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Deexcitation of Cardiac Cells

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ABSTRACT Excitation and deexcitation are fundamental phenomena in the electrophysiology of excitable cells. Both of them can be induced by stimulating a cell with intracellularly injected currents. With extracellular stimulation, deexcitation was never observed; only cell excitation was found. Why? A generic model with two variables (FitzHugh) predicts that an extracellular stimulus can both excite the cell and terminate the action potential (AP). Our experiments with single mouse myocytes have shown that short (2–5 ms) extracellular pulses never terminated the AP. This result agrees with our numerical experiments with the Beeler-Reuter model. To analyze the problem, we exploit the separation of time scales to derive simplified models with fewer equations. Our analysis has shown that the very specific form of the current-voltage (*I-V*) characteristics of the time-independent potassium current (almost no dependence on voltage for positive membrane potentials) is responsible here. When the shape of the *I-V* characteristics of potassium currents was modified to resemble that in ischemic tissues, or when the external potassium concentration (K_o) is increased, the AP was terminated by extracellular pulses. These results may be important for understanding the mechanisms of defibrillation.

GLOSSARY

Material constants

L (cm), R (Ω)	length and axial cytoplasmic resistance of the cell
C (μF)	capacitance of the cell membrane
D (cm^2/s)	effective diffusion coefficient

Potentials and currents

e_i, e_{ext}, e (mV)	internal, external, and transmembrane potentials
$\bar{e}$ (mV)	averaged membrane potential in a cell
E (V/cm)	extracellularly applied electric field
E_s (mV)	equilibrium potential for slow current
i_{K1}, i_{x1} (μA/cm^2)	time-independent and time-dependent potassium currents
i_{Na} (μA/cm^2)	initial fast inward Na current
i_s (μA/cm^2)	slow inward current (Ca)
IC	intracellularly applied electric current
EC	extracellularly applied electric current

Independent variables

Y_i, τ_i, $Y_{i\infty}$	gating variables, their characteristic times, and steady values
w	slow variable in FitzHugh equations

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INTRODUCTION

Excitation and deexcitation of an excitable cell when the cell is stimulated by an intracellular current (IC) injected through a microelectrode are well documented (see, e.g., Weidmann, 1951; Vassalle, 1966; Noble, 1975; McAllister et al., 1975; Beeler and Reuter, 1977). Cells are often stimulated by extracellularly applied current (EC). Because the cell membrane is relatively nonconducting, the interior of the cell remains almost isopotential (Knisley et al., 1993; Krassowska and Neu, 1994; Windisch et al., 1995). As a consequence, one end of the cell becomes locally hyperpolarized, whereas the other end is depolarized.

Separate effects of depolarization, [+], or hyperpolarization, [−], are well known: [+] results in excitation of a cell, [−] results in deexcitation, i.e., in termination of action potential (AP) (see Fig. 1 *A*). This phenomenon has been called "immediate repolarization," "forced repolarization," or "all-or-none repolarization" (Weidmann, 1951; Vassalle, 1966; Goldman and Morad, 1977; Beeler and Reuter, 1977). For brevity, we will refer to it here as "deexcitation." It has been investigated in detail with voltage clamp techniques, and with an internally injected current. The current should exceed threshold values in both amplitude and duration to induce excitation or deexcitation, which depend on the timing of the stimulus (Vassalle, 1966).

What happens when a cell is stimulated extracellularly, as is often the case in experiments and clinics? In this case, instead of a uniform depolarization or hyperpolarization of a cell, a diphasic potential spatial distribution of potential ([+, −]) is created in the cell (Plonsey and Barr, 1986a,b; see also Pumir et al., 1994). It is well known that a cell can be excited in this way (Bardou et al., 1990; Tung and Borderies, 1992; Trayanova and Roth, 1993; and references therein). Deexcitation by EC has not been observed (Dillon, 1991; Knisley et al., 1992; Fishler et al., 1996). The aim of this article is to consider the problem of deexcitation by an extracellular electric field, both experimentally and theoretically. The emphasis here is on short pulses, on the order of 2–20 ms, as used clinically in defibrillation. Different con-

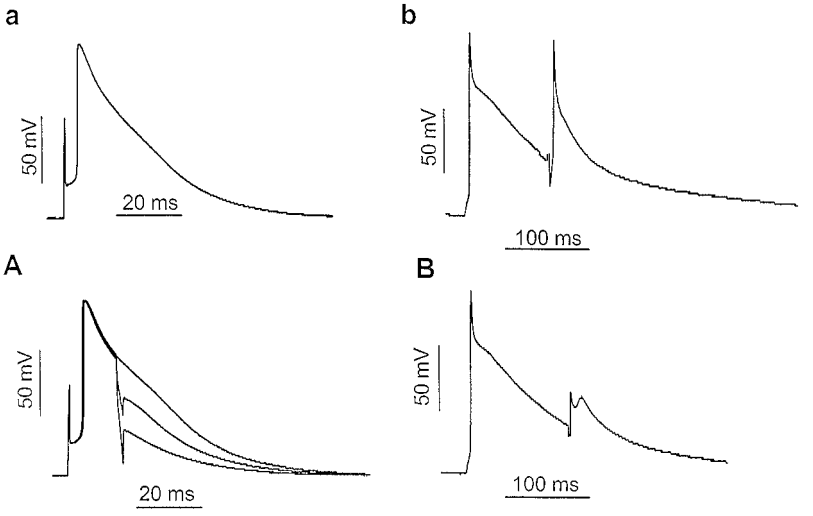

FIGURE 1 Mouse myocyte: effects of intracellularly (IC) and extracellularly applied currents (EC). Ten myocytes were tested in each condition of stimulation. Typical records are shown here. Left column (*a*, *A*), IC; right column (*b*, *B*), EC (*a*) IC excites myocyte; (*A*) IC terminates AP (three records are superimposed: 0, −50 pA, −100 pA, 2 ms). (*b*, *B*) EC excites myocyte but does not terminate AP. (*b*) An EC pulse (+20 V, 4 ms) was delivered at $t = 90$ ms after initiation of AP; (*B*) an EC pulse (−20 V, 4 ms) was delivered at $t = 115$ ms. Note that the spiky aspect of the records right after the stimulus is due to an imperfect bridge balance, unimportant for our purpose.

clusions could presumably be reached with longer pulses (Goldman and Morad, 1977).

One motivation for investigating this problem comes from theoretical analysis of defibrillation in a generic model of an excitable cell, the FitzHugh (FH) model (FitzHugh, 1961). Note that the term "generic" is used here in the sense of bifurcation and dynamical systems theory: a model is generic provided its predictions, its properties are robust (see Hirsch and Smale, 1974, for a precise mathematical definition). In the FH approach, for a wide class of current-voltage characteristics, an extracellular stimulus can terminate AP (Pumir and Krinsky, 1997). On the contrary, numerical studies of a more realistic model of cardiac membrane (obtained with the Luo-Rudy models (Luo and Rudy, 1991, 1994), the Beeler-Reuter (BR) model (Beeler and Reuter, 1977), and the Earm and Noble model (Earm and Noble, 1990)) showed that deexcitation with a pulse duration of 2–20 ms cannot be induced (Fishler et al., 1996). This suggests that some qualitatively important feature of the ionic dynamics in the cardiac cell is responsible for this behavior. This remark calls for a better understanding of the action of an electric field on myocytes.

In this paper, we consider the problem experimentally, numerically, and theoretically. Our experimental results, obtained with mouse myocytes, and the numerical results, obtained with the BR model, show that brief-duration EC does not terminate an AP. Our theoretical study does not purport to provide a precise description of the observed phenomena. In fact, the detailed properties of cardiac cells are both tissue- and species-dependent. Our goal here is merely to identify the important features necessary for qualitatively understanding the phenomenon, as well as modifications of the ionic currents that may result in a qualitatively different behavior. Our analysis of the BR equations, exploiting the time scale separation (see also Noble and Hall, 1963), shows that deexcitation by EC with brief-duration stimulation appears to be impossible because of the very specific (nongeneric) shape of the current-voltage characteristics of the slow repolarizing currents. We also show that in cells with modified potassium currents, as in ischemia or under increased external potassium concentration, EC results in deexcitation of a cell. These results lead to questions about defibrillation mechanisms under ischemia and other influences modifying the ionic currents.

