A novel role for HERG K^+ channels: spike-frequency adaptation

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1. The regular firing of a Hodgkin–Huxley neurone endowed with fast Na^+ and delayed K^+ channels can be converted into adapting firing by appending HERG (human *eag*-related gene) channels.

2. The computer model predictions were verified by studying the firing properties of F-11 DRG neurone × neuroblastoma hybrid cells induced to differentiate by long-term exposure to retinoic acid. These cells, which express HERG currents (I_{HERG}), show clear spike-frequency adaptation of their firing when current clamped with long depolarizations.

3. In agreement with the prediction, the selective blocking of I_{HERG} by class III antiarrhythmic drugs always led to the disappearance of the spike-frequency adaptation, and the conversion of adapting firing to regular firing.

4. It is proposed that, in addition to their role in the repolarization of the heart action potential, HERG channels may sustain a process of spike-frequency adaptation, and hence contribute to the control of burst duration in a way that is similar to that of the K^+ currents, I_{AHP}, I_C and I_M. In addition to the known cardiac arrhythmia syndrome (LQT2), genetic mutations or an altered HERG expression could lead to continuous hyperexcitable states sustained by the inability of nerve or endocrine cells to accommodate to repetitive stimuli. This might help in clarifying the pathogenesis of still undefined idiopathic familial epilepsies.

Spike-frequency adaptation is a property shown by a large number of neurones in the central and peripheral nervous system (Shepherd, 1992). Hypothalamic secreting cells, as well as pituitary and pancreatic β-cells, physiologically regulate hormone release by means of a spontaneous bursting pattern whose bursts and silent periods have a time scale of seconds (Cook, Satin & Hopkins, 1991). At least three K^+ currents (I_{AHP}, I_C and I_M) exert a remarkable action in producing spike-frequency adaptation (Pennefather, Jones & Adams, 1985; Jones & Adams, 1987; Brown, 1988). In brief, the role of these currents is to produce a gradual increase in a K^+ conductance that counteracts the Na^+ currents and thus leads to either a longer interspike interval or a complete block of the burst.

This role can equally be envisaged for the recently discovered I_{HERG} current (encoded by the human *eag*-related gene (*HERG*); Warmke & Ganetzky, 1994), which develops with slow kinetics during depolarization and contributes to the repolarization of the long action potentials typically present in the heart. I_{HERG} is one of the delayed rectifier currents ($I_{K(r)}$) of the heart, and *HERG* mutations are associated with one of the cardiac arrhythmia LQT syndromes (LQT2) (Curran, Splawski, Timothy, Vincent, Green & Keating, 1995; Sanguinetti, Jiang, Curran & Keating, 1995). We have previously discovered a novel current (I_{IR}) which is strongly expressed in neuroblastoma cells and has proved to be pharmacologically and biophysically equivalent to I_{HERG} (Arcangeli *et al.* 1993, 1995; Faravelli, Arcangeli, Olivotto & Wanke, 1996). Furthermore, it is encoded by a gene that is substantially the same as *HERG* (L. Bianchi *et al.* unpublished observations).

In this report, we present a theoretical and experimental approach to the assessement of the role of I_{HERG} in the firing activated by physiological patterns of membrane depolarization. The convergence of these approaches suggests that the conductance sustained by HERG channels is a novel means of inducing spike-frequency adaptation.

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METHODS

Cell cultures

F-11 clone cells (mouse neuroblastoma N18TG-2 × rat DRG; Platika, Boulos, Baizer & Fishman, 1985) were routinely cultured in Dulbecco's modified Eagle's medium (Hyclone, Milano, Italy), containing 4·5 g l^{-1} of glucose and 10% of fetal calf serum (Hyclone Milano, Italy). The cells were incubated at 37 °C in a humidified atmosphere with 10% CO_2. Retinoic acid (2 mM; Sigma) was applied for 48 h.

