Isoproterenol Antagonizes Prolongation of Refractory Period by the Class III Antiarrhythmic Agent E-4031 in Guinea Pig Myocytes

Mechanism of Action

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The mechanism by which isoproterenol (ISO) prevents the prolongation of action potential duration (APD) and refractory period (RP) by the class III antiarrhythmic agent E-4031 was studied. E-4031 (1 μM) increased RP by 50% with no effect on contractile force in papillary muscles isolated from guinea pig heart. ISO (1 μM) increased force of contraction more than fivefold and decreased RP by 25%. The prolongation of RP by E-4031 was prevented by pretreatment of muscles with ISO. The prolongation of APD in isolated guinea pig ventricular myocytes by 5 μM E-4031 also was antagonized by prior exposure of the cells to 1 μM ISO. Instantaneous currents and delayed rectifier K⁺ currents, I₆, were measured in isolated myocytes using the suction microelectrode voltage-clamp technique. Currents were measured in response to 225-msec depolarizing pulses from a holding potential of −40 mV. Previous studies have demonstrated that I₆ in these cells results from activation of two distinct outward K⁺ currents, Iₖs and Iₖr (specifically blocked by E-4031). ISO doubled the magnitude of Iₖs, without significant effect on Iₖr. The instantaneous current, putatively identified as a Cl⁻ current, also was doubled by ISO but was unaffected by E-4031. The augmented conductance of Iₖs and instantaneous current by ISO results in a decrease in RP. The small effect of E-4031 on APD and RP in the presence of ISO results from the smaller contribution of Iₖr, relative to the augmented repolarizing currents. (Circulation Research 1991;68:77–84)

Sudden cardiac death is believed to result primarily from ventricular fibrillation (VF), usually preceded by an episode of ventricular tachycardia (VT).1 Most currently used antiarrhythmic drugs do not adequately protect the myocardium from such events.2 In recent years much research has focused on the mechanisms underlying these malignant arrhythmias, and the current consensus is that they are caused by one (VT) or multiple (VF) self-sustaining reentrant circuits.3 The most promising group of drugs for prevention of VT and VF are the class III antiarrhythmic agents. These drugs, exemplified by d-sotalol, prolong the refractory period (RP) of cardiac muscle and thereby reduce the risk of reentrant-based arrhythmias.4,5 Analogues of d-sotalol with more potent class III activity and lacking β-adrenergic blocking actions recently have been reported. These newer benzenesulfonamides, such as E-4031,6,7 UK-66,914,8 and UK-68,798,9 are approximately 100 times more potent than sotalol for prolonging RP of isolated cardiac muscle. E-4031 and d-sotalol prolong RP of guinea pig ventricular muscle by blocking a specific component of delayed rectifier K⁺ current. The component blocked by both drugs, Iₖr, activates more rapidly than the classically described delayed rectifier K⁺ current and exhibits marked inward rectification at potentials greater than 0 mV.10 Thus, the delayed outward current (I₆) in guinea pig ventricular cells that traditionally has been ascribed to activation of a single current11–14 is actually the sum of two currents—I₆ and the more typical, slowly activating K⁺ current that we refer to as Iₖr.10 Fully activated Iₖr is more than 10 times larger than fully activated Iₖs, but during the time course of a normal action potential (<200 msec), the magnitude of the two components is roughly the same during the plateau phase of the action poten-
tial. Experiments specifically designed to show that $I_{K_C}$ and $I_{K_P}$ are two distinct currents are described in our previous study of $I_K$..

Catecholamines shorten action potential duration (APD) and thereby decrease the RP of ventricular muscle. This effect is caused by a $\beta$-adrenoceptor-mediated increase in the magnitude of $I_K^{15-18}$ and a time-independent $Cl^-$ current,$^{19,20}$ which acts to accelerate repolarization of the action potential. Thus, in guinea pig ventricle the potential exists that catecholamines could functionally antagonize the ability of the class III antiarrhythmic agents to prolong APD. In this study we have investigated the consequences and mechanisms of the interaction of a $\beta$-adrenergic-specific agonist, isoproterenol (ISO), with the potent class III antiarrhythmic agent E-4031 in guinea pig papillary muscles and isolated ventricular cells.

