Virus-Mediated Modification of Cellular Excitability

DAVID C. JOHNS, EDUARDO MARBAN,[a] AND H. BRADLEY NUSS

Section of Molecular and Cellular Cardiology, 844 Ross Building, Johns Hopkins University School of Medicine, 720 North Rutland Avenue, Baltimore, Maryland 21205, USA

Ion channels in the plasma membrane play a critical role in cellular function. These proteins are the gatekeepers that control ion homeostasis and shape excitability. Excitable cells use a variety of different ion channels to fashion their hallmark electrical signal, the action potential. Advances in molecular electrophysiology have led to the identification of more ion-channel genes than there are identified membrane currents. This excess is particularly striking with potassium channels, where a wide diversity of genes is compounded by variable levels of heteromultimerization, alternative splicing, and posttranslational modification. The classic methods of studying the roles of each gene rely either on exogenous expression in frog oocytes or pharmacological manipulation of native currents. While these techniques have yielded a wealth of information concerning ion-channel structure and function, they have come up short in linking individual genes and their products to physiology and disease.

Defects in ion channels have been linked to a number of inherited diseases, including epilepsy, periodic paralysis, cystic fibrosis, and long QT syndrome.[1] In addition, changes in cellular excitability are associated with several common disease states, including drug addiction, depression, and heart failure.[2–4] Finally, an enormous number of pharmaceutical

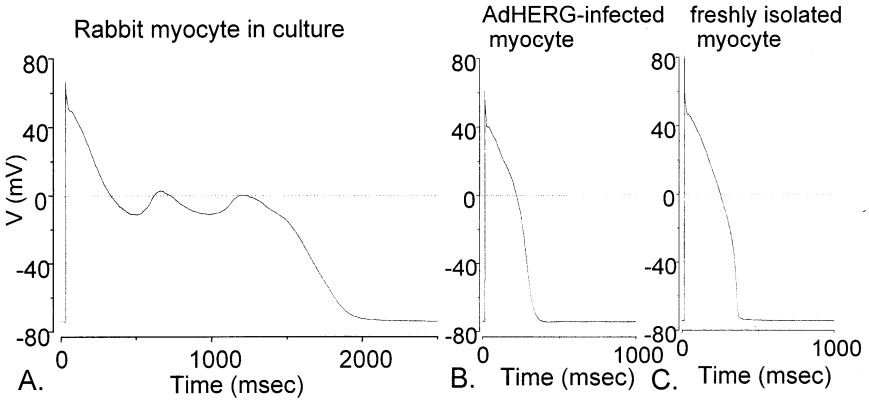


FIGURE l. Action potentials recorded in ventricular myocytes that were isolated and kept in primary culture for 48 h are markedly prolonged and exhibit frequent and multiple early after depolarizations **(A)**. Exogenous expression of *HERG* suppresses the frequency of EADs and normalizes the AP duration to close to normal values **(B,C)**.

[a]Corresponding author. Phone: 410-955-2776; fax: 410-955-7953; e-mail: marban@welchlink.welch.jhu.edu

agents either directly or indirectly affect cellular excitability. This may be by design, as in the case of local anesthetic block of sodium channels, or as an unwanted side effect that limits the potential usefulness of the agent, such as erythromycin-induced block of cardiac potassium channels, leading to fatal arrhythmias.

The use of viral vectors to modify cellular excitability genetically has been previously demonstrated.[5–8] Using this approach, we were able to reverse the salient phenotypic changes that occur in an animal model of heart failure.[9] However, the reversal was often accompanied by an excessive abbreviation of the action-potential waveform, which could be arrhythmogenic or at the very least could compromise contractile function. This suggested two areas for improvement: (1) screening of other channels whose ionic-flux properties may provide a more physiological modification of the action potential waveform, and (2) tighter control of the amount of channel expressed. Here we report our progress in addressing each of these issues.