Solutions

The standard extracellular solution contained (mM): NaCl, 130; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; Hepes–NaOH, 10; glucose, 5; pH 7·4. The standard pipette solution with an $[Ca^{2+}]_i$ of 10^{-7} M (pCa 7) contained (mM): potassium aspartate, 120; NaCl, 10; $MgCl_2$, 2; $CaCl_2$, 4; EGTA–KOH, 10; Hepes–KOH, 10; MgATP, 3; Na_2GTP, 0·2. WAY-123,398 (applied at 2 mM and kindly provided by Dr W. Spinelli, Wyeth-Ayerst Research, Princeton, NJ, USA; see Spinelli, Moubarak, Parson & Colatsky, 1993) was dissolved in distilled water to make 10 mM stock solutions.

Patch-clamp recordings

Firing was recorded under current clamp by means of a patch-clamp amplifier developed in our laboratory (Magistretti, Mantegazza, Guatteo & Wanke, 1996) or an Axopatch 200A amplifier in Ifast mode (Axon Instruments). The currents were recorded as previously described (Arcangeli *et al.* 1993, 1995; Faravelli *et al.* 1996) and series resistance errors were compensated for to a level of up to 85–95%. During acquisition and data analysis, the pCLAMP 6.0 (Axon Instruments) and Origin 4.1 (Microcal Inc., Northampton, MA, USA) software were routinely used.

Simulations

The Axon Engineer Pro program (AEON Software, Madison, WI, USA) was used to simulate cell firing in a model cell, expressing the delayed rectifier current, I_{DR}, the fast inward Na^+ current, I_{Na}, and I_{HERG}. For I_{DR}, we used the Hodgkin–Huxley scheme plus a slow inactivation variable to describe the I_{DR} data (neuroblastoma cells) shown in Arcangeli *et al.* 1995:

$$I_{DR} = G_{DR} n^4 h (V_m - E_K),$$

where G_{DR} is the maximal conductance, V_m is the membrane potential, E_K is the Nernst potential for K^+ and n and h are activation and inactivation, respectively. For I_{Na}, we used a Hodgkin–Huxley model with:

$$I_{Na} = G_{Na} m^3 h (V_m - E_{Na}),$$

where m is the activation and h is the inactivation. For I_{HERG} we used a model shown in Faravelli *et al.* 1996:

$$I_{HERG} = G_{HERG} n(V) R(V) (V_m - E_K),$$

where $n(V)$ is the activation and $R(V)$ the inactivation. The conductance ratios $G_{DR}/G_{Na}/G_{HERG}/G_{leak}$ were 2·5/15/4/0·05. The rate constants used in the simulation were, for I_{DR}:

$$\alpha_n = 0{\cdot}01(V + 60)/\{1 - \exp[-0{\cdot}1(V + 60)]\},$$
$$\alpha_h = 0{\cdot}001\exp[-0{\cdot}04(V + 70)],$$
$$\beta_n = 0{\cdot}125\exp[-0{\cdot}0125(V + 70)],$$
$$\beta_h = 0{\cdot}001\exp[-0{\cdot}0195(V + 40)],$$

for I_{Na}:

$$\alpha_m = 0{\cdot}1(V + 40)/\{1 - \exp[-0{\cdot}09(V + 40)]\},$$
$$\alpha_h = 0{\cdot}07\exp[-0{\cdot}05(V + 70)],$$
$$\beta_m = 4\exp[-0{\cdot}055(V + 70)],$$
$$\beta_h = 1/\{1 + \exp[-0{\cdot}09(V + 25)]\},$$

and for I_{HERG}:

$$\alpha_n = 0{\cdot}09/\{1 + \exp[0{\cdot}011(V + 100)]\},$$
$$\alpha_R = 30/\{1 + \exp[0{\cdot}04(V + 230)]\},$$
$$\beta_n = 0{\cdot}00035\exp[0{\cdot}07(V + 25)],$$
$$\beta_R = 0{\cdot}15/\{1 + \exp[-0{\cdot}05(V + 120)]\}.$$

RESULTS

Computer-simulated results: firing adaptation is produced by the addition of HERG conductances in regularly firing cells

Suitable computer simulations of regularly firing cells were obtained as indicated in the Methods section. Figure 1*A–C* shows a typical pattern of responses of neurones which do not express I_{HERG} to a series of depolarizing currents increasing in amplitude (see protocols below the traces; panel *A* corresponds to an injected current just over the threshold). The effects of including I_{HERG} (see panel *J*) under these conditions are illustrated by the traces shown in Fig. 1*D–F*. Figure 1*D* shows an almost complete inhibition of firing, whereas the firing shown in panels *E* and *F* clearly indicates the presence of an accomodation-inducing process. Comparison of the next-to-last action potentials (APs) from panels *B* and *E* (shown in panel *I*) suggests that HERG channels do not substantially change the fast AP waveform but add a supplementary after-hyperpolarization.

