Block of Delayed Rectifier Potassium Current, \( I_K \), by Flecainide and E-4031 in Cat Ventricular Myocytes

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The delayed rectifier outward potassium current, \( I_K \), is believed to play a major role in repolarizing the cardiac action potential. Selective block of \( I_K \) has been shown to underlie the uniform increase in action potential duration (APD) produced by the class III antiarrhythmic agents, whereas many class I antiarrhythmic agents, such as quinidine and cibenzoline, possess \( K^+ \) channel blocking properties that contribute to their antiarrhythmic drug action as well as to their subclassification as members of class IA.

Flecainide is an antiarrhythmic agent with pronounced \( Na^+ \) channel blocking properties that result in a marked depression of cardiac conduction at therapeutic drug concentrations. Ca\(^{2+}\) current is also inhibited by flecainide but at somewhat higher concentrations than those noted for \( Na^+ \) channel block. Although both of these actions would tend to shorten APD, as is typically observed in canine Purkinje fibers and at high concentrations in ventricular muscle, flecainide has been reported to increase ventricular APD in vitro and to prolong monophasic ventricular APD in humans. In clinical practice, however, flecainide is considered to produce only minor effects on refractoriness and large effects on conduction, leading to its classification as a class IC agent.

An increase in APD can be explained if flecainide also possesses \( K^+ \) channel blocking properties. In the present study, we examined the effects of flecainide on \( I_K \) and found activity similar to that of E-4031, a selective and potent blocker of delayed rectification in heart currently under development as a class III antiarrhythmic agent. The results support the notion that block of \( I_K \), as defined by E-4031 and flecainide, may be a common feature of many antiarrhythmic drugs, particularly those that increase APD (classes IA and III) or have little or no effect on APD because of concomitant block of \( Na^+ \) current (class IC). The data also raise the possibility that the combination of potent \( Na^+ \) and \( K^+ \) channel block may contribute to the proarrhythmic activity of flecainide by promoting slow conduction and heterogeneities in repolarization time course.

Methods

The methods used for single cell isolation have been described in detail elsewhere. Ventricular cells used for electrophysiological recordings were rod shaped and quiescent in Ca\(^{2+}\) containing HEPES buffer solution. Whole-cell single suction-pipette, voltage-clamp measurements were obtained by the
voltage-clamp circuit designed by M. Yoshii (Northwestern University, Chicago, Illinois). Electrodes were made from borosilicate glass (Kimax 151), and had tip resistances of 2–5 MΩ when filled with the K⁺ aspartate pipette solution. The electrodes were not fire polished. Voltage commands were applied by a PDP 11/73 (Digital Equipment, Pittsburgh, Pennsylvania) computer and a digital-analog converter (INDEC Systems, Sunnyvale, California). Pulse durations were 1 second or less to elicit currents over a time period appropriate to an action potential. Intervals between successive stimuli were 45–60 seconds to avoid residual activation of Iₚ. Current signals were output to the PDP 11/73 for storage after being filtered (1 or 3 kHz) with an eight-pole bessel filter (Frequency Devices, Havervill, Massachusetts). All current recordings were made at 32±0.5°C. Junction potentials between the pipette and external solution were about −8 mV. This potential was offset before the initiation of each experiment, and the data are uncorrected for this offset. Preparations were usually stable for at least 1 hour after gaining electrical access to the cell interior, with no obvious run-down of tail current amplitude.

The external bath solution for recording K⁺ currents was (mM): KCl 4, NaCl 135, MgCl₂ 2.5, CaCl₂ 2.4, HEPES 20, and glucose 11 (pH 7.3). In addition, the external solution contained 1 or 3 mM nitrendipine to block the slow inward current. Na⁺ current was inactivated by a holding potential of −40 mV. After the pH was balanced by using 1N NaOH, the total osmolarity was 300–310 mOsm. The pipette internal solution contained (mM): K⁺ aspartate 135, NaCl 10, MgATP 2, KH₂PO₄ 1, HEPES 5, and EGTA 5 (pH 7.3). The pH was balanced with 1N KOH, and the total osmolarity was 292–312 mOsm. Stock solutions of each compound were made in distilled water at concentrations of 10 (flecainide) or 3 mM (E-4031 and nitrendipine) and frozen until needed. E-4031 was synthesized by Dr. J. Butera, Wyeth-Ayerst Research, Princeton, New Jersey, and flecainide acetate was a gift from 3M Riker Laboratories, St. Paul, Minnesota.