MATERIALS AND METHODS

Cell culture

Primary cultures of cardiac cells from neonatal mouse were prepared as previously described (Steinhelper et al., 1990), with some modifications. Ventricles were dissected at 4°C and dissociated at room temperature for 20–30 min in 0.125% trypsin in Joklik's minimum essential medium (Steinhelper et al., 1990), with gentle agitation. Ventricles were then digested for 10 min with 0.05% collagenase in the same medium, also under gentle agitation. This was followed by mechanical dissociation with a Pasteur pipette. Cells released in the medium were centrifuged (1000 r.p.m. for 5 min), collected, and washed several times in Joklik's minimum essential medium. Cells obtained from three sequential collagenase (type II, Worthington, 246 U/mg) digestions were pooled and plated in collagen-coated Falcon culture dishes ($\phi = 35$ mm) at low density to obtain isolated cells. The culture medium was Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, bovine insulin (10 g/ml), bovin transferrin (10 g/ml), 1% chick embryo extract, and 10 nM dexamethasone. Half of the culture medium was changed every 2 days. Cells were used after 4 days in culture. Before electrophysiological experiments, the culture dish was placed on the warm stage (36°C) of an inverted microscope (Leitz-Diavert).

Electrophysiology

Cardiac action potentials were recorded with the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). The pipette solution contained 140 mM KCl, 1 mM EGTA, 4 mM $MgCl_2$, and 3 mM Na_2ATP; this solution was buffered at pH 7.3 with 10 mM HEPES/KOH. The external solution contained 140 mM NaCl, 2 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, and 5 mM glucose; this solution was buffered at pH 7.4 with 10 mM HEPES/NaOH. Patch pipettes (2–6 MΩ) were connected to the head stage of the recording apparatus (RK300; Bio-Logic, Grenoble, France). Stimulation and data acquisition were performed using pClamp software (Axon Instruments).

Extracellular electrical stimulation was performed via a bipolar electrode composed of a glass pipette (tip diameter 100 μm) filled with the

external solution and a platinum wire coiled around the pipette tip. The electric field generated on the axis of symmetry of the pipette is parallel to the axis, with a sizable gradient. The stimulating pipette was inclined at a small angle (~30° with respect to the long axis of the elongated cells). Cells were stimulated with rectangular pulses (1–10-ms duration, 10–100-V amplitude) delivered by a pulse generator connected to an isolation unit (WPI Instruments).

The field experienced by the cell is definitely not uniform, contrary to the idealized model analyzed (see Models, below). However, this is not a serious concern. Our experimental system induces a depolarization of one side of the cell and a hyperpolarization of the other. This is the most important feature of extracellular excitation, so only quantitative differences with the theoretical situation studied in the Models section are expected.

RESULTS

As already known, cardiac cells can be excited by both intracellular (IC) and extracellular (EC) stimuli (Fig. 1, *a* and *b*). Also in agreement with the theoretical models (FitzHugh, 1961; Beeler and Reuter, 1977), AP was quickly terminated after short intracellular currents of negative polarity were applied during the plateau phase (phase 3) of the AP (Fig. 1 *A*). During the plateau phase, the inward depolarizing currents, such as the Ca^{2+} currents, balance the outward repolarizing currents, such as the K^+ current. Pulses of negative (hyperpolarizing) currents applied during the plateau phase presumably result in the deactivation of the voltage-dependent Ca^{2+} current, leading to a shorter AP. In our experiments, IC with positive polarity resulted in prolongation of AP (not shown; note, however, that it may also result in an earlier termination of AP; see figure 8 of Beeler and Reuter, 1977).

On the contrary, extracellular stimulation never resulted in termination of AP (see Fig. 1, *b*, *B*). Stimuli applied at the beginning of the repolarization have no effect on AP, whereas stimuli applied later evoked a new AP (Fig. 1 *b*) at phase 3 of the previous one. A natural explanation is that large-amplitude local hyperpolarization resulted in removal of inactivation of the Na channels.

The electric field used for extracellular stimulation did not create a symmetrical pattern of depolarization and hyperpolarization in the stimulated cell (see Materials and Methods). Therefore, we repeated every experiment with two opposite polarities of the current. Neither of them resulted in termination of AP. The results are summarized in Table 1.

MODELS

In this section we briefly describe the models of excitable cells investigated here and the effect of the electric field.

TABLE 1 Mouse myocyte: stimulation by electric current

Stimulation	Intracellular	Extracellular
Excitation	Yes	Yes
Termination of AP	Yes	No

Models of excitable myocytes

Theoretical studies of myocytes are based either on models taking into account ionic currents experimentally measured, in the spirit of the Hodgkin-Huxley equations, or on much simpler, generic models of excitable media. We have considered here the Beeler-Reuter (BR) model (Beeler and Reuter, 1977), with eight variables, along with the simpler FitzHugh (FH) model (FitzHugh, 1961), with two variables only.

The BR model describes the membrane potential e:

$$\dot{e} \equiv \partial e/\partial t = -(i_{Na} + i_{K1} + i_{x1} + i_s - i_{IC})/C + D\nabla^2 e \qquad (1)$$

Here e is defined as the difference between the internal potential (e_i) and the external potential (e_{ext}): $e = e_i - e_{ext}$, and i_{IC} is the stimulating intracellular current (positive inward).

The ionic currents, positive outward, are i_{Na}, the initial fast inward sodium current; i_{K1}, the time-independent potassium current; i_{x1}, the time-dependent outward current (also transported mainly by potassium); and i_s, the slow inward current, mostly carried by calcium ions. They are described in the form

$$\begin{cases} i_{Na} = (g_{Na} m^3(t) h(t) j(t) + g_{NaC})(e - E_{Na}) \\ i_{K1} = i_{K1}(e) \\ i_{x1} = x_1(t) g(e) \\ i_s = g_s d(t) f(t)(e - E_s) \end{cases} \qquad (2)$$

with

$$\begin{cases} E_s = -82.3 - 13.0287 \ln[\mathrm{Ca}](t) \\ \mathrm{d}[\mathrm{Ca}]/\mathrm{d}t = -10^{-7} i_s + 0.07(10^{-7} - [\mathrm{Ca}](t)) \end{cases} \qquad (3)$$

Here, E_{Na} and E_s are the equilibrium potentials for Na and Ca, $[\mathrm{Ca}](t)$ is the intracellular calcium ion concentration, $i_{K1}(e)$ and $g(e)$ are functions of e, and $m(t)$, $h(t)$, $j(t)$, $x_1(t)$, $d(t)$, $f(t)$ are the gating variables, described by

$$\dot{Y}_i(t) \equiv \partial Y_i/\partial t = [Y_{i\infty}(e) - Y_i(t)]/\tau_i(e); \quad i = 1\text{–}6 \qquad (4)$$

Indices $i = 1$–6 relate to the gating variables $m(t)$, $h(t)$, $j(t)$, $x_1(t)$, $d(t)$, $f(t)$, respectively. The time constants $\tau_i(e)$ vary over orders of magnitude (Fig. 4 *c*). The steady-state values of the activation and inactivation variables, $Y_{i\infty}$, depend monotonically on e, and approach 0 or 1 when $e \to -90$ mV or $e \to 50$ mV.

The BR model dates back two decades. A number of its deficiencies have been identified (e.g., incorrect fast sodium dynamics and rectification of the potassium currents, see Luo and Rudy, 1991, 1994; too slow dynamics of the calcium currents, see Courtemanche and Winfree, 1991) and corrected in more recent models. Note in particular that the time-dependent potassium current, i_{x1}, is a composite current, resulting from several different channels. As pointed out by Fishler et al. (1996), the BR description of the interaction between an external electric field and a cardiac cell is qualitatively similar to the description obtained with the help of more recent models.