In order to gain a further insight into the accommodation process, the traces of currents (I_{Na}, I_{DR}, I_{HERG}) during the second and last AP (from the burst shown in Fig. 1*E*) were superimposed in panels *G* and *H*, respectively (note that the vertical scales are logarithmic). It is clear that the only current that was profoundly modified during the long depolarization was I_{HERG}, and further inspection indicates that the effect is at the level of the small currents underlying the AP threshold and the after-hyperpolarization phase. On the whole, these effects resemble those produced by classical simulations of firing when the conductances associated with Ca^{2+}-activated K^+ channels or M-channels are involved (see Shepherd, 1992).

Spike-frequency adaptation is converted into regular firing by blocking the HERG currents in neuroblastoma cells

In these experiments, F-11 hybrid neuroblastoma cells were used after long-term exposure to retinoic acid (Platika *et al.* 1985), a treatment which we have found to be capable of differentiating the cells up to the production of many APs in response to long depolarizing currents. The typical responses obtained in a differentiated cell are shown in Fig. 2. The *I–V* plot illustrated in Fig. 2*A* shows the presence of fast inward Na^+ currents in the region of −20 to 10 mV, while outward K^+ currents appear to be activated beyond 15–20 mV. Under voltage clamp (see Fig. 2*B*), we verified

the fact that I_{HERG} is still present after differentiation, as it is in undifferentiated cells (Faravelli *et al.* 1996). The current-clamp stimulation of cell firing (Fig. 2*D* and *E*) was characterized by APs with high threshold levels and poor sensitivity to injected current amplitude (Fig. 2*C*). In contrast, after the application of WAY-123,398 (an I_{HERG} blocker; Faravelli *et al.* 1996), the firing pattern was characterized by regular firing (Fig. 2*G* and *H*) at frequencies clearly related to the amount of the injected stimuli, while below or around threshold (Fig. 2*F*) firing is not present, as in control. No change in the holding current was necessary to maintain the holding potential (−70 mV) after the drug application.

As these data suggest that I_{HERG} plays a significant role in the excitability of neuroblastoma cells, additional experiments were carried out in order to further our understanding of the effects of this current on the frequency of firing and adaptation. We repeated the stimuli of Fig. 2 in a more differentiated neurone, and examined the frequency of firing before (Fig. 3*A–C*) and after (Fig. 3*D–F*) the application of the I_{HERG} blocker WAY-123,398. As shown in panel *H* (data derived from panels *B* and *E*), the instantaneous firing frequency was similar under both conditions at the beginning of stimulation (7–8 Hz) but became much larger, after drug application, at the end the pulse (5·3 *vs.* 1·8 Hz). Notice also that the post-pulse hyperpolarizations present in the control disappear after drug application, mimicking the model prediction shown in Fig. 1 (panels *D–F versus A–C*). Moreover, the direct superposition of the next-to-last APs of Fig. 3*B* and *E*, shown in panel *G*, resembles the model prediction shown

