evoked action potential was also not affected by \( I_{K(ERG)} \) block, as expected from the slow activation kinetics of ERG channels, which necessitates repetitive firing to accumulate enough open-state ERG channels to participate in 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, \( I_{K(ERG)} \) does not contribute to the repolarization of the only action potential fired. However, one Purkinje neuron was capable of producing a sustained discharge, and in this cell an analysis of the tenth action potential of evoked firing suggested a contribution of \( I_{K(ERG)} \) in repolarization. In contrast, in adult Purkinje neurons action potential repolarization was not affected by block of \( I_{K(ERG)} \), 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 that of immature ones (2.5 ms). Previous studies, which found a contribution of \( I_{K(ERG)} \) in spike repolarization, were conducted on cells that fired action potentials with durations markedly longer than those of typical CNS 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 suggest 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 such as neuron-neuroblastoma hybrid cells (Chiesa et al. 1997). In vivo, during active movements, the mossy and the climbing fiber afferrences to Purkinje neurons evoke brief bursts of action potential discharge, which constitute the signal that, through deep cerebellar nuclei, impinges 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 also likely present 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 and 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 attributed to an \( I_{K(A)} \) current (Hounsgaard and Midtgaard 1988). It is interesting to note that, in Purkinje neurons, similarly to \( I_{K(ERG)} \), the subthreshold activated \( I_{K(AP)} \) which might be responsible for spike acceleration, also seems not to be involved in action potential repolarization (Sacco and
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 of several spikelets and has a duration longer than that of simple spikes, similar to Ca$^{2+}$ action potentials in endocrine cells. These properties could allow a sufficient activation of ERG channels (Schoenherr et al. 1999). Our results show for the first time that a single postsynaptic event—the Purkinje neuron complex spike—is also large and long enough to produce an accumulation of open ERG channels sufficient to exert an effect on membrane repolarization. Because complex spikes fired by Purkinje neurons are involved in the dynamic control of movements (Welsh et al. 2000), 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, given that the complex spike–elicited intradendritic [Ca$^{2+}$]$_i$ 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 also likely to influence 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 CNS neurons.

**DISCLOSURES**

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Cui J, Melman Y, Palma E, Fishman GI, and McDonald TV. Cyclic AMP activates ERG channels (Schoenherr et al. 2001). 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.

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