HERG SUPPRESSES EADS

The identification of mutations in the human *ether-a-go-go*-related gene (*Herg*) underlying a form of congenital long QT syndrome (LQT2) makes exogenous expression of the wild-type *Herg* gene a logical strategy for prevention of arrhythmias. *Herg* is particularly

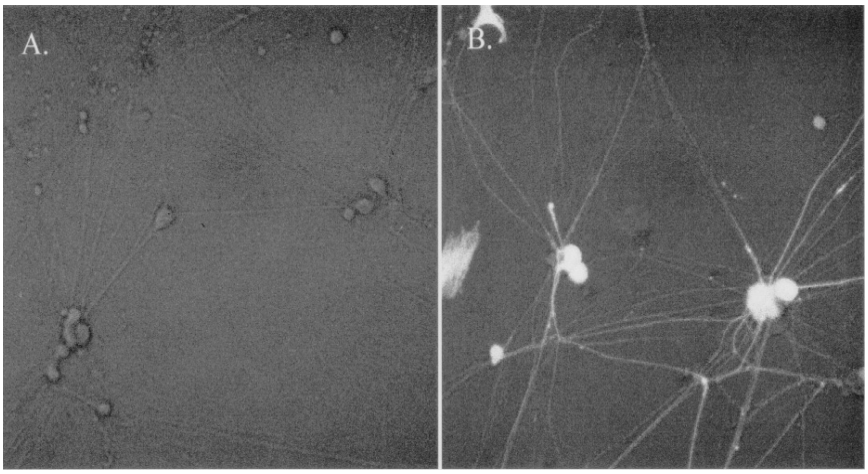
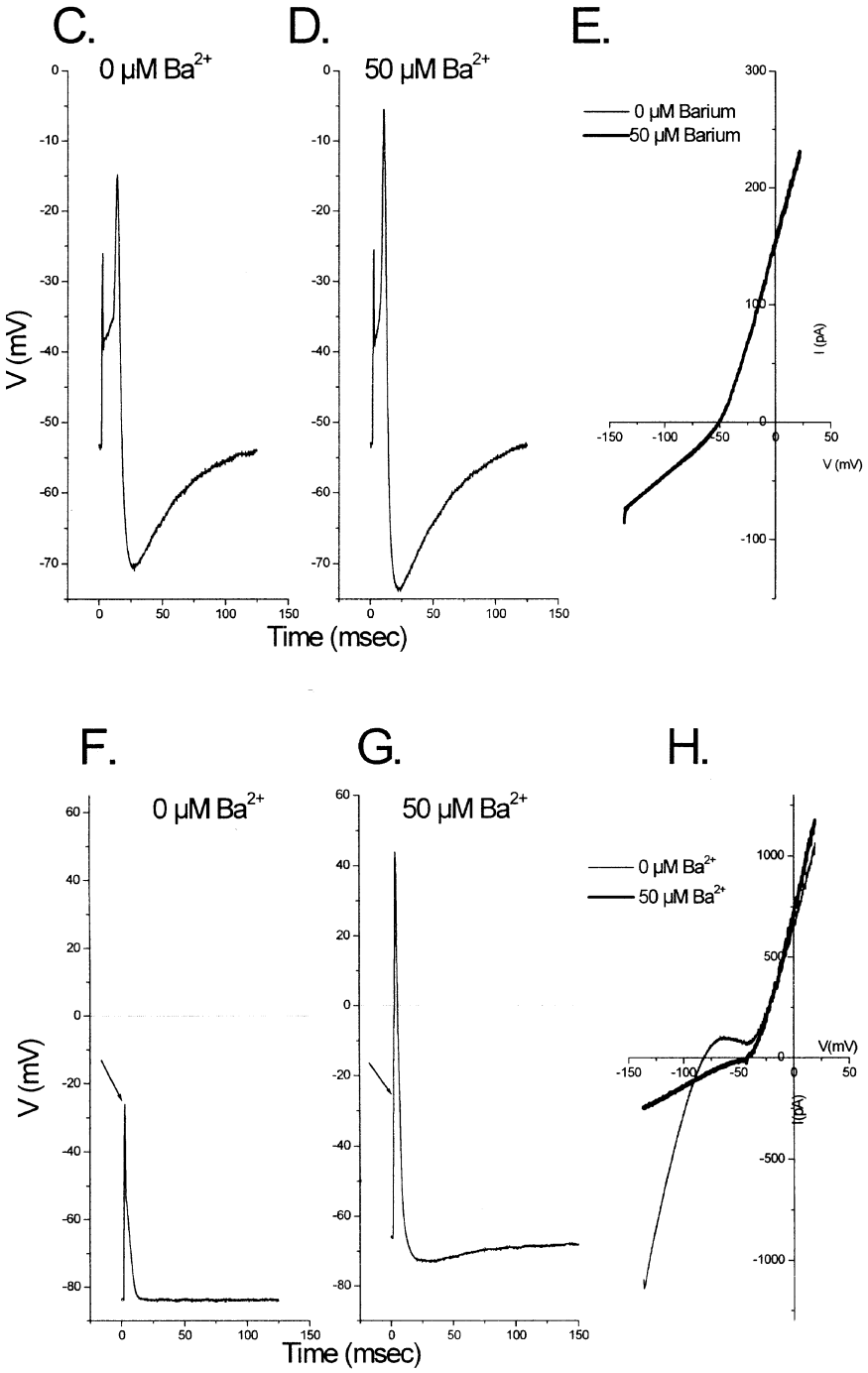


