Reversal of Lidocaine Effects on Sodium Currents by Isoproterenol in Rabbit Hearts and Heart Cells

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Abstract

We demonstrated recently that isoproterenol enhanced the cardiac voltage-dependent sodium currents (I_{Na}) in rabbit ventricular myocytes through dual G-protein regulatory pathways. In this study, we tested the hypothesis that isoproterenol reverses the sodium channel blocking effects of class I antiarrhythmic drugs through modulation of I_{Na}. The experiments were performed in rabbit ventricular myocytes using whole-cell patch-clamp techniques. Reversal of lidocaine suppression of I_{Na} by isoproterenol (1 μM) was significant at various concentrations of lidocaine (20, 65, and 100 μM, $P < 0.05$). The effects of isoproterenol were voltage dependent, showing reversal of I_{Na} suppression by lidocaine at normal and hyperpolarized potentials (negative to −80 mV) but not at depolarized potentials. Isoproterenol enhanced sodium channel availability but did not alter the steady state activation or inactivation of I_{Na} nor did it improve sodium channel recovery in the presence of lidocaine. The physiological significance of the single cell I_{Na} findings were corroborated by measurements of conduction velocities using an epicardial mapping system in isolated rabbit hearts. Lidocaine (10 μM) significantly suppressed epicardial impulse conduction in both longitudinal (θ_L, 0.430±0.024 vs. 0.585±0.001 m/s at baseline, $n = 7$, $P < 0.001$) and transverse (θ_T, 0.206±0.012 vs. 0.257±0.014 m/s at baseline, $n = 8$, $P < 0.001$) directions. Isoproterenol (0.05 μM) significantly reversed the lidocaine effects with θ_L of 0.503±0.027 m/s and θ_T of 0.234±0.015 m/s ($P = 0.014$ and 0.004 compared with the respective lidocaine measurements). These results suggest that enhancement of I_{Na} is an important mechanism by which isoproterenol reverses the effects of class I antiarrhythmic drugs. (*J. Clin. Invest.* 1993. 91:693–701.) Key words: β-adrenergic stimulation • sodium channels • antiarrhythmic drugs • cardiac myocytes • conduction velocities

Introduction

Approximately 80% of the 350,000 deaths each year from sudden cardiac arrest in this country are due to ventricular tachyarrhythmias (1). Sodium channel–blocking antiarrhythmic drugs (class I) are the most commonly used pharmacological agents for the treatment of life-threatening ventricular arrhythmias. However, recurrence of ventricular tachyarrhythmias remains high (up to 32% in 2 yr) in patients treated with class I antiarrhythmic drugs that show suppression of ventricular tachyarrhythmias induced by programmed electrical stimulation (2). The decline in drug efficacy during long-term follow-up is probably multifactorial and may be due to inconsistent patient compliance in drug administration, change in myocardial substrate in patients with coronary artery disease, concurrent disease states that may affect drug metabolism, proarrhythmic and adverse hemodynamic effects of antiarrhythmic drugs (3), and interaction with drug metabolites or with other concurrent drugs that may reduce the effects of antiarrhythmic drugs (4). In addition, β-adrenergic catecholamines, whether in blood circulation or released locally from sympathetic nerve endings, may be important in the modulation of antiarrhythmic drug effects.

It is well known that β-adrenergic stimulation is important in the pathogenesis of ventricular tachyarrhythmias (5). Isoproterenol has been shown to facilitate induction of ventricular tachyarrhythmias in patients whose clinical arrhythmias could not be induced by programmed electrical stimulation (6, 7). Beta-adrenergic blockade is known to protect patients against sudden cardiac death after a myocardial infarction (8) and addition of β-blockers to class I antiarrhythmic drugs has been demonstrated to provide further protective effects against induction of ventricular tachycardia and its recurrence (9). More recently, clinical electrophysiological studies reported β-adrenergic catecholamines could reverse the protective effects of class I antiarrhythmic drugs that have been shown to be efficacious by programmed electrical stimulation (10, 11). Patients presented with sudden cardiac arrest and recurrent syncope due to rapid ventricular tachycardia showed a high propensity for isoproterenol-induced antiarrhythmic reversibility (80 and 62.5%, respectively) (10). The mechanism by which isoproterenol reverses the antiarrhythmic effects of sodium channel–blocking drugs is unknown.