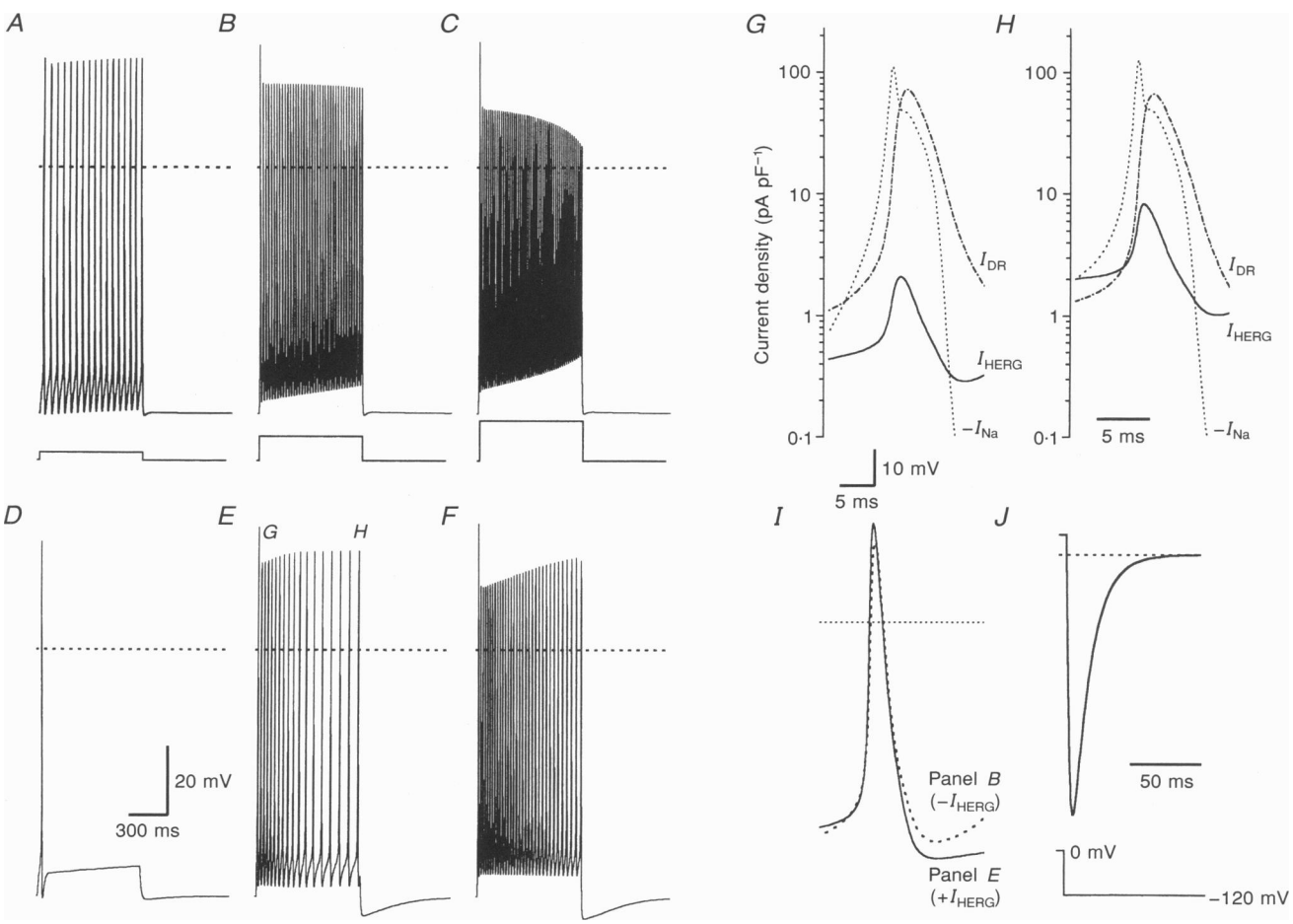

Figure 1. Prediction of firing in a model neurone with and without HERG channels

A–C, firing in a model neurone with I_{DR} and I_{Na}, but without I_{HERG} under three increasing depolarizations (to 10, 30 and 50 pA pF^{-1}; see protocols below the traces; holding potential, −72 mV). Notice the absence of adaptation. *D–F*, firing in the same model neurone as that in *A–C* but with I_{HERG} (see Methods). Note the spike-frequency adaptation. *G* and *H*, the active current densities (log scale) during the action potentials labelled *G* and *H* shown in *E*. Note the much higher I_{HERG} in the last spike (*H*) than in the second (*G*), suggesting the origin of the adaptation process. *I*, the superimposed and expanded waveforms of the next-to-last APs shown in *B* and *E*. *J*, the I_{HERG} elicited by the protocol shown below under voltage clamp (computer simulated; compare with Fig. 2*B*).

in Fig. 1*I*, suggesting that I_{HERG} underlies an after-hyperpolarization that is less strong after the drug application.