FIGURE 2 *(above and overleaf).* Confocal images of SCG neurons infected with AdEGI-Kir2.1 and AdVgRXR in the absence **(A)** and presence **(B)** of muristerone A. The effects of a suprathreshold stimulus on a control cell do not change with the addition of barium **(C,D)**. The membrane current recorded from the same cell under these conditions using a ramp protocol from −38 mV to +22 mV over 500 ms **(E)**. The addition of barium had little or no detectable effect on the outwardly rectifying I–V curves in control cells. The response to a subthreshold stimulus on a Kir2.1-infected cell **(F)** is markedly changed by the addition of barium **(G)**; in both panels the *arrow* indicates the cessation of the applied stimulus. The I–V curve for this cell is much different upon addition of barium **(H)**. (Reprinted by permission from Johns *et al.*[12])


C.
0 µM Ba2+
D.
50 µM Ba2+
E.
0 µM Barium
50 µM Barium
V (mV)
Time (msec)
I (pA)
V (mV)
F.
0 µM Ba2+
G.
50 µM Ba2+
H.
0 µM Ba2+
50 µM Ba2+
V (mV)
Time (msec)
I (pA)
V(mV)

attractive because its ion flux is greatest during repolarization, so that it may not interfere with normal excitation. To test the effects of exogenous expression of the *Herg* channel in cardiac myocytes, we engineered an adenovirus containing the gene under the control of the Rous sarcoma virus promoter. This virus directed high-level expression of *Herg* currents when infected into CHO-K1 cells.

Changes that occur when heart cells are maintained in primary culture recapitulate many of the changes that have been noted in heart failure. In particular, there is an enhanced frequency of occurrence of spontaneous early afterdepolarizations (EADs) in cultured rabbit myocytes (FIG. 1A). EADs are the cellular triggers of long QT arrhythmias. We have used the cultured cells as a model system to test the hypothesis that *Herg* expression would suppress EADs without dramatically affecting the action-potential waveform. Indeed, there was a marked suppression in the frequency of EADs (40 ± 17% of AdGFP infected cells vs. 9 ± 5% AdHerg infected cell: $p < 0.05$) without significant changes in the waveform or duration (FIG. 1B) as compared to freshly isolated cells (FIG. 1C). These results show that exogenous expression of *Herg* (or pharmacologic agonists of the channel) may have therapeutic value for suppressing long QT-related anhythmias, including those that cause sudden death in heart failure patients.

INDUCIBLE EXPRESSION

Control of gene expression was achieved by use of an ecdysone-regulatable promoter[10] either directly or in concert with an internal ribosome entry site (IRES).[11] To demonstrate the utility of this system we constructed viruses that express GFP alone, a GFP-Kir2.1 fusion protein, or a bicistronic message containing GFP and Kir2.1. When superior cervical ganglion (SCG) neurons were infected with these vectors, expression (as judged by green fluorescence) was only seen in the presence of the ecdysone analog muristerone A (FIG. 2A and 2B). FIGURE 2C shows an action potential elicited from a cell infected with a GFP control virus that is not changed by superfusion with 50-μM barium (Ba^{2+}), to specifically block the introduced Kir2.1 channels (FIG. 2D). Membrane voltage recordings from this cell are superimposable in the absence and presence of Ba^{2+} (FIG. 2E). FIGURE 2F–2H show a similar experiment performed on a cell that was infected with AdEGI-Kir2.1. Here a sub-threshold stimulus was given prior to application Ba^{2+}, to illustrate the effects on excitability of exogenous expression of this ion channel.