The simpler FitzHugh model involves only the membrane potential e and the recovery variable w:

$$\dot{e} = f(e) - w + i_{IC} + D\nabla^2 e, \quad \dot{w} = \epsilon(e - kw) \qquad (5)$$

(we assume $C = 1$ here.) The variable w lumps together the time-dependent activation of the potassium current with the time-dependent inactivation of the sodium current, i.e., the two slow variables of the Hodgkin-Huxley equation. The FitzHugh model can be derived in some limits from more faithful models, thanks to the very different time scales involved in the problem (Krinsky and Poroticov, 1973; see Fig. 4 *c* and the Theoretical Analysis below).

Effect of an externally applied electric field

The coupling term $D\nabla^2 e$ in Eqs. 1 and 5 describes the current flowing through the cytoplasmic resistance. As myocytes are very elongated ($L \approx 100$ μm long and $l \approx 10$ μm wide), we simply describe the cell by a one-dimensional medium, with coordinate x. The diffusion constant $D = L^2/RC$, where R is the resistance of the cell and C the capacitance of the membrane. In a continuous description, and in the presence of an applied electric field (external potential $e_{ext}(x) = Ex/L$, E being the voltage drop along the cell), the boundary conditions at the borders of the cell, which we take at $x = \pm L/2$, read

$$\frac{\partial e}{\partial x}(-L/2) = \frac{\partial e}{\partial x}(+L/2) = E/L \qquad (6)$$

This relation expresses the continuity of the current and reflects the fact that the membrane resistance is much larger than the intracellular resistance, thus permitting one to neglect the currents at the edges of the cell. To simulate an extracellular stimulation, one simply sets boundary conditions as in Eq. 6.

Equation 1, in the absence of any ionic current, reduces to a diffusion equation, the solution of which is simply a linear potential:

$$e(x) = e_{lin}(x) + \bar{e}, \quad \text{with} \quad e_{lin}(x) = E(x - L/2)/L \qquad (7)$$

where $\bar{e}$ is the averaged membrane potential in the cell.

The time scale necessary to establish the potential gradient inside the cell, $\tau \equiv L^2/(4\pi^2 D) \approx 1$ μs, is fast (Krassowska and Neu, 1994). This is a key feature of a number of asympotic analyses (Krassowska and Neu, 1994; Pumir and Krinsky, 1996; Keener, 1996; see also Theoretical Analysis below).

NUMERICAL RESULTS

We briefly discuss here our numerical results obtained with the BR equations. In particular, we show that the behavior obtained is qualitatively similar to the experimental results, reported in Materials and Methods and the Results.

Numerical implementation

To study the problem numerically, the cell was discretized by N uniformly spaced grid points (typically, $N = 16$). The discretized equations with the proper boundary conditions at the edges of the cell (see, e.g., Pumir et al., 1994) were time stepped by a Crank-Nicholson algorithm, second order in time. We always checked our numerical results by comparing various time steps and spatial resolutions.

Numerical results for the Beeler-Reuter system

Fig. 2, *a-A*, summarizes the numerical results on the effect of a brief current pulse uniformly spread over the cell. Fig. 2 *a* shows an AP induced by a short injected current pulse (duration 2 ms and amplitude 13.2 μA/cm^2). Fig. 2 *A* illustrates how an applied current may shorten AP: at time $t = 200$ ms after the beginning of the AP, a short current pulse (duration 2 ms) is applied, with an intensity as indicated in the figure. This results in a shortening of the AP, the more so as the current becomes more negative.

Fig. 2, *b*, *B*, illustrates the effect of an external current (EC). The AP, shown in Fig. 2, *b*, *B*, was induced by an EC pulse of duration 2 ms and intensity 7.75 V/cm, very close to the excitation threshold. Subsequently, a short-duration pulse (2 ms) was applied at $t = 320$ ms (amplitude 25 V/cm; Fig. 2 *b*) and at $t = 250$ ms (amplitude 20 V/cm; Fig. 2 *B*) after the beginning of the AP. As Fig. 2, *b*, *B*, illustrates, we could never observe a shortening of AP by an EC stimulation. The only effect we could observe was instead a prolongation of the AP. Note that in Fig. 2, *b*, *B*, the averaged membrane potential over the entire cell is shown. In general, except when the EC pulse is on, and for a very short while thereafter, the membrane potential does not depend on the precise position inside the cell (see, e.g., figure 2 of Fishler et al., 1996). In comparison, the membrane potential is always constant throughout the cell when stimulated by an IC.

The main conclusion of this short numerical study is that the results obtained with the BR model agree qualitatively with the experimental results. This points to a serious discrepancy between the generic two-variable FH model (Pumir and Krinsky, 1997) and the experimental or the BR results, as summarized in Table 2.

THEORETICAL ANALYSIS

The purpose of this section is to understand qualitatively the behavior observed experimentally and numerically.

One of the important results concerns the origin of the discrepancy between the qualitative description of deexcitation in the FH and BR models. One may expect that these discrepancies are due to the increased complexity of the BR model, because many important features of cardiac cells are not included in the FH model (in particular, we have in mind the presence of the calcium currents, and the fact that the time scale of activation of the potassium currents and inactivation of calcium currents are comparable; see Fig. 4 *c*).