The measurements obtained in different neurones were normalized by calculating the 'percentage adaptation', *A* (the variation of firing frequency *versus* the successive spikes), according to the following equation:

$$A(i) = 100(f_{max} - f(i))/f_{max},$$

where f_{max} is the maximal firing frequency at the beginning of the pulse and $f(i)$ is the instantaneous frequency of the ith spike. The data, obtained from three other neurones, similar to those shown in Fig. 3*H*, were thus normalized to obtain the percentage adaptation as a function of the ith AP, as shown in Fig. 3*I*. In this case too the selective blocking of I_{HERG} produced neurone behaviour that was characterized by poor adaptation (20–25%), whereas the presence of I_{HERG} generated firing with more than 70% adaptation.

Taken as a whole, the data presented here suggest that the activation of I_{HERG} begins to affect firing between 0·5 and 1 s after the onset of the depolarizing plateau (which is centred around the −35 to −50 mV region); these values fit well with the I_{HERG} gating time constant, which spans a bell-shaped region around 1–10 s (see Fig. 1*C* of Arcangeli *et al.* 1995).

DISCUSSION

In this paper we present evidence supporting the proposition that a novel conductance should be included among those considered responsible for spike-frequency adaptation. Starting from the prediction generated by a model reconstruction of the effect of HERG channels in a theoretical Hodgkin–Huxley neurone, we found that HERG-expressing F-11 cells both with and without block of the HERG channels behave exactly as predicted by theory.