Beta-adrenergic catecholamines are known to modulate the activities of a number of membrane ionic currents in cardiac cells (12). These include the slow-inward calcium current (13), the pacemaker current in the sinoatrial node, I_f (14), the chloride current (15, 16), the transient outward potassium current (17), the delayed rectifer potassium current (18), and an Na^+-dependent inward current (19). Although β-adrenergic modulation of these ion currents may significantly alter the electrophysiological properties of the heart and may facilitate development of arrhythmias, direct demonstration that such β-adrenergic effects modulate antiarrhythmic drug action has not been previously reported. Recently, we demonstrated that the voltage-dependent sodium currents (I_{Na})[1] in rabbit cardiac

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1. *Abbreviations used in this paper:* HP, holding potential; I_{Na}, sodium current.

myocytes are enhanced by isoproterenol (20). The enhancement of I_{Na} by isoproterenol is mediated both through direct stimulatory effects of $G_{s\alpha}$ and indirect cAMP-dependent phosphorylation mechanisms. In this study, we tested the hypothesis that the enhancement of I_{Na} by isoproterenol is responsible for the reversal of suppression of I_{Na} by class I antiarrhythmic drugs and that the reversal of such effects should be reflected by changes in impulse conduction velocities in the ventricular myocardium. We measured the effects of lidocaine and isoproterenol on I_{Na} in isolated rabbit ventricular myocytes using patch-clamp techniques. The effects of lidocaine and isoproterenol on epicardial impulse conduction velocities were measured using an epicardial mapping system in Langendorff-perfused isolated rabbit hearts.

Methods

Isolation of ventricular myocytes. Isolated rabbit ventricular myocytes were obtained by enzymatic dissociation through retrograde coronary perfusion with 0.017 mg/ml protease (type XXIV; Sigma Chemical Co., St. Louis, MO) as described previously (20). After 10 min of enzyme perfusion at 37°C, 2 mm × 2 mm pieces of myocardium were removed from the ventricles and further digested with collagenase (type I, 0.6 mg/ml; Sigma Chemical Co.) in a nominally zero-$CaCl_2$ solution with the following composition (mM): 140 NaCl, 4.5 KCl, 1.0 $MgCl_2$, 10 Hepes, and 5.55 glucose, pH 7.4. After 5 min of incubation at 35°C, the tissue segments were rinsed with at least five aliquots of zero-$CaCl_2$ solution and single cells were dissociated by mild mechanical trituration. Elongated, striated, and Ca^{++}-tolerant single myocytes were used for the experiments. These cells were quiescent without spontaneous contractile activities and had resting membrane potentials between −75 and −89 mV. All cellular experiments were performed within 6 h of cell isolation.

Cellular electrophysiological techniques. Voltage-dependent I_{Na} in isolated cardiac ventricular myocytes were measured using patch-clamp techniques as described previously (20, 21). Bath solutions were superfused using a direct current–powered pump (Cole-Palmer Instrument Co., Chicago, IL) at a rate of 1 to 2 ml/min and solution exchanges were complete within 30 to 60 s. Whole-cell I_{Na} was recorded with an integrating amplifier (Axopatch 1C or Axopatch 200; Axon Instruments, Foster City, CA), visualized on-line using a digital oscilloscope, filtered with a four-pole low pass Bessel filter with a bandwidth (−3 dB) of 2 kHz and sampled at 25 kHz using a 12-bit resolution A/D converter. pClamp software (Axon Instruments) was used for generating voltage-clamp protocols and for the acquisition and analysis of I_{Na} using an IBM-compatible 80386–based personal computer. Whole-cell studies were performed with a bath solution with the following composition (mM): 20 NaCl, 100 tetramethylammonium chloride, 20 tetraethylammonium chloride, 4.5 KCl, 1.0 $MgCl_2$, 2.0 $CaCl_2$, 1.0 $BaCl_2$, 2.0 $CoCl_2$, 10.0 Hepes, and 5.55 glucose, pH 7.35. The pipette solution for whole-cell recording had the following composition (mM): 130 CsCl, 5 Na_2ATP, 0.5 GTP, 5 EGTA, 0.5 $CaCl_2$, 2 $MgCl_2$, and 10 Hepes, pH 7.25. All cellular electrophysiology experiments were performed at room temperature (21–23°C). Time-dependent changes of I_{Na} consisting of shifts of inactivation in the hyperpolarizing direction were frequently observed (22–25). These shifts typically occurred in the first 20 min after rupture of the sealed membrane patch. We therefore waited ≥ 20 min for the time-dependent shifts of I_{Na} to reach steady state. No noticeable change in I_{Na} amplitude, time to peak amplitude, and inactivation rate (holding potential [HP] = −100 mV, testing potential [TP] = −30 or −35 mV) were observed over a 5-min period before data were taken.