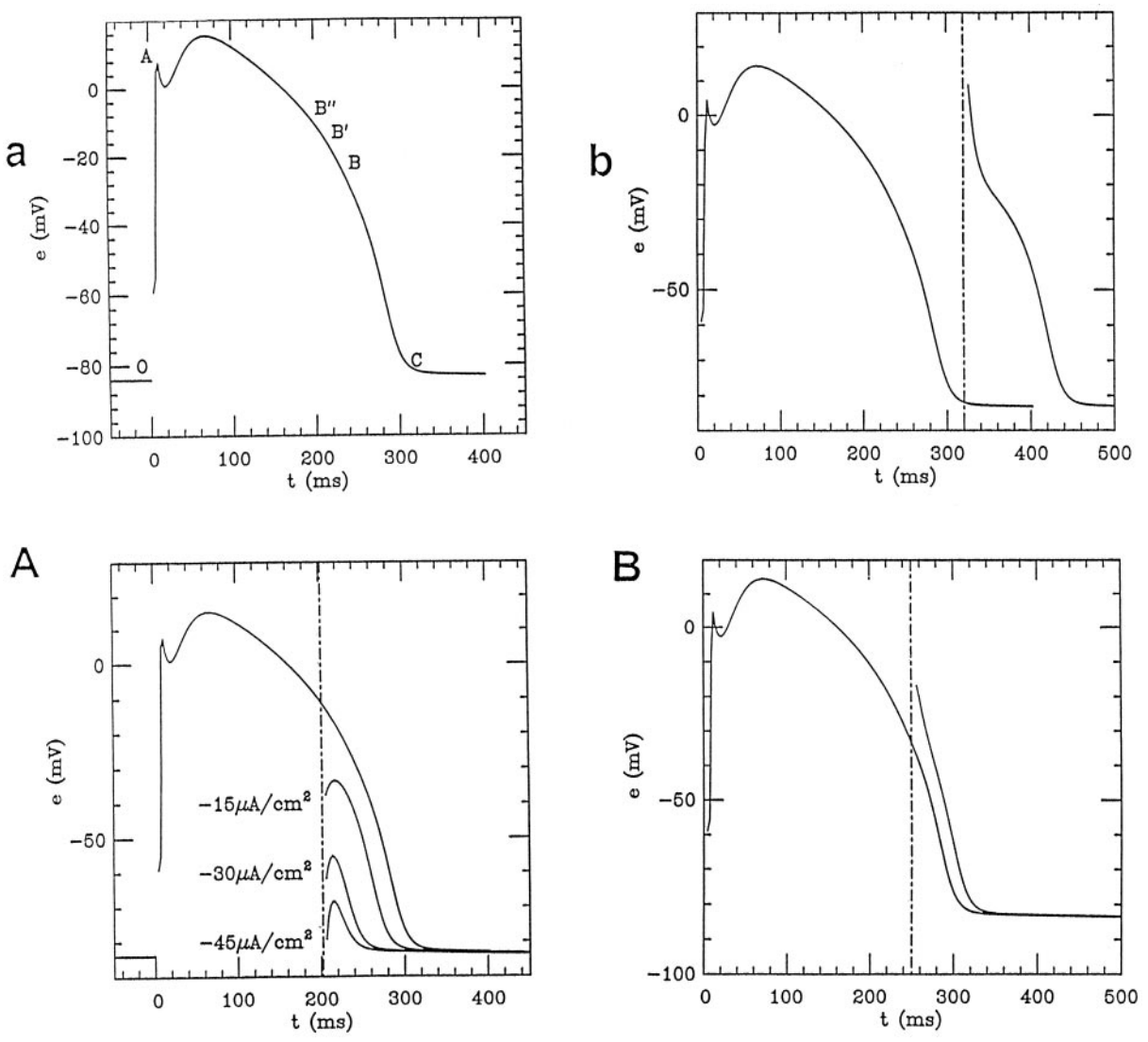

FIGURE 2 BR equation: different effects of IC and EC. Numerical results with the Beeler-Reuter model, to be compared with Fig. 1. (*a*, *A*) Effect of an IC in the Beeler-Reuter model. (*a*) An AP, induced by an IC (duration 2 ms, intensity = 13.2 μA/cm^2), applied uniformly throughout the cell. (*A*) A brief duration (2 ms) negative current pulse applied uniformly on the cell at t = 200 ms after the initiation of the AP terminated the AP. (*b*) An EC pulse (25 V/cm, 2 ms) was delivered at t = 320 ms after initiation of AP; (*B*) an EC pulse (20 V/cm, 2 ms) was delivered at t = 250 ms after initiation of AP. The results were obtained with N = 16 grid points in the cell, and a time step of dt = 0.05 ms when the electric field was off, and dt = 0.004 ms when the electric field was on. The averaged membrane potential in the cell is shown.

Remarkably, our analysis shows that the reason for the discrepancy is quite different: it is the flat (nongeneric) dependence of the *I-V* characteristics of time-independent potassium currents (see below, when can an EC deexcite AP?).

The FitzHugh model

As is well known, an important aspect of the FH model is the existence of two very different time scales. The ratio between the fast time scale, associated with the variable *e*, and the slow time scale, associated with the variable *w*, is described by the small parameter ϵ in Eq. 5. In the phase plane, because of the time scale separation, the motion is much faster along the horizontal (*e*) direction than along the vertical (*w*) one. The fast movements in the vector field are shown by arrows in Fig. 3 *a*.

Intracellular current: excitation, deexcitation

Fig. 3 *a* shows the nullclines $\dot{e} = 0$ of the fast equation of Eq. 5 with $D = 0$ for various values of the intracellularly

TABLE 2 Effects of electric current

	FH	BR	Myocyte
IC, excitation	Yes	Yes	Yes
IC, deexcitation	Yes	Yes	Yes
EC, excitation	Yes	Yes	Yes
EC, deexcitation	Yes	No	No

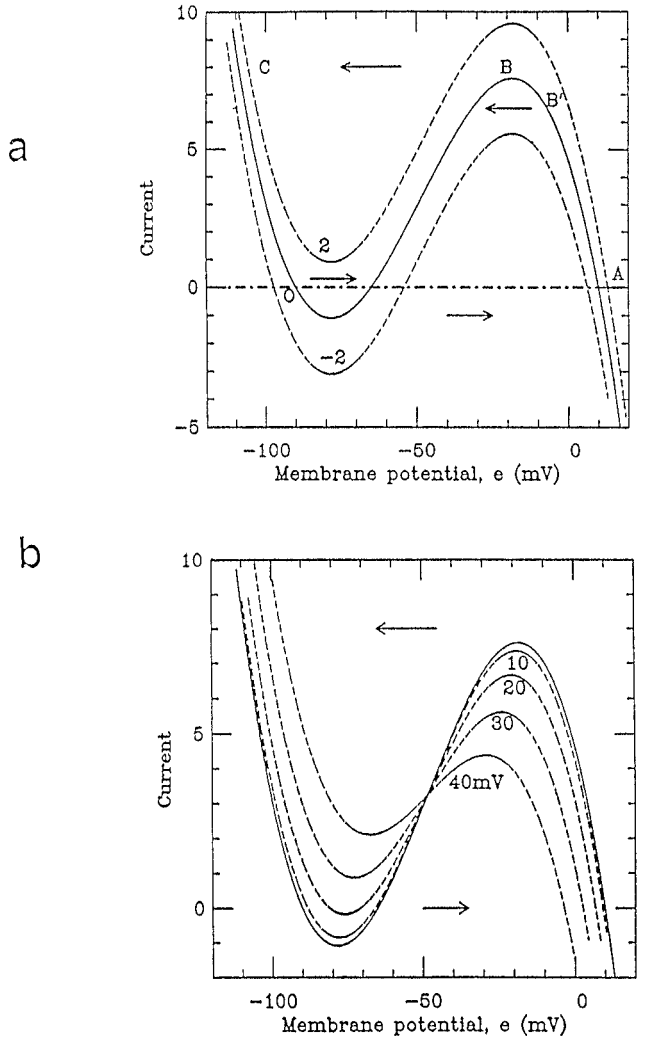

FIGURE 3 FitzHugh model: same effects of IC and EC. In the FH model, IC and EC affect the e equation (fast variable) only (see Eqs. 8 and 9). (*a*) Effects of IC. Dashed lines, Nullclines $\dot{e} = 0$ affected by IC. (*b*) Effects of EC. Dashed lines, Nullclines $\dot{e} = 0$ affected by EC (mV per cell are indicated). It is seen that, contrary to the experiment and BR model (Figs. 1 and 2), EC terminates AP in the generic FH model. All other phenomena (*a*, *b*, *A* in Fig. 1) are the same in BR and FH models. The function $f(e)$ in Eq. 5 is the cubic: $f(e) = \mathcal{A}(e + 90)(e + 65)(e - 10)$. The membrane potential is expressed in mV, and $\mathcal{A} = 8.10^{-5}$ μA/(cm^2 (mV)^3).

applied stimulating current (IC), i_{IC}. The solid line corresponds to a zero current. The point *O* represents the resting state of the cell. An action potential (AP) is represented in the phase plane (Fig. 3 *a*) by a trajectory lying close to *OABCO*, where *OA* and *BC* are the fast movements, and *AB* and *CO* are the slow movements. It can be initiated, for example, if an IC of 2 units is applied (see the arrow going from the resting state *O*).

During the plateau of the AP, a negative current may terminate AP. In the phase plane, this can be seen in the following way. In the plateau phase, the point describing the system in the (e, w) plane moves upward along the right branch of the nullcline $\dot{e} = 0$. When an IC of -2 units is applied, this nullcline jumps from the position shown by the solid line (Fig. 3 *a*) to the position beneath shown by the dashed line -2. Then the fast movement (see *arrow*) brings the point describing the state of the system from B' to the left branch of the nullcline, therefore terminating the AP.