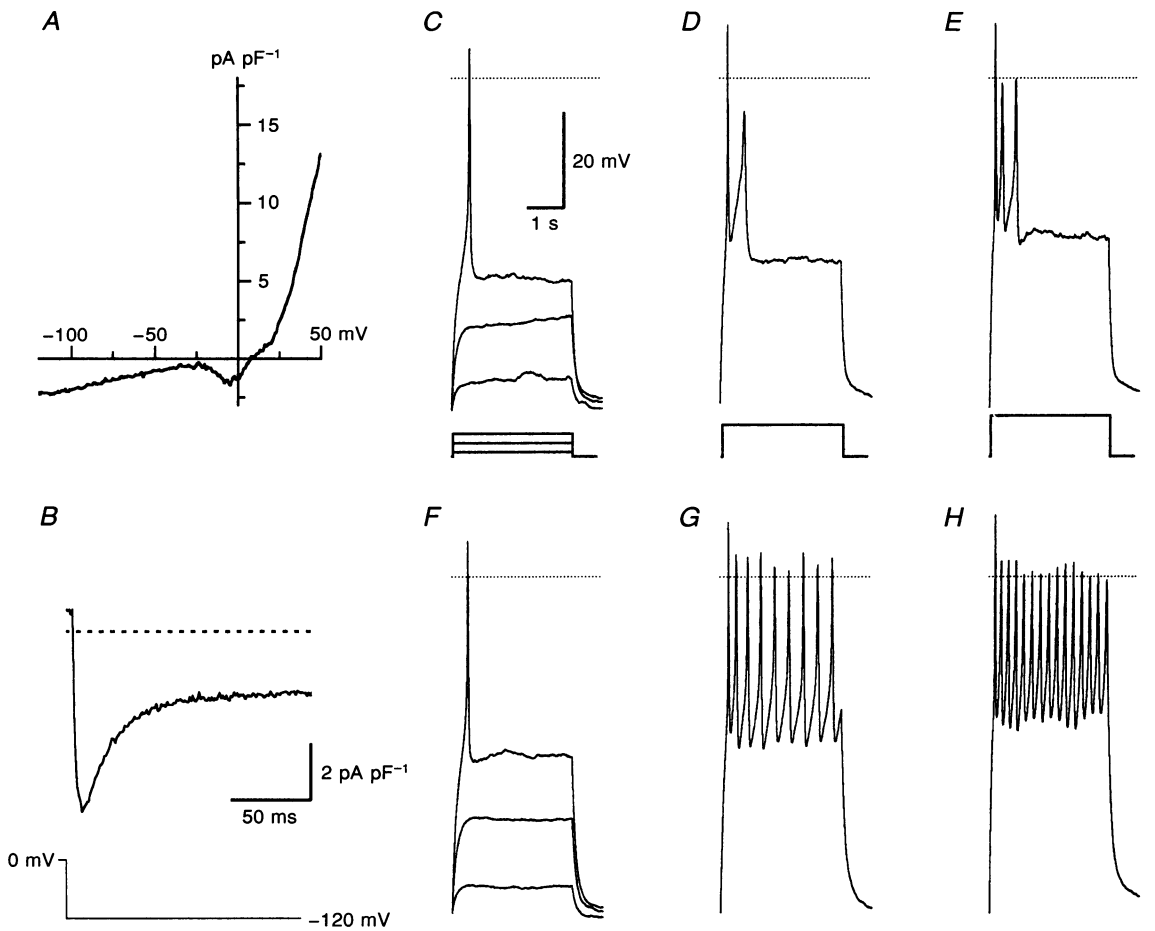

Figure 2. Firing and currents in an F-11 cell before and after the block of I_{HERG}

A, *I–V* plot of a neurone (2 s ramp stimulation) obtained under voltage clamp in control conditions. *B*, inward current elicited using the voltage protocol shown below the trace. *C–E*, firing under control conditions following the injection of current pulses of increasing amplitude (5, 15, 25, 35 and 45 pA; see protocol below traces). *F–H*, firing after a 2 min application of WAY-123,398, using the same holding potential (−70 mV) and the same current pulses. *A–H* are results from the same neurone. Cell capacitance, 61 pF.

M-channels have been described as underlying firing adaptation in a manner similar to HERG channels, the activation gating of HERG and M-channels being substantially the same (in the range −40 to −10 mV; Brown, 1988). Another channel with gating properties that fit the same voltage range is the eag K^+ channel (Ludwig *et al.* 1994), which has been reasonably hypothesized as belonging to the M-channel class (Stansfeld, Ludwig, Roeper, Weseloh, Brown & Pongs, 1997). We have investigated in the theoretical model the effects of the addition of a conductance similar to that described for the eag channel, and we obtained the qualitative prediction that these channels should also produce spike frequency adaptation, as has already been reported for M-channels (Pennefather *et al.* 1985; Jones & Adams, 1987; Brown, 1988). It is of interest to mention here that, since the discovery of the *eag* gene, it has emerged that mutations of this gene cause spontaneous repetitive firing in motor axons, and learning and memory assessments in *eag* mutants consistently indicate abnormally short retention and normal conditioning (Ganetzky & Wu, 1986).

It has been very recently reported that, in *Drosophila*, a homologue of the HERG K^+ channel is encoded by the *sei* (seizure) locus (Titus, Warmke & Ganetzky, 1997; Wang, Reynolds, Deak & Hall, 1997), providing an alternative explanation for the severe neuronal hyperactivity described in *sei* mutants which has so far been attributed to Na^+ channel mutations. This recent finding and the fact that HERG is abundantly expressed in brain (Wymore, Gintant, Wymore, Dixon, Mckinnon & Cohen, 1997) are also well in line with the role for HERG channels which emerges from the present study and opens the possibility that some human seizure-based diseases may be caused by the null or defective expression of HERG channels.