Materials and Methods

Papillary Muscle Preparation

Papillary muscles were dissected from guinea pig hearts and placed in 20-ml organ baths containing a Krebs-Henseleit solution (pH 7.4, 37°C) of the following millimolar composition: NaCl 118, KCl 4.7, NaH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaH$_2$CO$_3$ 23, CaCl$_2$ 2.0, and glucose 11; and were continually gassed with 95% O$_2$–5% CO$_2$. The tendonous end of each muscle was attached to an isometric force transducer, while the proximal end was impaled on a rigid stainless steel pin. Resting tension was adjusted to produce the maximum twitch tension for each muscle, followed by a 1.5-hour equilibration period. Muscles were stimulated with 1-msec electrical pulses applied by bipolar platinum electrodes at a voltage 30% greater than threshold. The muscles were paced at a constant rate of 1 pulse/sec, and the RP for each muscle was determined by a standard single premature pulse protocol. RP was defined as the minimum interpulse interval (in milliseconds) between a basal stimulus and a single premature stimulus that resulted in a measurable contractile response. A single premature stimulus was applied after 10–20 pulses at the basal rate (1 pulse/sec).

RP and isometric force were determined before and 8–10 minutes after addition of 1 $\mu$M ISO or phenylephrine to the tissue baths. The Krebs-Henseleit solution contained ascorbic acid (0.2% wt/wt, pH readjusted to 7.4 with NaH$_2$CO$_3$) to retard oxidation of ISO that otherwise would occur during the 2–2.5 hours required for these experiments. Some of the tissues then were exposed to E-4031 at concentrations ranging from 0.1 to 10 $\mu$M (in 0.5 log molar steps) in the continued presence of ISO. The muscles were exposed to each concentration of E-4031 for 30 minutes before RP was redetermined.

Isolation of Single Ventricular Cells

Cells were isolated as described previously$^{10}$ using a slightly modified method from that first described by Mitra and Morad.$^{21}$ Guinea pig hearts wereperfused in a retrograde manner at 10 ml/min with an oxygenated (100% O$_2$), Ca$^{2+}$-free HEPES-buffered solution of the following millimolar composition: NaCl 132, KCl 4.8, MgCl$_2$ 1.2, HEPES 10, and glucose 5 (pH 7.2, 37°C). After 5 minutes of perfusion with this solution, the heart was perfused with the same solution containing 300 units/ml of type II collagenase (Worthington Biochemical Corp., Freehold, N.J.) plus 0.5–1.0 units/ml type XIV protease (Sigma Chemical Co., St. Louis) for 8 minutes. Finally, the heart was perfused with HEPES-buffered solution containing 0.2 mM CaCl$_2$ for an additional 5 minutes. The enzyme-digested heart was cut into small pieces, placed in 20 ml of HEPES-buffered solution containing 0.2 mM CaCl$_2$, and shaken until single cells were dissociated from the tissue pieces. The cells then were filtered through 200- $\mu$m nylon mesh and resuspended in HEPES-buffered solution containing 1.8 mM CaCl$_2$ at 24–26°C. Cells were used within 8 hours after isolation.

Measurement of Action Potentials and Membrane Currents

The techniques used in this study were the same as in our previous study of the effects of E-4031 on delayed rectifier K$^+$ currents.$^{10}$ Briefly, the suction microelectrode technique described by Giles and Shibata$^{22}$ was used to measure currents in the isolated ventricular cells. Square-bore capillary tubing (Glass Company of America, Millville, N.J.) was used to make very small tipped microelectrodes that were filled with 1 M potassium gluconate and 50 mM KCl. These electrodes had resistances of 4–7 MΩ when filled with this solution. Perfusion of the cell by the pipette solution was minimized by maintaining constant negative pressure with a gas-tight syringe.$^{10,22}$

Action potentials were elicited by 10-msec current pulses applied at a rate of 30 pulses/min. APD was measured at 90% repolarization (APD$_{90}$). For the voltage-clamp experiments, Ca$^{2+}$ currents were blocked by addition of 0.2 $\mu$M nisoldipine to the bathing solution, and Na$^+$ currents were voltage-inactivated by maintaining the holding potential at –40 mV. The cells were superfused with the bathing solution at a constant rate of 1.5 ml/min while the temperature was maintained constant at 35°C. Cells were exposed for 2 minutes to 1 $\mu$M ISO and for 2 minutes to ISO plus 5 $\mu$M E-4031 before currents were recorded. Preliminary experiments showed that this was ample time to reach steady state with either treatment.