Stimulus protocols. To examine the effects of isoproterenol on the tonic and use-dependent blocks of I_{Na} by lidocaine, trains of depolarizing current pulses were used. Three holding potentials at −120, −100, and −80 mV were examined. The test potential was at −35 mV. The pulse duration was 40 ms and each train consisted of 20 pulses at cycle lengths of 250 ms. Intertrain intervals were > 30 s. Peak current amplitudes, which were elicited with each pulse during the train of pulses, were plotted against time.

To examine the effects of lidocaine and isoproterenol on I_{Na} throughout the activation range, current–voltage relations were measured with external Na^+ reduced to 20 mM and at room temperature. The holding potentials at −120 mV and the testing potentials ranged from −60 to 20 mV in 5-mV steps. The effects of lidocaine and lidocaine plus isoproterenol were compared with control values. The activation (m_∞) curve was derived from the current–voltage data using the equation

$$m_\infty = I_{Na}/[g_{Na}(V_m - E_{rev})],$$

where I_{Na} represents the current amplitude at the test potential (V_m) and g_{Na} is the maximal conductance value obtained from a linear-regression line of each current–voltage relation extrapolated through the reversal potential (E_{rev}). The curves were then fitted to a conventional Boltzmann function as shown below:

$$m_\infty = \{1 + \exp[(V_{1/2} - V_m)/k]\}^{-1},$$

where $V_{1/2}$ is the voltage of half-activation ($m_\infty = 0.5$) and k is the slope factor. Isoproterenol (1 μM) did not affect any background current under our recording conditions ($n = 5$), therefore, leak subtraction was not performed.

Channel availability was determined using the following protocol: a conditioning pulse of 1-s duration with potentials from −130 to −50 mV in 5 mV increments was followed by a 1-ms interval at −100 mV before a 40 ms testing pulse from −100 to −35 mV was elicited. A 5-s recovery period at −100 mV was introduced between each double-pulse episode. The effects of lidocaine and lidocaine plus isoproterenol were compared with those of control. The steady state inactivation (h_∞) curve was obtained by normalizing currents to the maximal I_{Na} obtained at −130 mV. The curve was then fitted using a conventional Boltzmann distribution equation as follows:

$$h_\infty = \{1 + \exp[(V_m - V_{1/2})/k]\}^{-1},$$

where $V_{1/2}$ represents the voltage at half-inactivation ($h_\infty = 0.5$) and k is the slope factor.

To assess the recovery of I_{Na} from steady state inactivation, experiments were performed using a two-pulse paradigm. A 500-ms conditioning pulse to 0 mV was followed by a recovery period of variable durations from 1 ms to 4 s at −120, −100, or −80 mV. A test pulse of 40-ms duration to −35 mV was then elicited. The amplitude of the peak I_{Na} during the test pulse was normalized to the value of I_{Na} after complete recovery from inactivation, I_{Na} max, and this ratio was plotted against the recovery interval. Recovery curves from I_{Na} inactivation had been reported to display two exponential components (26). The data were therefore analyzed using a two-exponential fit with an equation of the following form:

$$f = \text{Amp } 1(1 - e^{-t/\tau_1}) + \text{Amp } 2(1 - e^{-t/\tau_2}),$$

where Amp 1 + Amp 2 = 1. Curve fitting was performed using a Marquardt-Levenberg least-square fitting procedure from Sigmaplot software (Jandel Sci., Corte Madera, CA). The relative contribution of the fast and slow recovery components, Amp 1 and Amp 2, and their respective time constants, τ_1 and τ_2, were compared for control, lidocaine, and lidocaine plus isoproterenol. The control current at HP = −120 mV was determined statistically to be better fit by one exponential.