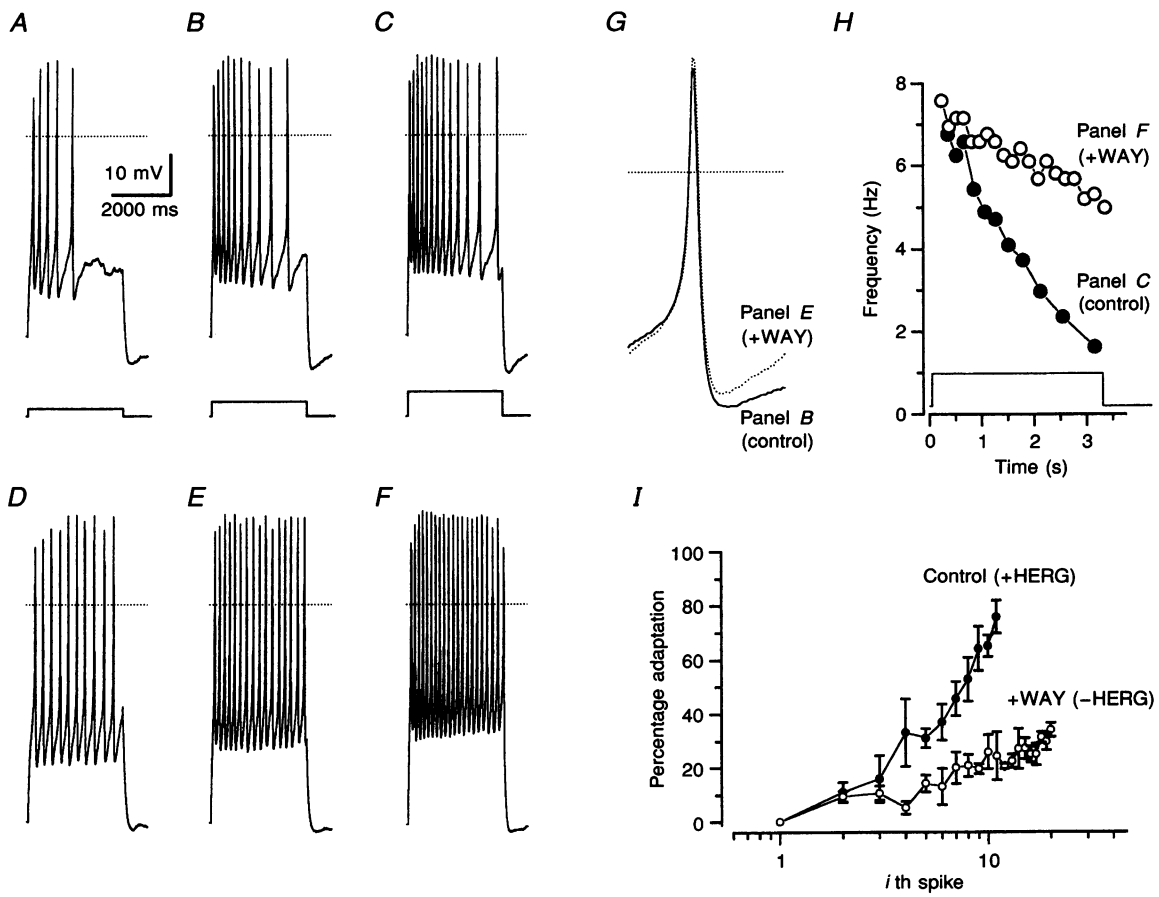

Figure 3. The properties of the spike-frequency adaptation present in a differentiated F-11 cell before and after I_{HERG} blockade

Current-clamp recording using pulse amplitudes to 30, 42 and 55 pA and holding potentials in the range −52 to −54 mV. *A–C*, spike-frequency adaptation of firing in control conditions. *D–F*, firing after the application of WAY-123,398. *G*, superposition of the next-to-last APs from panels *B* and *E*. *H*, plot of the instantaneous frequency of firing *versus* time of the recordings shown in *C* and *F*. The firing frequencies were calculated from the reciprocal of the interspike interval. Cell capacitance, 64 pF. *I*, percentage adaptation *versus* the number of spikes. The data were obtained from other cells ($n = 3$) under stimulus conditions similar to those shown in this figure (9 sets of data were averaged).

Arcangeli, A., Becchetti, A., Mannini, A., Mugnai, G., De Filippi, P., Tarone, G., Del Bene, M. R., Barletta, E., Wanke, E. & Olivotto, M. (1993). Integrin-mediated neurite outgrowth in neuroblastoma cells depends on the activation of potassium channels. *Journal of Cell Biology* **122**, 1131–1143.

Arcangeli, A., Bianchi, L., Becchetti, A., Faravelli, L., Coronnello, M., Mini, E., Olivotto, M. & Wanke, E. (1995). A novel inward-rectifying K^+ current with a cell cycle-dependence governs the resting potential of neuroblastoma cells. *Journal of Physiology* **489**, 455–471.