Cells were current- or voltage-clamped using a List EPC-7 amplifier (List-Electronic, Darmstadt, FRG). Series resistance was compensated 40–70%, and currents were low-pass filtered at 2 kHz before digitization using a microcomputer and PCclamp software (Axon Instruments, Foster City, Calif.).

Data Analysis

All data are expressed as mean±SEM (n=number of cells or papillary muscles). In many of the figures
the standard error bars are not smaller than the symbols used for mean data and are not visible. When appropriate (papillary muscle data), tests of statistical significance were performed using a two-way analysis of variance (ANOVA).23 RS/EXPLORER (BBN Software Products Corp., Cambridge, Mass.) was used to perform a repeated measures analysis of the voltage-clamp data. When differences between groups were detected, this analysis was followed by a nonsimultaneous multicomparison test (NMCT) of significance. A value of p<0.05 was considered statistically significant. Curve fitting of data to Boltzmann distributions was performed using the Marquardt least-squares method of linear regression analysis.24

Materials

Isoproterenol HCl was prepared as a 1 mM stock solution in distilled water containing ascorbic acid (0.1%) and was stored on ice until used. E-4031 [1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonylaminobenzoyl)piperidine] was synthesized by Dr. H. Selnick of the Department of Medicinal Chemistry at Merck Sharp & Dohme Research Laboratories (West Point, Pa.). Purity was characterized by thin-layer chromatography and high performance liquid chromatography. The HCl salt of E-4031 was prepared as a 10 mM stock solution in distilled water.

Results

Isolated Papillary Muscles

The effects of E-4031 and ISO on the RP and force of contraction of isolated guinea pig papillary muscles are summarized in Figure 1.

Refractory period. E-4031 significantly lengthened the RP of isolated guinea pig papillary muscles in a concentration-dependent manner, to a maximum of 50% at 1 μM (Figure 1A, solid circles, n=6). Treatment of papillary muscles with 1 μM ISO shortened RP by an average of 25% (n=15). Six of these tissues then were exposed to increasing concentrations of E-4031, from 0.1 to 10 μM, in the continued presence of ISO. Even in the presence of 10 μM E-4031, the RP of these tissues was 13% less than the predrug values (open triangles). Thus, pretreatment with ISO greatly reduced the change in RP normally caused by E-4031. The modest prolongation of RP by E-4031 in ISO-pretreated tissues (an increase of 20 msec with 10 μM E-4031) was caused only in part by the class III agent, because in the nine tissues treated with ISO alone, the RP increased 10 msec during the equivalent time required to expose the tissues to the full range of E-4031 challenges (open circles). Nonetheless, a significant increase in RP still was caused by E-4031 relative to controls treated only with ISO. In a separate group of tissues, the functional antagonism by ISO of the ability to prolong RP by E-4031 was completely prevented by pretreatment of the tissues with 1 μM timolol, a β-adrenergic blocker (filled triangles, n=4).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Effect of isoproterenol (ISO) and E-4031 on refractory period (RP) (panel A) and force (panel B) of isolated guinea pig papillary muscles. Muscles were treated with E-4031 (n=6); 1 μM ISO (n=9); 1 μM ISO, then E-4031 (n=6); or 1 μM ISO plus 1 μM timolol, then E-4031 (n=4). E-4031 or E-4031+ISO+timolol significantly increased (ANOVA, p<0.05) RP at all concentrations relative to tissues treated with ISO+E-4031. In the presence of ISO alone, E-4031 also significantly increased (ANOVA, p<0.05) RP relative to muscles treated with ISO alone. ISO significantly (ANOVA, p<0.05) increased force, unless timolol was present. con, Control.