Extracellular current: excitation, deexcitation

On time scales larger than $\tau = (L/2\pi)^2/D \approx 1$ μs, one may assume that the membrane potential is described by $e(x, t) = e_{lin}(x) + \bar{e}(t)$ (see Effect of an externally applied electric field, above, particularly Eq. 7). As shown by Krassowska and Neu (1994), Pumir and Krinsky (1996), or Keener (1996), the mean value of the membrane potential $\bar{e}(t)$ is sensitive to the averaged ionic current I flowing into the membrane: $I = (1/L)\int_{-L/2}^{L/2} i(x')dx'$. The stimulating pulses applied during an experiment are typically much shorter (a few ms) than the slow time scale in the problem (on the order of 100 ms). Therefore, in the FH model, the ionic currents involved in the dynamics depend only on e. As the dependence of e on x inside the cell is linear, the averaged ionic current flowing into the cell is

$$I = F(\bar{e}, E) - w, \quad F(\bar{e}, E) = \frac{1}{E}\int_{\bar{e}-E/2}^{\bar{e}+E/2} f(e')de' \qquad (8)$$

The equation for the membrane potential, Eq. 5, is therefore replaced by

$$\dot{\bar{e}}(t) = F(\bar{e}, E) - w \qquad (9)$$

The corresponding nullclines are shown in Fig. 3 *b* for several values of EC (extracellularly applied electric field). It is seen that EC can excite a cell in a fashion similar to that of IC (see previous section).

Similarly, an external electric field may also induce termination of an action potential. In fact, the FH model, with a cubic function $f(e)$, has a symmetry resulting in exactly the same properties of excitation and deexcitation. This symmetry is not essential: for nonsymmetrical functions $f(e)$, both excitation and deexcitation would be possible, although the thresholds for excitation and deexcitation would be different. In the BR model, deexcitation appears impossible. This suggests that the repolarization phase of an AP in the BR model presents some features that differ significantly from the FH analysis presented above. In fact, we will demonstrate that the difference can be attributed to some peculiar, nongeneric voltage dependence of the outward ionic currents.

The Beeler-Reuter model

The existence of several variables makes the analysis much more complicated than in the FH case. Properly identifying the relevant time scales allows one to carry out a theoretical analysis of the equation, as noted in a somewhat similar context by Noble and Hall (1963). We explain here why an IC or an EC pulse applied to a quiescent cell has similar

effects in the BR and the FH models, and why an EC cannot terminate an AP in the BR model, contrary to the FH case.

The BR model involves eight variables, with widely different time scales. Fig. 4 *c* shows the various times constants, $\tau_i(e)$ (see Eq. 4), as a function of the membrane potential, *e*. The main idea of the theoretical analysis presented below consists of taking advantage of the widely different time scales. During a pulse of duration of ~5 ms, the gating variables with a time constant $\tau_i(e) \gg 5$ ms remain essentially frozen, whereas the fast variables ($\tau_i \ll 5$ ms) respond instantaneously, permitting simplification of the system.

This allows us to derive several simplified models from the full BR system, with a smaller number of variables, which we will denote as BR_1, BR_2, BR_3, where the subscript refers to the number of variables (BR_8 is the full system). The simplified equations derived in this way depend on the details of the gating variables. For this reason, we should create different BR_1 equations for analyzing excitation and deexcitation. We will call them BRe_1, BRd_1, respectively. The approximate systems derived are valid for a finite time only. Interestingly, once the proper time scales have been identified, the analysis of the BR case is qualitatively very similar to the analysis of the FH model.

Excitation by an intracellular current

During excitation of a quiescent cell by a short pulse of ~5 ms duration, the gating variables associated with the currents i_{x1} and i_s are essentially frozen. The time scale of the sodium current activation variable *m* is very fast: $\tau_m \approx 0.1$ ms $\ll 5$ ms, so *m* can be replaced by its asymptotic value $m_\infty(e)$. The inactivation variable of the sodium current is equal to $h = 1$ at a resting potential. For sufficiently depolarized membrane, the inactivation variable *h* is diminishing to its steady-state value $h_\infty(e) \approx 0$, but the characteristic time of this transition is large: $\tau_h(e) > 5$ ms (for -80 mV $\lesssim e \lesssim -50$ mV). For this reason, we may assume that *h* remains unchanged and equal to its initial value, $h_0 \approx 1$.

The dynamics of *j* is slow compared to the duration of the shock: $\tau_j \gtrsim 10$ ms in the range of membrane potential of interest, so *j* remains unchanged: $j \approx j_0 \approx 1$. As a consequence, the sodium current i_{Na} reduces to

$$i'_{Na} = (g_{Na} m_\infty^3(e) + g_{NaC})(e - E_{Na}) \qquad (10)$$

As a result, the equation of evolution for *e* reduces to BRe_1:

$$\dot{e} = -I(e)/C, \quad \text{with} \quad I(e) = (i'_{Na} + i'_{K1} + i'_{x1} + i'_s - i_{IC}) \qquad (11)$$

where $i'_{K1,x1,S}$ are the values of the currents obtained by freezing the gating variables to their values in the quiescent state. All of these currents depend explicitly on *e*.

Fig. 4 *a* shows the total current $I(e)$ for several values of the stimulating current i_{IC}. The resting state corresponds to point *O*. This state is stable: when *e* is larger (smaller) than the resting value, the current is positive (negative), bringing the system back to its original state. When the intracellularly applied current i_{IC} increases, the total current (*dashed lines*) diminishes. At a value of $i_{IC} \gtrsim 2$ μA/cm^2, the total current

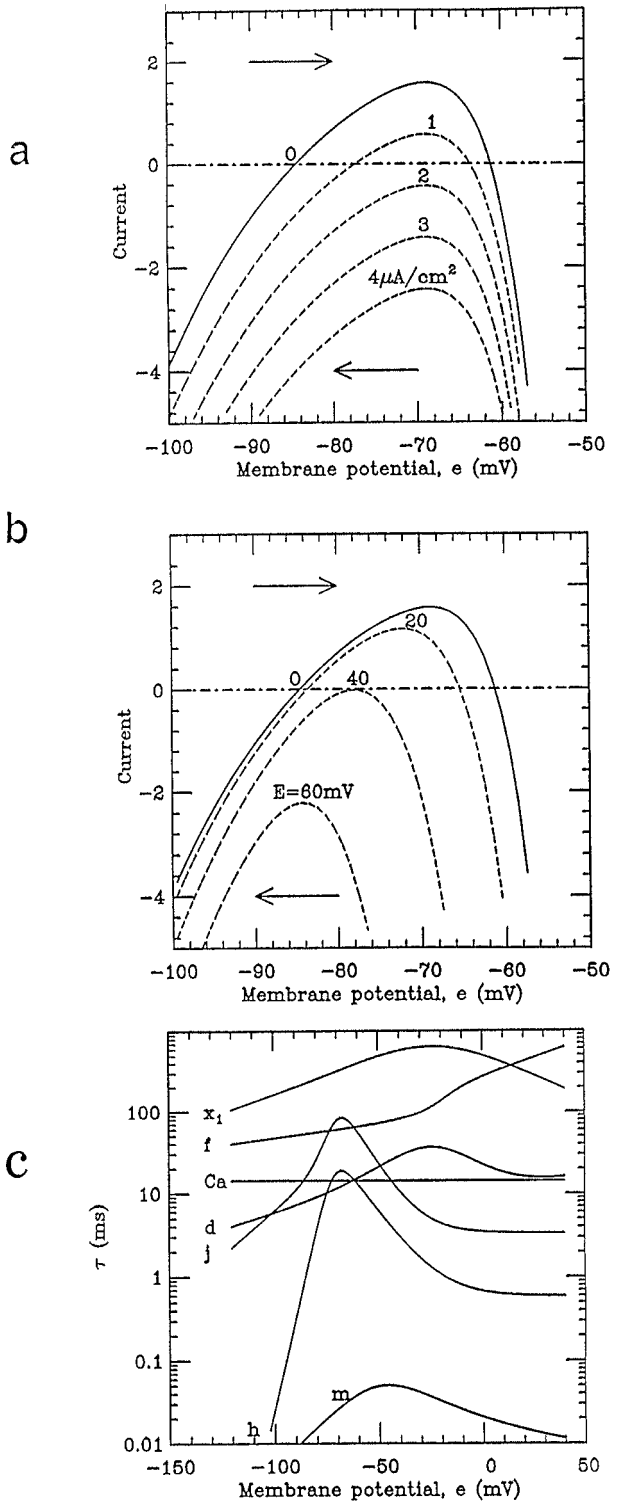

FIGURE 4 Analysis of excitation in the BR model. Current-voltage characteristics (see Eqs. 11 and 12) are affected by IC (*a*) and EC (*b*). The arrows indicate the direction of the motion of the point describing the state of the system. (*c*) Characteristic time scales in the BR equations. Both IC and EC can excite a cell.

becomes negative throughout the entire range of e: the membrane potential may then grow to large values; the cell becomes excited.