In summary the use of viral gene transfer to modify cellular excitability genetically has powerful applications in both clinical and basic science. Through logical design of the vector, both in the choice of the gene to be expressed and the cis-acting elements that control its expression, we have modified excitability of cardiac and neuronal cells in ways that are physiologically relevant.

ACKNOWLEDGMENTS

This work was supported by Tanabe Seiyaku Co. Ltd. We thank Drs. R.E. Mains and R. Marx for help with culturing SCG neurons, and Dr. B. O'Rourke for his help and advice with this work.

REFERENCES

1. CURRAN, M.E., I. SPLAWSKI, K.W. TIMOTHY, G.M. VINCENT, E.D. GREEN & M.T. KEATING. 1995. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell **80**(5): 795–803.
2. NESTLER, E.J., M.T. BERHOW & E.S. BRODKIN. 1996. Molecular mechanisms of drug addiction—Adaptations in signal transduction pathways. Mol. Psychiatry. **1**(3): 190–199.
3. SANGUINETTI, M.C., C. JIANG, M.E. CURRAN & M.T. KEATING. 1995. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell **81**(2): 299–307.
4. HOFFMAN, P.L. & B. TABAKOFF. 1994. The role of the NMDA receptor in ethanol withdrawal. Exper. Suppl. **71:** 61–70.
5. JOHNS, D.C., H.B. NUSS, N. CHIAMVIMONVAT, B.M. RAMZA, E. MARBAN & J.H. LAWRENCE. 1995. Adenovirus-mediated expression of a voltage-gated potassium channel in vitro (rat cardiac myocytes) and in vivo (rat liver). A novel strategy for modifying excitability. J. Clin. Invest. **96**(2): 1152–1158.
6. KARSCHIN, A., J. AIYAR, A. GOUIN, N. DAVIDSON & H.A. LESTER. 1991. K^+ Channel expression in primary cell cultures mediated by vaccinia virus. FEBS Lett. **278**(2) 229–233.
7. LEONARD, R.J., A. KARSCHIN, S. JAYASHREE-AIYAR, N. DAVIDSON, M.A. TANOUYE, L. THOMAS, G. THOMAS & H.A. LESTER. 1989. Expression of Drosophila Shaker potassium channels in mammalian cells infected with recombinant vaccinia virus. Proc. Natl. Acad. Sci. USA **86**(19): 7629–7633.
8. EHRENGRUBER, M.U., C.A. DOUPNIK, Y. XU, J. GARVEY, M.C. JASEK, H.A. LESTER & N. DAVIDSON. 1997. Activation of heteromeric G protein-gated inward rectifier channels overexpressed by adenovirus gene transfer inhibits the excitability of hippocampal neurons. Proc. Natl. Acad. Sci. USA **94:** 7070–7075.
9. Nuss, H.B., D.C. Johns, S. Kaab, G.F. Tomaselli, D. Kass, J.H. Lawrence & E. Marban. 1996. Reversal of potassium channel deficiency in cells from failing hearts by adenoviral gene transfer: A prototype for gene therapy for disorders of cardiac excitability and contractility. Gene Therapy **3**(10): 900–912.
10. NO, D., T.P. YAO & R.M. EVANS. 1996. Ecdysone-inducible gene expression in mammalian cells and transgenic mice. Proc. Natl. Acad. Sci. USA **93**(8): 3346–3351.
11. DIRKS, W., M. WIRTH & H. HAUSER. 1993. Dicistronic transcription units for gene expression in mammalian cells. Gene **128**(2): 247–249.
12. JOHNS, D.C., R. MARX, R.E. MAÏNS, B. O'ROURKE & E. MARBAR. 1999. Inducible genetic suppression of neuronal excitability. J. Neurosci. In press.