Force. Prolongation of RP by E-4031 did not lead to an increase in force of contraction (Figure 1B). The force of contraction was increased 5.4-fold by 1 μM ISO (n=15). In tissues treated with ISO, a gradual decline in force was observed over the course of the experiment. E-4031 did not significantly increase contractile force beyond that attained with ISO alone (open triangles). The slow but incomplete recovery from the effects of ISO on RP and force may result from slow oxidation of this catecholamine that occurs even in the presence of ascorbic acid.

Action Potentials in Isolated Myocytes

The interaction between ISO and E-4031 on action potentials was studied using isolated guinea pig ventricular myocytes. At a maximally effective concentration (5 μM), E-4031 lengthened APD_{90} from 212±12 to 268±19 msec (n=6), an average increase of 26% (Figure 2A).

A separate group of cells (n=12) was first exposed to 1 μM ISO, then 5 μM E-4031 in the continued presence of ISO. ISO decreased APD_{90} in six of these cells (from 233±21 to 211±18 msec) and increased APD_{90} in the six other cells (from 219±14 to 245±17 msec). Examples of these two responses are depicted in Figures 2B and 2C. When cells were exposed to 5 μM E-4031 in the continued presence of ISO, APD_{90} was increased by an average of only 6%, regardless of the initial response to ISO. Cells that responded to ISO with an increase in APD were lengthened 6±3% by E-4031 (n=6). Cells that responded to ISO with a decrease in APD were lengthened 6±2% by E-4031 (n=6).
Effects of Phenylephrine on Papillary Muscles and $I_K$ in Isolated Myocytes

$\alpha$-Adrenergic agonists increase APD of certain cardiac cells, an effect recently demonstrated to result from an $\alpha_1$-adrenoceptor-mediated decrease in transient outward current, $I_{to}$. $\alpha_2$-Adrenoceptor agonists have little effect on APD of guinea pig ventricular preparations, consistent with the small $I_{to}$ present in these cells. We confirmed the lack of effect of phenylephrine (10 $\mu$M) on effective RP of guinea pig papillary muscles ($n=4$) and also demonstrated that it has no significant effect on either $I_{Ks}$ or $I_{Kc}$ in isolated ventricular myocytes. Exposure of cells to 10 $\mu$M phenylephrine caused a slight decrease ($-16\pm6\%$; $n=8$, data not shown) in $I_{Kc}$ (defined by current blocked by 5 $\mu$M E-4031). Thus, the potential effects of endogenous catecholamines on $K^+$ currents in guinea pig ventricular cells are limited to those mediated through $\beta$-adrenoceptor stimulation.

Mechanism of Functional Antagonism Between Isoproterenol and E-4031

Whole-cell voltage-clamp experiments were performed to determine the effects of ISO and E-4031 on the outward currents responsible for repolarization of the action potentials of guinea pig ventricular muscle. In these experiments outward currents were measured during 225-msec depolarizing pulses applied in 10-mV steps to test potentials ranging from $-30$ to $+40$ mV from a holding potential of $-40$ mV. Currents were recorded before and 2 minutes after exposure of the cell to 1 $\mu$M ISO and then again after addition of 5 $\mu$M E-4031. ISO increased both time-independent ("instantaneous current") and time-dependent outward currents ($I_{Ko}$, composed of $I_{Ks}$ and $I_{Kc}$) during the 225-msec depolarizing steps, as well as tail currents measured on repolarization to the holding potential of $-40$ mV. This increase in outward currents was voltage-dependent. As shown in Figure 3, ISO enhanced outward currents more at $+50$ mV than at $-10$ mV. Addition of 5 $\mu$M E-4031 in the continued presence of ISO reduced the tail currents after test pulses to $-10$ mV and $+50$ mV, but reduced the time-dependent outward current only during the pulse to $-10$ mV.