Epicardial conduction velocity recordings. Isolated rabbit hearts were maintained on a Langendorff apparatus as described previously (27). The hearts were perfused at 25 ml/min with a buffer of the following composition (mM): 140 NaCl, 4.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 20 glucose, 0.5 NaH_2PO_4, 10 Hepes, pH 7.40 at 37°C equilibrated with 100% O_2. A 30-min equilibration period was instituted before any mapping procedure or experimental intervention was performed. For epicardial conduction velocity recordings, a custom-made electrode array was mounted onto the anterolateral surface of the left ventricle using an

adjustable girdle. The electrode array consisted of 25 gold-plated coaxial bipolar electrodes arranged in a square lattice spaced 2 mm from each other. Pacing was performed via bipolar electrode sites on the periphery of the electrode array with 2-ms current pulses at twice the diastolic threshold using an electronic stimulator (Bloom Associates, Reading, PA) at a rate sufficient to overdrive spontaneous activities (cycle lengths 250–300 ms). Pacing sites were selected according to the isochronal activation maps generated that allowed analysis of transverse and longitudinal conduction velocities. The average ventricular effective refractory period was 177.5±5.4 ms. Perfusion of drugs was allowed to reach steady state (10–15 min for lidocaine and 5–10 min for lidocaine plus isoproterenol) before mapping was performed. After drug intervention, the heart was perfused with control buffer for 30 min and mapping performed to ensure reversibility of drug effects on wash out. Activation times at the recording sites were acquired using a mapping system (BARD Electrophysiology; C. R. Bard, Billerica, MA). Data were stored on the computer hard disk. Analysis of activation times was performed using the BARD software. The activation times for all the beats were automatically assigned by the computer program and marked as the maximum voltage deflection of the bipolar electrogram. All activations were subsequently edited by the same investigator to maintain consistency in activation time measurements. Activation sequence maps with isochronal activation lines at 5- or 10-ms intervals were generated by the BARD software, displayed on the monitor, and printed for future reference. Conduction velocities were calculated by measuring the distance between sites along the longitudinal and transverse axes divided by the difference in activation times. The concentrations of drugs used in these experiments were within the therapeutic ranges used in humans (10 μM of lidocaine was equivalent to 1.7 μg/ml; 0.05 μM of isoproterenol was equivalent to 0.3 μg/min).

Statistical analysis. All data were expressed as mean±SEM and significance was determined by paired t test at $P < 0.05$.

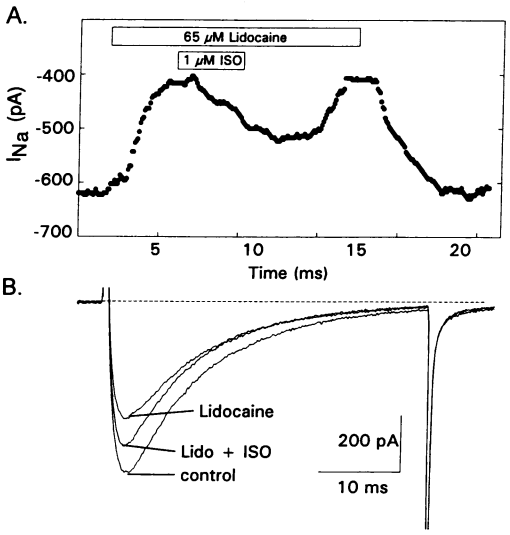

Figure 1. Reversal of lidocaine suppression of sodium currents by isoproterenol. *A* represents the peak whole-cell sodium current amplitudes in 20 mM $[Na^+]_o$ at room temperature plotted versus time. HP = −100 mV, testing potential (TP) = −35 mV with pulse durations of 40 ms, elicited at 5-s intervals. Bath application of lidocaine (20 μM) and isoproterenol (1 μM) are represented by the bars above. *B* represents raw current tracings from the same cell before (*control*) and after application of 20 μM lidocaine (*Lidocaine*), and reversal with the addition of 1 μM isoproterenol to 20 μM lidocaine (*Lido + ISO*).