Excitation by an extracellular current

The time it takes to establish the potential gradient in the cell and τ_m are much shorter than the time scales of the other relevant gating variables in the problem. We restrict ourselves to the intermediate range of time scales, $\tau_m \ll t \ll \tau_{d,j}$. In this range, it is legitimate to assume that a potential gradient is established instantaneously inside the cell, as described by Eq. 7.

We use again the fact that the averaged membrane potential inside a cell, $\bar{e}$, is sensitive to the averaged ionic current, I (see Eq. 8). Because of the time scales of the problem, one has to deal only with Eq. 11, with $i_{IC} = 0$. As in the FH case, the effect of an extracellular electric field replaces the current by its averaged value, so the dynamics is described by BRe_1, the currents being averaged:

$$\dot{\bar{e}} = I_E/C \quad \text{with} \quad I_E(\bar{e}) = \frac{1}{E}\int_{\bar{e}-E/2}^{\bar{e}+E/2} -(i'_{Na} + i'_{K1} + i'_{x1} + i'_s)(e')de' \tag{12}$$

The averaged current, defined by Eq. 12, can be computed numerically, as a function of $\bar{e}$ and E (the extracellularly applied electric field). When $E = 0$, the quiescent state is stable: $\dot{e} < 0$ ($\dot{e} > 0$) when e is larger (smaller) than the resting state. As E increases, the maximum value of the averaged current, I, diminishes. For $E \gtrsim 40$ mV/cell (4 V/cm), the averaged current is negative throughout the whole range of e, implying that excitation is possible (Fig. 4 *b*).

Deexcitation of an excited tissue

In the repolarization phase of the AP, $i_{Na} \approx 0$, because the time scale for the inactivation variable is short, and the value of $h_\infty(e)$ is extremely small for $e \geq -40$ mV. Therefore, the ionic current reduces to $i = i_s + i_{K1} + i_{x1}$.

The potassium currents i_{x1} and i_{K1} are positive and tend to favor repolarization. On the other hand, the slow current, i_s, is negative and hinders repolarization, until it is deactivated. Previous work on deexcitation of cardiac cells has demonstrated that a voltage clamp pulse may induce forced repolarization (Beeler and Reuter, 1977). This is exactly what is expected, because voltage clamp is achieved by IC. The response depends on the type of stimulus (see Figs. 1 and 2), as we now analyse in detail.

Reduction of the Beeler-Reuter system to a single equation (BRd_1)

For $e \approx 0$ mV, the time scales associated with the potassium (i_{K1} and i_{x1}) and with the calcium (i_s) currents are all longer than 5 ms, the shock duration. As a first approximation, we freeze all of these gating variables, so the BR system reduces to the simple ordinary differential equation BRd_1:

$$\partial_t e = -\frac{1}{C}(i''_{x1}(e) + i''_{K1}(e) + g_s d_0 f_0(e - E''_{s0}) - i_{IC}) \tag{13}$$

where the gating variables and the calcium concentrations are all frozen in the expressions of the currents i''_{K1}, i''_{x1}, and E''_{s0}. To analyze the effect of the shock, one has to understand how the total current on the right-hand side of Eq. 13 is modified.

Reduction of the Beeler-Reuter system to a system of three equations (BRd_3)

Equation 13 is a convenient zeroth-order approximation for the study of the influence of an electric shock. It has some serious limitations, however. One problem is that Eq. 13 gives no hint about how a cell is repolarized. We discuss here a more elaborate description of the repolarization phase.

The time scales of the calcium concentration (Eq. 3) and of the gating variable d (see Eq. 4) are much smaller than the time scales of the other gating variables, f and x_1. We therefore simply assume that the variables f and x_1 are frozen on intermediate time scales of up to 5 ms (typical duration of a shock), and that only e, d, and [Ca] evolve. This allows us to reduce the system of eight ordinary differential equations to a system of only three ordinary differential equations BRd_3:

$$\dot{e} = -\frac{1}{C}(i''_{x1}(e) + i''_{K1}(e) + g_s d f_0(e - E_s) - i_{IC}) \tag{14}$$

$$\dot{d} = \frac{-(d - d_\infty(e))}{\tau_d(e)} \tag{15}$$

$$E_s = -82.3 - 13.0287 \ln[\mathrm{Ca}](t)[\dot{C}a] = -10^{-7} i_s + 0.07(10^{-7} - [\mathrm{Ca}](t)) \tag{16}$$

where the gating variables are frozen in the expression of i''_{x1} and i''_{K1}. The resulting description (Eqs. 14–16) is valid for $\gtrsim$5 ms. We have explicitly checked, by comparing the solutions to the system BRd_3 to the solutions of the full system with identical initial conditions, that the results of the model BRd_3 effectively capture the qualitative features of the full BR model.

Reduction of the Beeler-Reuter system to a system of two equations (BRd_2)

A less faithful but more tractable description of the repolarization phase can be obtained by noticing that the dependence of the current i_s on the calcium concentration is moderate, as it enters through a logarithm in the value of the potential E_s (see Eq. 3). For the sake of simplicity, we

therefore freeze the [Ca] concentration, an assumption we have checked to be qualitatively correct, independently of the precise value of the calcium concentration. This leaves us with a system BRd_2 of two variables only, e and d:

$$\dot{e} = -\frac{1}{C}\left(i''_{x1}(e) + i''_{K1}(e) + g_s d f_0(e - E''_s) - i_{IC}\right) \quad (17)$$

$$\dot{d} = \frac{-(d - d_\infty(e))}{\tau_d(e)} \quad (18)$$

where the gating variables in the expression of the currents $i''_{x1,K1}$ and the calcium concentration in the expression of the potential E''_s are all frozen.

The dynamics of the system can be understood with the help of geometric methods. In the (e, d) plane, Fig. 5 *A* shows the nullclines of the system (Eqs. 17 and 18), i.e., the location of the points where $\dot{e} = 0$ and $\dot{d} = 0$. The arrows shown in Fig. 5 *A* indicate the direction of motion, induced by the flow. The state point of the system at the time where the shock is applied is marked by a cross, in the upper right corner. The resting, final state corresponds to $d \approx 0$ and $e \approx -90$ mV, in the lower left corner. The evolution takes the state point of the system through the narrow neck between the $\dot{e} = 0$ and $\dot{d} = 0$ nullclines. This slow motion through this "bottleneck" corresponds to the end of the plateau region. On longer time scales, the bottleneck opens up, as a result of the evolution of the slow variables. Any effect resulting in the opening of this narrow region, where the motion is slow, will result in a hastening of the recovery process, terminating AP.