The net outward currents measured during depolarizing pulses represent the sum of instantaneous currents plus $I_K$ ($I_{Ks}$ and $I_{Kc}$). The effects of ISO and E-4031 on instantaneous outward currents and both delayed rectifier $K^+$ currents were quantified.

Effects on time-dependent $I_K$ ($I_{Ko}$ and $I_{Kc}$). ISO increased time-dependent outward current ($I_{Ko}$) in a voltage-dependent manner. Significant (NMCT, $p<0.05$) increases were noted for depolarizations to 0 mV or more (Figure 4). Possible differential effects on $I_{Kc}$ or $I_{Ks}$ were determined by adding 5 $\mu$M E-4031 in the continued presence of ISO. Addition of 5 $\mu$M E-4031 significantly (NMCT, $p<0.05$) reduced these currents at potentials less than $+40$ mV. E-4031-sensitive currents were measured by digital subtraction of currents measured in the presence of ISO plus E-4031 from currents measured in the presence of ISO alone. In some cells the slowly activating, time-dependent current continued to increase slightly during exposure to E-4031 plus ISO. This resulted in an underestimation of the average E-4031-sensitive currents when measured simply as the difference in time-dependent current. A more quantitative measure of E-4031-sensitive current was obtained by digital subtraction of the currents recorded before

![Figure 2](http://circres.ahajournals.org/)

**FIGURE 2.** Action potentials recorded from isolated myocytes. Panel A: Prolongation of action potential duration at 90% repolarization ($APD_{90}$) from 205 msec in control (1) to 269 msec with 5 $\mu$M E-4031 (2). Panel B: Lengthening of $APD_{90}$ from 198 msec in control (1) to 231 msec with 1 $\mu$M isoproterenol (2); 5 $\mu$M E-4031 had no effect in the presence of isoproterenol (3). Panel C: Shortening of $APD_{90}$ from 216 msec in control (1) to 192 msec with 1 $\mu$M isoproterenol (2); 5 $\mu$M E-4031 lengthened $APD_{90}$ by 14 msec (3).

![Figure 3](http://circres.ahajournals.org/)

**FIGURE 3.** Increase in outward currents by isoproterenol (ISO) and partial block by E-4031. Currents were recorded during 225-msec depolarizing pulses to $-10$ and $+50$ mV, followed by 750-msec pulses back to the holding potential of $-40$ mV. Note that a slower sampling rate was used for the repolarizing step. Currents were recorded during control (CON), 2 minutes after exposure to 1 $\mu$M ISO, and again after 2 minutes of exposure to 5 $\mu$M E-4031 in the continued presence of ISO.
and after exposure to E-4031 and measuring the peak difference (Figure 5). The currents measured in this manner had the same voltage dependence and similar magnitude as E-4031-sensitive currents (I_K) in cells not treated with ISO when currents are expressed relative to cell size. This result indicates that ISO augments I_K by increasing I_K, and is without measurable effect on I_K.

**Effects on instantaneous currents.** Instantaneous current is composed of several components, including inward rectifier K⁺ current, leak current, and a recently described Cl⁻ current.¹⁹,²⁰ Cells in which the instantaneous current did not increase during the control period (n=5) were analyzed for changes in this component of outward current caused by ISO.

The instantaneous currents measured for the three conditions (control, 1 μM ISO, and 1 μM ISO plus 5 μM E-4031) are plotted in Figure 6A. ISO significantly increased this current component over the entire voltage range tested, and especially at positive potentials. Addition of E-4031 caused no further change. The instantaneous current enhanced by ISO (Figure 6B) rectified in the outward direction and by extrapolation of this data had a reversal potential of about −50 mV. In a separate set of experiments, the reversal potential of the ISO-induced instantaneous current was determined with a protocol that included pulses from +40 to −120 mV. The reversal potential of the ISO-induced instantaneous currents in these experiments was −52±5 mV (n=4).