Results

To ensure adequate voltage control, whole-cell I_{Na} studies were performed with reduced external Na^+ as previously described (20). Under these conditions, 65 μM of lidocaine showed rapid suppression of I_{Na} and the lidocaine effects were significantly reversed by 1 μM of isoproterenol (Fig. 1). Upon removal of isoproterenol, I_{Na} returned to the previous suppressed current amplitude with lidocaine application alone. In addition, I_{Na} returned to the original drug-free baseline on wash out of lidocaine (Fig. 1). Lidocaine suppressed rabbit cardiac I_{Na} in a dose-dependent manner. From a holding potential of −100 mV and a test potential of −30 mV in the presence of 20, 65, and 100 μM of lidocaine, the peak amplitudes of I_{Na} were 86.8±2.2 (n = 4), 76.1±6.6 (n = 4), and 67.5±2.1% (n = 3) of baseline currents, respectively. The ability of isoproterenol to reverse the lidocaine suppression of I_{Na} was also dependent on lidocaine concentration. Addition of 1 μM isoproterenol to 20, 65, and 100 μM of lidocaine resulted in reversal of the lidocaine effects to 100.2±4.4, 88.6±5.7, and 77.8±2.5% of drug-free baseline current, respectively. The reversal by isoproterenol on the suppressed I_{Na} was statistically significant at all lidocaine concentrations ($P < 0.05$). Also, the amounts of both tonic and use-dependent block of I_{Na} increased with increasing lidocaine concentrations.

Tonic I_{Na} blockade by lidocaine was voltage dependent (Fig. 2). At holding potentials of −120, −100, and −80 mV, the peak I_{Na} elicited by a test potential to −35 mV, in the presence of 65 μM of lidocaine, was 82.5±6.9, 80.0±7.6, and 60.9±6.3% of the respective baseline I_{Na} (n = 5, $P < 0.05$ for comparison between −80 and −120 or −100 mV; tonic blocks between −120 and −100 mV were not significantly different). Use-dependent blockade of I_{Na} by lidocaine in rabbit ventricular myocytes was also dependent on membrane potential, as shown in Fig. 2. At a holding potential of −120 mV, a train of 20 depolarizing pulses (testing potentials of −35 mV and pulse durations of 48 ms) at a cycle length of 250 ms elicited little but discernible use-dependent I_{Na} block in the presence of 65 μM of lidocaine (Fig. 2, *top*). At a holding potential of −80 mV, the same pulse protocol resulted in the development of significant use-dependent I_{Na} block. These results showed that the amount of block from use dependence was greater than the amount of tonic block (Fig. 2, *bottom*), which was represented by the peak I_{Na} amplitude elicited by the first pulse of the train with intertrain intervals of > 30 s. The same pulse train at a holding potential of −100 mV elicited intermediate amounts of use-dependent I_{Na} block (Fig. 2, *middle*). These results are consistent with the current concepts that antiarrhythmic drug actions of sodium channel blockade are voltage dependent (28). This effect is probably due to the voltage dependence of sodium channel repriming as previous reported (26). The frequency-dependent reduction of I_{Na} observed during baseline at a holding potential of −80 mV can also be explained by the kinetics of sodium channel repriming (Fig. 2, *lower*). At −80 mV, I_{Na} does not recover completely from inactivation with a recovery interval of 250 ms (see Fig. 5, *bottom*), resulting in frequency-dependent accumulation of channel inactivation. The ability of isoproterenol (1 μM) to reverse lidocaine blockade of cardiac I_{Na} is also dependent on membrane potential (Fig. 2). At a holding potential of −120 mV, 1 μM of isoproterenol completely reversed the tonic block and significantly reversed the use-dependent block of I_{Na} by 65 μM of lidocaine (Fig. 2, *top*) whereas at a holding potential of −80 mV, 1 μM of isoproterenol showed little reversal of the tonic or use-dependent I_{Na} block by lidocaine (Fig. 2, *bottom*). At a holding potential of