Brown, D. A. (1988). M currents. In *Ion Channels*, vol. 1, ed. Narahashi, T., pp. 55–94. Plenum, New York.

Cook, D. L., Satin, L. S. & Hopkins, W. F. (1991). Pancreatic B cells are bursting, but how? *Trends in Neurosciences* **14**, 411–414.

Curran, M. E., Splawski, I., Timothy, K. W., Vincent, G. M., Green, E. D. & Keating, M. K. (1995). A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* **80**, 795–804.

Faravelli, L., Arcangeli, A., Olivotto, M. & Wanke, E. (1996). A HERG-like channel in rat F-11 DRG cell line: pharmacological identification and biophysical characterization. *Journal of Physiology* **496**, 13–23.

Ganetzky, B. & Wu, C. F. (1986). Neurogenetics of membrane excitability in Drosophila. *Annual Review of Genetics* **20**, 13–44.

Jones, S. W. & Adams, P. R. (1987). The M-current and other potassium currents of vertebrate neurons. In *Neuromodulation*, ed. Kaczmarek, L. & Levitan, I. B., pp. 159–186. Oxford University Press, New York.

Ludwig, J., Terlau, H., Wunder, F., Brueggeman, A., Pardo, L. A., Marquardt, A., Stuehmer, W. & Pongs, O. (1994). Functional expression of a rat homologue of the voltage gated *ether á gogo* potassium channels reveals differences in selectivity and activation kinetics between the *Drosophila* channel and its mammalian counterpart. *EMBO Journal* **13**, 4451–4458.

Magistretti, J., Mantegazza, M., Guatteo, E. & Wanke, E. (1996). Action potentials recorded with patch-clamp amplifiers: are they genuine? *Trends in Neurosciences* **19**, 531–534.

Pennefather, P., Jones, S. W. & Adams, P. R. (1985). Modulation of repetitive firing in bullfrog sympathetic ganglion cells by two distinct K^+ currents, I_{AHP}, I_M. *Society for Neuroscience Abstracts* **11**, 148.

Platika, D., Boulos, M. H., Baizer, L. & Fishman, M. C. (1985). Neuronal traits of clonal cell lines derived by fusion of dorsal root ganglia neurons with neuroblastoma cells. *Proceedings of the National Academy of Sciences of the USA* **82**, 3499–3503.

Sanguinetti, M. C., Jiang, C., Curran, M. E. & Keating, M. T. (1995). A mechanistic link between an inherited and acquired cardiac arrhythmia: HERG encodes the Ikr potassium channel. *Cell* **81**, 299–307.

Shepherd, G. M. (1992). *The Synaptic Organization of the Brain*, chaps 2 and 3. Oxford University Press, USA.

Spinelli, W., Moubarak, I. F., Parson, R. W. & Colatsky, T. J. (1993). Cellular electrophysiology of WAY 123,398, a new class III antiarrhythmic agent: specificity and frequency-independence of Ik block in cat ventricular myocytes. *Cardiovascular Pharmacology* **27**, 1580–1589.

Stansfeld, C., Ludwig, J., Roeper, J., Weseloh, R., Brown, D. & Pongs, O. (1997) A physiological role for *ether-a-go-go* K^+ channel. *Trends in Neurosciences* **20**, 13–14.

Titus, S. A., Warmke, J. W. & Ganetzky, B. (1997). The Drosophila erg K^+ channel polypeptide is encoded by the seizure locus. *Journal of Neuroscience* **17**, 875–881.

Wang, X., Reynolds, E. R., Deak, P. & Hall, L. M. (1997). The *seizure* locus encodes the *Drosophila* homolog of the Herg potassium channel. *Journal of Neuroscience* **17**, 882–890.

Warmke, J. W. & Ganetzky, B. (1994). A family of potassium channel genes related to *eag* in *Drosophila* and mammals. *Proceedings of the National Academy of Sciences of the USA* **91**, 3438–3442.

Wymore, R. S., Gintant, G. A., Wymore, R. T., Dixon, J. E., Mckinnon, D. & Cohen, I. S. (1997). Tissue and species distribution of mRNA for the I-Kr-like K^+ channel ERG. *Circulation Research* **80**, 261–268.

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