**Effects on tail currents.** The effects of ISO and E-4031 on tail currents are plotted in Figure 7. ISO significantly (NMCT, p<0.05) increased tail currents at potentials of −10 mV or greater. In contrast to the effects on time-dependent current, E-4031 decreased tail current amplitudes by an equal amount at potentials between 0 and +40 mV. This is seen more clearly in Figure 8, in which the E-4031-sensitive tail current was normalized relative to its maximum (0.8 pA/pF) and was plotted as a function of test potential (V_t). This relation was fitted by a Boltzmann distribution:

\[ y = \frac{1}{1 + \exp[(V_{1/2} - V_t)/k]} \]

The half-point, V_{1/2}, of the curve was −20.4 mV, and the slope factor, k, was 7.9 mV. This relation is very similar to the activation curve of I_K, measured previously in the absence of ISO, where V_{1/2} and k were determined to be −21.5 and 7.5 mV, respectively.¹¹,¹³

Figure 4. *Time-dependent currents measured during 225-msec pulses to the indicated test potentials: effects of isoproterenol (ISO) and E-4031 (n=6).* Significantly different (non-simultaneous multicomparison test, p<0.05) from control (*) or from ISO (#) at same potential.

Figure 5. *E-4031-sensitive currents measured in the presence of isoproterenol (ISO) (n=6, filled circles) and from another group of cells not exposed to ISO (n=12; taken with permission from Reference 10). Inset shows currents measured during pulse to 0 mV (225-msec test pulse, 750-msec return to holding potential) in the presence of ISO, and again after exposure to E-4031, as well as the difference record (DIF) obtained by digital subtraction of the two records.
Furthermore, the voltage range over which ISO exerted its augmentation (≥ -10 mV) is the same as the activation range for \( I_{Kr} \). Thus, ISO increases outward \( K^+ \) current in guinea pig ventricular cells by augmenting \( I_{Kr} \) without influencing the other component of time-dependent outward current, \( I_{Kr} \).

**Discussion**

ISO at 1 \( \mu \)M decreased RP and increased force of contraction in isolated guinea pig papillary muscles. E-4031, a class III antiarrhythmic agent, increased RP by 50% at maximum effective concentrations but had no significant effect on force of contraction. A new finding of this study was that pretreatment of papillary muscles or isolated cells with ISO caused a functional antagonism of the increase in RP or APD usually observed with E-4031. The magnitude of increase in \( ADP_{90} \) by E-4031 in isolated cells was about one half that measured for RP in the multicellular papillary muscle preparations. E-4031 increased RP by a maximum of 50% in the papillary muscles but only lengthened APD\(_{90} \) in single cells by 26%, similar to our previous study. In the presence of ISO, E-4031 lengthened RP by about 10% and APD\(_{90} \) by 6%. Possible reasons for this difference include the influence of cell-to-cell coupling that is absent in the single cells and potential unknown effects of the cell isolation procedure on ion channel properties. The direction of change in APD of isolated cells in response to ISO varied considerably. Previous studies have shown that the effect \( \beta \)-adrenergic agonists have on APD is determined by the relative augmentation of \( I_{Kr} \) versus \( Ca^{2+} \) current. If ISO caused a greater increase in \( Ca^{2+} \) current than in outward currents in a particular isolated cell, then an increase in APD would be expected. The fact that ISO always decreased RP in papillary muscles indicates that in whole tissue the net response to ISO is a shortening of APD. It is interesting to note that the RP and \( ADP_{90} \) measured in the presence of maximal effective concentrations of E-4031 were approximately the same (about 270 msec in the absence of ISO). We measured the outward currents responsible for repolarization of the action potential from isolated guinea pig ventricular myocytes to characterize the mechanism of this functional antagonism.