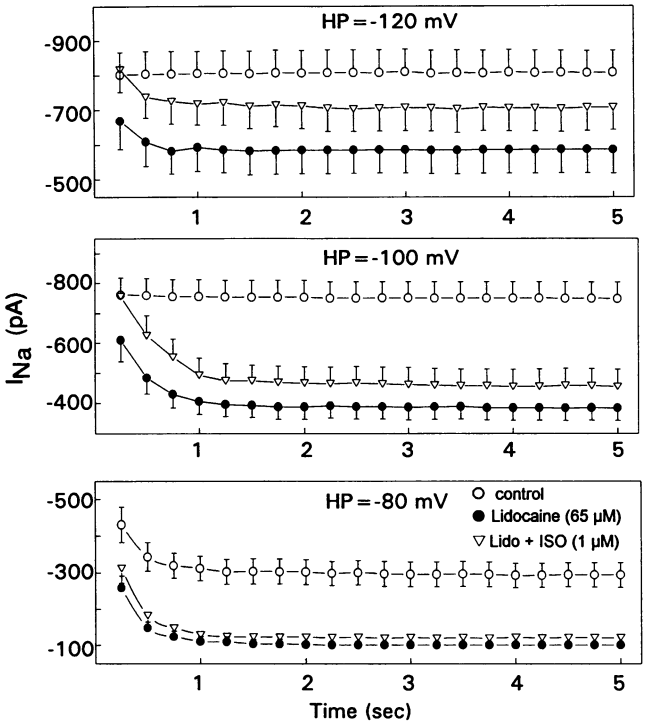

Figure 2. Effect of holding potential on isoproterenol reversal of lidocaine suppression of sodium currents. The peak whole-cell sodium current amplitudes in 20 mM $[Na^+]_o$ at room temperature are plotted against trains of 20 depolarizing current pulses at a cycle length of 250 ms (n = 5). The HPs are −120 (*top*), −100 (*middle*), and −80 mV (*bottom*). The testing potential is −35 mV of 40-ms duration for all pulse trains. For each holding potential, the sodium currents from five cells at baseline (*open circles*), with 65 μM lidocaine (*closed circles*), and with 1 μM isoproterenol plus 65 μM lidocaine (*open triangles*) are plotted against time during pulse trains. Values represent mean±SEM and symbols without error bars indicate that error bars are smaller than the symbol size.