Results

Figure 1A shows the membrane currents elicited by a 1-second voltage-clamp step from −40 to +20 mV in the same cell before and after exposure to 10 μM flecainide (left panel) and 1 μM E-4031 (right panel). Under control conditions (trace C), a small and slowly activating outward current flowed during depolarization, followed by a large outward tail current that has been shown to represent the gradual decay of Iₚ.¹⁻⁴,⁶⁻⁷,¹⁷ Flecainide (10 μM, trace F) markedly reduced the time-dependent outward current at +20 mV and also abolished the tail current on repolarization. These effects were largely reversible on wash-out (trace C, right panel). Subsequent exposure of the same myocyte to 1 μM E-4031 (trace E) produced effects similar to those described for flecainide. These results suggest that both flecainide and E-4031 are potent blockers of Iₚ in cat ventricular myocytes.

Figure 1B summarizes the results of several complete experiments in which the effects of flecainide (left panel) and E-4031 (right panel) on tail current amplitude were evaluated for a series of 750-msec step depolarizations to test potentials between −30 and +40 mV. Control curves were obtained immediately before drug exposure in each experiment. In the absence of flecainide (left panel, trace C), the data were well fitted by a simple Boltzmann relation with a slope factor of 6.2 mV and a midpoint at +6 mV, reaching a maximum near +30 mV. Flecainide (10 μM) reduced the maximum peak tail current by 93±4% with no significant change in midpoint or slope (n=3). Similar curves were constructed for the E-4031 experiments (right panel, Figure 1B). Under control conditions, previous exposure to E-4031, Iₚ activated near −20 mV, reached its one-half maximal value near +1.4 mV, and saturated around +30 mV. The tail currents were nearly completely blocked by 1 μM E-4031 (96±3%, n=3).

The small size of the outward current component blocked by both flecainide and E-4031 at positive potentials relative to the large amplitude of the tail current reflects the marked inward rectification properties of the delayed rectifier channel. In addition, the absence of an effect of flecainide or E-4031 on holding current at −40 mV suggests little effect on the background rectifier potassium channel, IₚK. Both of these effects can be demonstrated more clearly with slow (30.7 second) ramp depolarizations from −100 to +40 or +60 mV. Figure 2A shows that under control conditions (trace C), the membrane current-voltage relation was nonlinear with two distinct regions of inward rectification. Between −50 and −20 mV, the current represents IₚK, the inwardly rectifying background potassium current. A second region of rectification occurred near +20 mV where the current-voltage relation was flat. This area is associated with the activation of IₚK.

After exposure to 10 μM flecainide (trace F, Figure 2A), the same voltage ramp typically elicited a current response similar to that seen under control conditions but without the area of rectification near +20 mV. Subtraction of the two traces yielded a difference current that activated near −20 mV, reached a peak near +10 to +20 mV, and rectified inwardly to zero at potentials near +40 mV (Figure 2B). This change in the current-voltage relation is expected from the step depolarization results and confirms the inward rectification properties of IₚK. Similar results were obtained in a total of four experiments using 10 μM flecainide and in one experiment using a lower concentration (3 μM). In two other experiments at 10 μM, flecainide appeared to block an additional component of outward current at positive potentials, so that the difference current did not exhibit a negative slope. In no case was an effect on IₚK seen. Similar effects on the membrane current-voltage relation were observed in four sepa-
rate experiments using 1 μM E-4031, and a representative example is illustrated in Figure 2C and D.

**Discussion**

The present study demonstrates that flecainide and E-4031 selectively block $I_K$ in cat ventricular myocytes. This suppression of $I_K$ most likely underlies the reported increase in APD produced by flecainide and E-4031 in ventricular myocardium.\(^6,9,12-14\) In addition, the rapid and selective block produced by the two compounds may provide a means of pharmacologically isolating $I_K$ from other outward membrane currents over a wide voltage range to permit further study of its gating characteristics. That the “pure” class III antiarrhythmic agent E-4031 and the class IC agent flecainide appear to block the same current implies that $I_K$ may be a common site of action for antiarrhythmic drugs that prolong APD, particularly class III, IC, and IA agents.