ISO increased both an instantaneous current and a time-dependent current about twofold during 225-msec pulses to potentials of 10 mV or greater. Thus, the relative increase in the instantaneous current and the time-dependent outward current by 1 \( \mu \)M ISO was nearly equal using this pulse protocol. The enhancement of time-dependent currents by ISO was much larger when longer test pulses were applied (not shown). We chose to measure current magnitudes during times that are relevant to the normal
duration of a cardiac action potential. Catecholamines also cause a large increase in Ca$^{2+}$ current, an action that would lengthen APD in the absence of any effects on K$^+$ and Cl$^-$ currents. Moreover, β-adrenergic agonists can either increase or decrease APD of cardiac cells, dependent on the concentration-dependent, dominant effect on either inward Ca$^{2+}$ or outward K$^+$ and Cl$^-$ currents.\(^{17,27}\) Ca$^{2+}$ current was blocked withnisoldipine in these experiments to focus attention on the dominant repolarizing outward currents. We previously have reported that 5 μM E-4031 does not affect Ca$^{2+}$ current in these cells.\(^{10}\) Thus, the functional antagonism between ISO and E-4031 results from effects on outward currents. Although we did not try to rigorously identify the nature of the instantaneous current induced by ISO, recent reports have demonstrated that this current is carried by Cl$^-$.\(^{19,20}\) In favor of this interpretation was our finding that the ISO-induced instantaneous current had a reversal potential close to the estimated equilibrium potential (E$_{Cl^-}$) for Cl$^-$. The intracellular Cl$^-$ activity (aCl$^-_i$) of our isolated guinea pig myocytes is unknown. In rabbit papillary muscles bathed in a HEPES-buffered solution, aCl$^-_i$ was reported to be 24 mM.\(^{20}\) If we assume this to be a reasonable estimate of the aCl$^-_i$ in the guinea pig cells, then the E$_{Cl^-}$ in undialyzed cells would be −47 mV. Diffusion of glutamate from the high-resistance pipettes into the cell would shift E$_{Cl^-}$ to more negative potentials. The reversal potential of the ISO-induced instantaneous current (−52 mV) is consistent with the interpretation that this current is carried largely by Cl$^-$, as reported by others.\(^{19,20}\)

ISO also enhanced the component of I$_K$ that we refer to as I$_{Kr}$ but had no measurable effect on I$_{Ks}$. Thus, in cells exposed to ISO, I$_{Kr}$ is small relative to the total outward current. For example, I$_{Kr}$ contributed 22% to the total outward current measured at the end of a 225-msec pulse to +20 mV, but only 10% in the presence of ISO. We conclude that the functional antagonism by ISO of the class III activity of E-4031 results from the relatively small role that I$_{Kr}$ has in repolarization of cells that have an augmented conductance of I$_{Ks}$ and instantaneous (probably Cl$^-$) currents.

In guinea pig ventricular muscle, α-adrenoceptor stimulation has relatively little effect on APD or K$^+$ currents. Therefore, in these cells the major effect of catecholamines on K$^+$ currents would be a β-adrenergic-mediated increase in I$_{Kr}$.

The intent of this study was to determine the mechanism of the interaction between ISO and the class III antiarrhythmic agent E-4031. K$^+$ currents have been recorded from isolated human atrial cells,\(^{30}\) but to our knowledge there are no published studies of ionic currents using human ventricular cells. Extrapolation of our results to clinical conditions is only speculative, because we do not know which currents are responsible for repolarization of action potentials in human ventricular cells. Assuming there is some similarity between the two species with respect to the relative contributions of the two components of I$_K$, there may be therapeutic consequences to the interaction of E-4031 and β-agonists. For example, does the functional antagonism by ISO of the therapeutically desired effect of these antiarrhythmic agents, lengthening of RP, predict that episodic release of endogenous catecholamines would reduce the effectiveness of this class of drugs to prevent sudden cardiac death? The efficacy of β-adrenergic blockers in the prevention of sudden cardiac death has been demonstrated both in animal models\(^{31}\) and in clinical trials.\(^{32}\) Comparison of the efficacy of a pure class III agent with a drug such as sotalol, which is both a β-adrenergic blocker and an I$_{Kr}$ blocker, in a malignant arrhythmia model that
incorporates a challenge with a catecholamine\textsuperscript{32} could be useful in determining the relevance of our findings. Alternatively, an increase in $I_K$ and instantaneous current (neither blocked by E-4031) by endogenous catecholamines might be beneficial. Such activity could prevent excessive action potential prolongation by class III antiarrhythmic agents. Ultimately, the relevance of these findings must await the outcome of similar experiments in human cardiac myocytes.

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