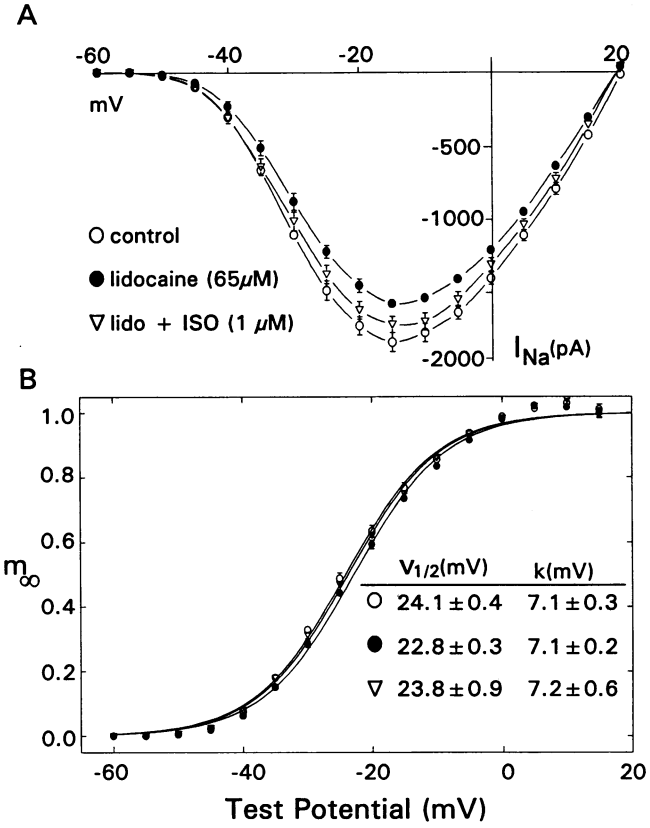

Figure 3. The effect of lidocaine and isoproterenol on the whole-cell sodium current–voltage relation and activation (m_∞) curves in 20 mM $[Na^+]_o$ at room temperature. *A* represents the current–voltage relation from four cells at baseline (*control, open circles*), after application of 65 μM of lidocaine (*closed circles*), and after application of 65 μM of lidocaine plus 1 μM of isoproterenol (*open inverted triangles*). Holding potentials are at −120 mV with pulse durations of 40 ms. Pulses are repeated at 5-mV increments at 2-s intervals. Values represent mean±SEM and symbols without error bars indicate the error bars are smaller than symbol size. *B* represents the whole-cell activation curve constructed from the same data used in *A*. Values are mean±SEM. The data are curve fitted using a conventional Boltzmann equation. $V_{1/2}$ is the half-activation value and k is the slope factor; values between control, lidocaine, and lidocaine plus isoproterenol are not significantly different.

−100 mV, isoproterenol completely reversed tonic I_{Na} block and achieved intermediate levels of reversal of use-dependent I_{Na} block by lidocaine (Fig. 2, *middle*).

Fig. 3 *A* shows the effects of lidocaine (65 μM) and lidocaine plus isoproterenol (1 μM) throughout the I_{Na} activation range. From a holding potential of −120 mV, lidocaine (65 μM) significantly suppressed I_{Na} at all potentials (from −45 to 20 mV with $P < 0.05$ compared with control currents). Addition of isoproterenol (1 μM) significantly reversed the lidocaine effects at potentials between −45 and 0 mV ($P < 0.05$ compared with currents in the presence of lidocaine alone). Isoproterenol also consistently enhanced I_{Na} in the presence of lidocaine at the other potentials, though statistical significance was not reached (Fig. 3 *A*). To determine the mechanism underlying the I_{Na} changes, the activation process of I_{Na} was investigated. The activation (m_∞) curves of I_{Na} under control conditions in the presence of lidocaine (65 μM) or in the presence of lidocaine plus isoproterenol (1 μM) are shown in Fig. 3 *B*. Neither lidocaine nor lidocaine plus isoproterenol significantly altered the half-activation value ($V_{1/2}$) or the slope factor (k) (P = NS) when compared with control values (n = 4).

The effects of prepulse potential on the isoproterenol reversal of lidocaine suppression of I_{Na} are shown in Fig. 4. Lidocaine (65 μM) reduced the maximum available I_{Na} by 20% and significantly reduced the steady state I_{Na} availability at all potentials (−130 to −60 mV, $P < 0.05$ compared with control currents, n = 8). In addition, lidocaine shifted the voltage-dependent I_{Na} availability curve in the hyperpolarizing direction by ~ 5 mV but did not change the steepness of the curve. Isoproterenol (1 μM) increased I_{Na} availability in the presence of lidocaine at resting or hyperpolarized potentials (negative to −80 mV, $P < 0.05$ compared with current in the presence of lidocaine alone, n = 8) but not at depolarized potentials (Fig. 4 *A*). The normalized steady state inactivation curves are shown in Fig. 4 *B*. Lidocaine shifted the half-inactivation value, $V_{1/2}$, from −80.0±0.8 to −84.9±0.5 mV (n = 8, $P < 0.00001$) but did not change the slope factor, k, (5.3±0.2 vs. 5.2±0.2 mV in controls, n = 8, P = NS). Neither the hyperpolarizing shift of the inactivation curve by lidocaine nor the slope factor of the inactivation curve was altered by isoproterenol ($V_{1/2}$ = −83.3±0.8 mV and k = 5.4±0.2 mV, n = 8, P = NS compared with the respective values of lidocaine).

To further assess the interaction between lidocaine and isoproterenol on I_{Na}, we measured the effects of isoproterenol on