**E-4031 Block of $I_K$**

The selective prolongation of APD without slowing conduction defines class III antiarrhythmic activity and has been shown to be a very effective means of preventing or terminating ventricular arrhythmias.\(^18-20\) Preliminary studies indicate that E-4031 is one of the most potent class III agents available. In anesthetized dogs, E-4031 (30–300 μg/kg i.v.) markedly increases ventricular refractoriness and effectively prevents the induction of ventricular arrhythmias.\(^21\) In isolated guinea pig myocytes, selective block of $I_K$ occurs at concentrations in the nanomolar range, an effect that most likely underlies the class III activity observed in vivo.\(^4\) The present study is
consistent with other preliminary reports and confirms that E-4031 is a potent and selective blocker of $I_K$.

**Flecainide Block of $I_K$**

The antiarrhythmic efficacy of flecainide has primarily been attributed to its effects on the fast inward Na$^+$ current. Results of the present study suggest that $K^+$ channel block should also be considered. In these experiments, 10 $\mu$M flecainide produced about 93% block of $I_K$, yielding an apparent $K_T$ of 0.75 $\mu$M, assuming a 1:1 stoichiometry for the drug-receptor binding reaction. Because the clinically effective serum concentration of flecainide ranges between 0.25 and 1.3 $\mu$M after correction for protein binding, the estimated amount of $I_K$ block during the normal course of therapy could approach 25–63%. Therefore, the current observations are relevant to the clinical situation and suggest that block of $I_K$ may play a significant role in the electrophysiological effects of flecainide on the heart.

The clinical significance of $I_K$ block by flecainide may be manifested in its ability to prolong the duration of premature responses elicited early in diastole, an effect that may contribute to the efficacy of flecainide in programmed electrical stimulation. For example, suppression of sustained ventricular tachycardia during electrophysiological testing has been associated with a significantly greater prolongation of refractoriness in responders compared with nonresponders, data that are consistent with a recent clinical report suggesting that a lesser slowing of conduction and greater prolongation of refractoriness with the class IA agent procainamide tend to abolish reentry while a greater slowing of conduction and lesser increase in refractoriness tend to stabilize the reentrant circuit and promote arrhythmia induction. It is also possible that prolonging ventricular APD (block of $I_K$) while decreasing Purkinje fiber APD (block of plateau Na$^+$ channels) might tend to reduce disparities in ventricular repolarization and remove a potential substrate for arrhythmogenesis. However, the marked slowing of conduction typically seen with flecainide may overcome the potential benefits of a decreased dispersion of refractoriness and instead favor arrhythmogenesis, particularly under conditions such as ischemia, in which conduction may already be impaired. Moreover, effects on repolarization are likely to be nonuniform and unpredictable, given that block of $I_K$ and the plateau Na$^+$ current will each exhibit their own time and voltage dependence, thereby potentially enhancing any preexisting local heterogeneities in repolarization time course and facilitating reentry. Thus, the difference between flecainide and E-4031 in their effects on the cardiac Na$^+$ channel may help to explain the observed differences in the spectrum of antiarrhythmic activity for these two agents.

**Role of Inward Rectification of $I_K$**

The inward rectification of $I_K$ in cat ventricular myocytes resembles that previously described for $I_{Kr}$.
in sheep Purkinje fibers\(^1\) and in chick cell aggregates\(^2\) and for \(I_k\) in rabbit sinoatrial node cells\(^2\) but is discordant with measurements of \(I_k\) in cultured embryonic chick ventricular myocytes,\(^2\) which show a linear membrane current-voltage relation for \(I_k\) at both single channel and whole cell levels. The property of rectification provides some insight into how \(I_k\) might modulate APD in ventricular myocardium. In cat ventricular myocytes, \(I_k\) is very small and activates slowly at potentials near the plateau (+10 to +20 mV). Therefore, its contribution to repolarization would be small compared with that for inward \(Ca^{2+}\) or \(Na^+\) current inactivation. However, the inward rectifying properties of \(I_k\) ensure a major contribution to repolarization by allowing the conductance of \(I_k\) to increase as the membrane potential becomes more negative from the plateau voltage through −40 mV. The rectification, therefore, acts as a positive feedback for repolarization, contributing a great deal of outward current between 0 and −40 mV and a lesser amount as the membrane potential approaches the resting potential. It should be kept in mind, however, that the present studies used cycle lengths (45–60 seconds) that permitted full recovery of the tail currents. In the normally beating heart, slow deactivation of \(I_k\) during diastole will provide additional (residual) outward current to the repolarization process that would further shorten APD.

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