Deexcitation by an intracellular current

The starting point of the present analysis is the single ordinary differential equation (Eq. 13), BRd_1. The *I-V* characteristics obtained for a set of values of the intracellularly applied current, i_{IC}, are shown in Fig. 5 *a*. Making the current i_{IC} more negative significantly pushes the *I-V* char-

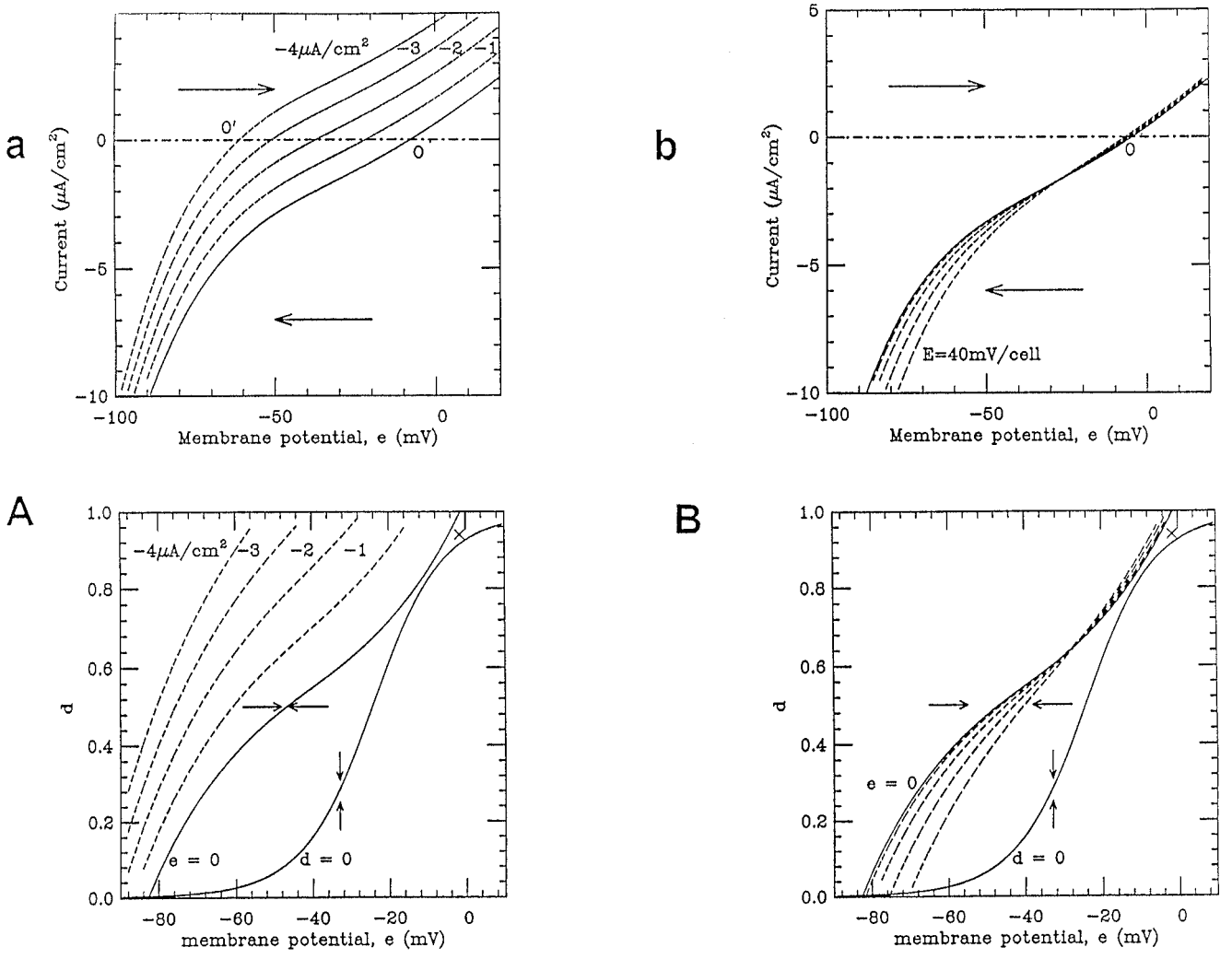

FIGURE 5 Analysis of deexcitation in BR model. An electric pulse of duration 2 ms is applied at $t = 160$ ms after the beginning of an AP. Left column (*a*, *A*), IC; right column (*b*, *B*), EC. First row (*a*, *b*), *I-V* characteristics (see Eq. 3). Second row (*A*, *B*), Nullclines in the (*e*, *d*) plane (see Eqs. 17 and 18). It is seen that EC cannot terminate AP.

acteristics toward the negative e region. As a result, during the shock, the value of e moves toward negative values of e, effectively repolarizing the system.

A more precise picture of the effect of an intracellular current may be obtained with the help of the reduced description with two variables BRd_2 (Eqs. 17 and 18). A negative intracellular current, IC, shifts the e-nullclines so as to make the neck wider, and results in a faster repolarization of the cell. This is illustrated in Fig. 5 *A*, which shows the nullclines for a set of values of the intracellular current. The more negative the current, the wider the neck becomes, so the quicker the plateau phase terminates, and the cell repolarizes, as observed experimentally (Weidmann, 1951; Vassalle, 1966; Beeler and Reuter, 1977; see also the Numerical Results, above).

The behavior described in this subsection is in qualitative agreement with the FH picture.

Effect of an extracellular current

The effect of EC consists of averaging the current $i = i''_{K1}(e) + i''_{x1}(e) + i_s(e)$ on the right-hand side of Eq. 17. The slow current $i_s = g_s d f_0(e - E''_s)$ depends linearly on e, so it is unaffected by the averaging process. Remarkably, both the i_{K1} and i_{x1} currents vary very slowly with e in the domain $-70\ \text{mV} \lesssim e \lesssim 50\ \text{mV}$ (see, e.g., Fig. 6 *a* for i_{K1}). As a result, the currents i_{x1} and i_{K1} are almost unchanged by the averaging process induced by the extracellular field. These features explain why the total current in model BRd_1 (right-hand side of Eq. 13) is barely modified by an extracellular current (Fig. 5 *b*).

The more elaborate two-dimensional system, BRd_2 (Eqs. 17 and 18), shows a qualitatively similar picture. Because of the shapes of the currents, either independent or linearly dependent on e, the nullcline $\dot{e} = 0$, shown in Fig. 5 *B* for several values of the extracellular potential, hardly changes when an electric field is applied.

Our analysis explains why EC never terminated excitation in our numerical experiments. We emphasize that the main reason for this effect, as the results of the following subsection demonstrate, is the very weak dependence on e of the ionic currents inducing repolarization.

In this work we have assumed that i_{Na} remains identically zero. This is possibly a limitation, because decreasing the membrane potential may terminate the deactivation of the sodium currents and hence permit the cell to repolarize. As our work is mainly concerned with the possibility of deexcitation of cells by an electric field, this is not a major concern. However, the analysis presented below has to be modified so as to also incorporate the fast sodium current dynamics to reproduce the numerical observations (see Fig. 2). This can easily be done.

When can an EC deexcite AP?

The purpose of this subsection is to show that with a modification of the *I-V* characteristics of the potassium

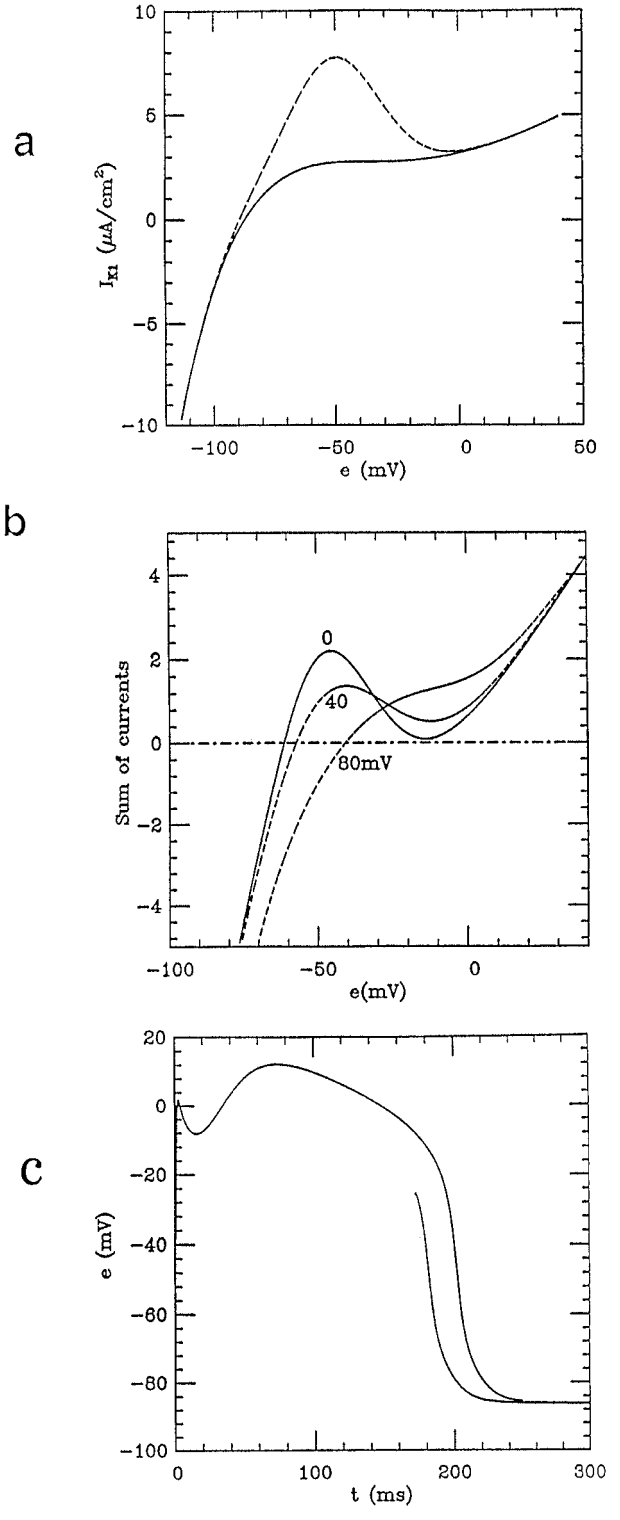

FIGURE 6 After modification of I_{K1}, EC terminates AP in BR model. (*a*) *I-V* characteristics of I_{K1} (modified, *dashed line*; unmodified, *solid line*). The modified characteristics are $I_{K1}(e) + 5\exp(-((e + 50)/35)^2)$, where e is expressed in mV and I in μA/cm^2. (*b*) *I-V* characteristics (see Eq. 13) affected by EC. (*c*) Termination of AP by EC. The number of points within one cell and the time step are the same as in Fig. 2.

currents, an EC may deexcite a cell. The modifications of the current-voltage characteristics used here are inspired by some known features of myocardium (see below) not fully taken into account by the Beeler-Reuter model.

In the BR model deexcitation appears impossible only because of a nongeneric feature of the potassium currents description. Generic perturbations of the *I-V* characteristics will make deexcitation by an EC possible (although with a very high threshold, if the perturbation is weak).

Here we consider a modified time-independent current, i_{K1}. Indeed, electrophysiological experiments have shown that the current-voltage characteristics of the potassium channel can be significantly modified by changing, e.g., the external potassium concentration (Sakmann and Trube, 1984). The shape of the current-voltage characteristics of i_{K1} may also be modified in a number of ways (Nichols et al., 1996). We demonstrate here that it is possible in principle to obtain qualitatively different results, namely that under certain conditions, EC may induce termination of an AP.

Fig. 6 *a* shows both the current i_{K1} involved in the BR description (*solid line*) and the modified current studied in this subsection (*dashed line*). Analytically, it was obtained by adding a hump with a Gaussian shape on the unperturbed current voltage characteristics. Fig. 6 *b* shows the sum of the currents, appearing on the right-hand side of Eq. 13, obtained by using the modified i_{K1} current (*solid line*). This curve has an inverted N shape, like in the FH case. Similar changes are known to make possible action potential-like responses when an intracellular current is applied (Tourneur, 1986). An extracellularly applied electric field (EC) modifies the current-voltage characteristics, as indicated in Fig. 6 *b* (*dashed line*). As a consequence, when an extracellular current is applied, the cell, initially at a potential $e \approx 0$, may jump to a much lower membrane potential.

This effect is illustrated in Fig. 6 *c*, which shows an action potential (*solid curve*), terminated by EC, at time $t =$ 160 ms (the duration of the electric pulse was 12 ms, and its intensity was $E = 10$ V/cm). We emphasize that the analysis made with the most simplified BR_1 model turns out to predict qualitatively the behavior obtained with the full model (BR_8). Note that deexcitation by an IC is also made easier by this modification of the i_{K1} current-voltage characteristics.

A similar effect was obtained by making the time scale of the *d* variable, τ_d, 50 times smaller than its nominal value. When τ_d is very short, the slow current, i_s, can no longer be considered as frozen, as *d* relaxes immediately to its asymptotic value, $d_\infty(e)$. This modifies the effective current-voltage characteristics, giving a shape as shown in Fig. 6 *a*. This demonstrates that it may be possible, under appropriate conditions, to terminate action potential propagation with an extracellularly applied electric field.

We emphasize that the perturbations added to the model are most likely to be too large to really describe cardiac cells under normal conditions. The hump observed by Sakmann and Trube (1984) in the current-voltage characteristics of the potassium currents and taken into account in the Luo-Rudy model (Luo and Rudy, 1991) does not seem to result in any qualitative difference in the premature repolarization of cardiac cells (Fishler et al., 1996). Likewise, the factor 50 in the speed-up of the dynamics of the calcium currents is significantly higher than what is generally accepted (Courtemance and Winfree, 1991). The results of this subsection are merely intended to show the possibility of inducing repolarization, under conditions that are most likely to be abnormal.

DISCUSSION

The contemporary point of view in the physiological literature is that extracellular stimulation may result in excitation only, whereas intracellularly injected current may result in both excitation and deexcitation. Excitation and prolongation of AP with EC were found in tissue experiments (Dillon, 1991; Zhou et al., 1991; Knisley et al., 1992). However, it is difficult to draw firm conclusions from tissue experiments, because large zones of depolarization or hyperpolarization may be induced by the heterogeneities in the tissue (Gillis et al., 1996). Experiments with a single cell are easier to interpret and to compare with models. Deexcitation was also not reported in such experiments (Knisley et al., 1993; Windisch et al., 1995), although none of these authors explicitly looked for the effect. We have conducted a number of experiments ourselves, to search for deexcitation of single cells by extracellular stimulation. In agreement with the previously cited authors, we have not been able to deexcitate cells in this way. These experimental facts are in agreement with the numerical results of Fishler et al. (1996), who systematically investigated the conditions under which the action of an EC induces a new depolarization of the cell.

The experiments of which we are aware (Knisley et al., 1993; Windisch et al., 1995; see also Materials and Methods and Results here) were performed under standard conditions. Could one guarantee that the possibility of terminating AP with an EC pulse is always ruled out? If not, under what circumstances could the effect be expected? In particular, could it be observed in other types of myocytes, or when the ionic conductivities are changed? Our analysis of the ionic model of cardiac tissue (BR model) has demonstrated that it may be possible to terminate AP by an EC, and the current lack of evidence for this effect is due to a very specific shape of the current-voltage characteristics of the potassium currents, i_{K1} and i_{x1}: these currents are essentially independent of membrane potential in the range $-70 \text{ mV} \lesssim e \lesssim 50 \text{ mV}$. It is known that increased potassium concentrations (Sakmann and Trube, 1984) or ischemia (see the contributions of Kléber et al., pp. 174–182, and Zipes, pp. 441–453, in Zipes and Jalife, 1995) modify the current-voltage characteristics of the potassium currents (i_{K1} and i_{x1}), so that they show significant inward rectification. Our analysis suggests that under these conditions, deexcitation by EC might take place.

The problem analyzed here is of potential importance for clinical applications. The results discussed in the present work are devoted exclusively to short electric stimuli, of duration 2–20 ms, as used clinically in defibrillation. Among the different mechanisms of defibrillation induced by electric shocks, deexcitation is seldom taken into consideration. The results presented in this article (see also the analysis in Pumir and Krinsky, 1997) mean that different mechanisms may coexist for ischemic and normal tissues, and deexcitation may play a role in defibrillation.

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