the recovery of steady state inactivation of sodium channels from lidocaine block. These experiments were performed using a two-pulse paradigm as described in Methods. The recovery curves of I_{Na} from inactivation in control with lidocaine (65 μM) and with lidocaine plus isoproterenol (1 μM) at three different holding potentials (−120, −100, and −80 mV) are displayed in Fig. 5. The parameters of I_{Na} recovery (Amp 1, τ_1, Amp 2, and τ_2) are shown in Table I. Recovery of I_{Na} from inactivation was dependent on holding potential. At a holding potential of −120 mV, recovery of I_{Na} can be fitted with a single exponential with a time constant of 8.7±1.0 ms (n = 4). However, at a holding potential of −100 mV, the time course of I_{Na} recovery showed two exponential components: a fast component with an amplitude (Amp 1) of 0.95 and a time constant (τ_1) of 11.1±0.2 ms followed by a slow component with an amplitude (Amp 2) of 0.05 and a time constant (τ_2) of 99.2±9.0 ms (n = 4). At a holding potential of −80 mV, Amp 1 was 0.61 with τ_1 of 111.3±12.3 ms and Amp 2 was 0.40 with τ_2 of 249.3±35.1 ms (n = 6) (Fig. 5 and Table I). Hence, at more depolarized potentials, both time constants became more prolonged and the contribution of the fast component became more diminished, indicating that repriming of the sodium channel is voltage dependent, which is similar to previous observations (26). Lidocaine (65 μM) significantly reduced the amplitude of the fast component and increased the amplitude of the slow component of sodium channel recovery ($P < 0.005$ for Amp 1 and Amp 2 at all three potentials compared with respective values at baseline). This is consistent with the current concept that the fast component represents recovery of unblocked channels and the slow component represents recovery of blocked channels (29). Lidocaine did not alter τ_1 at −120 and −100 mV but significantly prolonged τ_1 at −80 mV (P = 0.015 compared with baseline, n = 6) and lidocaine significantly prolonged τ_2 at −100 and −80 mV ($P < 0.01$ compared with control values). Isoproterenol had no significant effect on the kinetics of sodium channel recovery in the presence of lidocaine (Amp 1, τ_1, Amp 2, τ_2 were not significantly different from those of lidocaine alone, P = NS for comparisons with lidocaine alone at all three potentials). These results suggest that isoproterenol reversal of lidocaine effects is not through facilitation of sodium channel recovery from blockade by lidocaine.

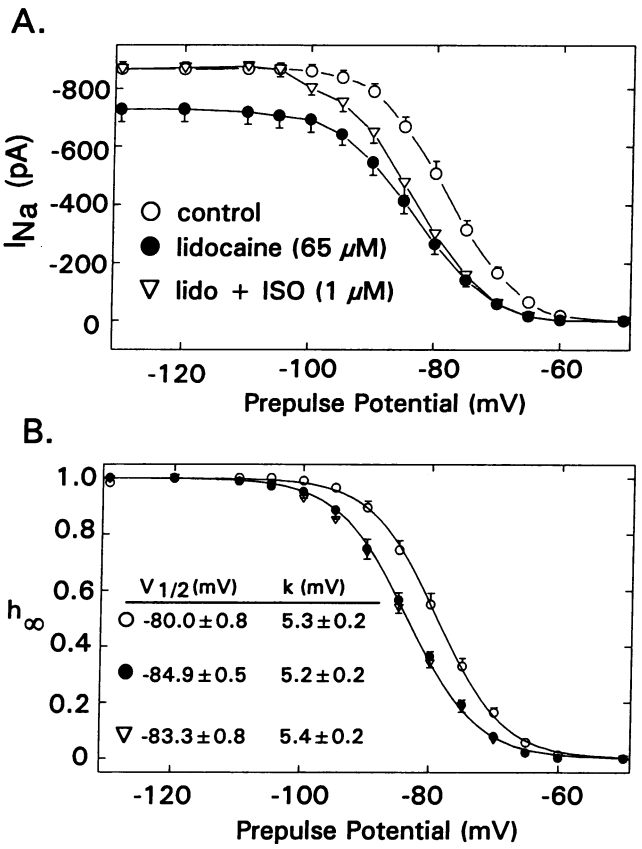

Figure 4. Effect of prepulse potential on isoproterenol reversal of lidocaine suppression of the sodium current. (*A*) The amplitudes of the sodium current (testing potentials of −35 mV) are plotted against the indicated prepulse potentials (500 ms) before (*open circles*) and after (*closed circles*) the application of 65 μM lidocaine and in the presence of 1 μM isoproterenol plus 65 μM lidocaine (*open inverted triangles*). Each point represents the mean±SEM from eight experiments. (*B*) The effect of lidocaine and isoproterenol on the whole-cell inactivation (h_∞) curve. The data from the experiments shown in the *A* are normalized to maximal sodium current and plotted versus prepulse potentials for control (*open circles*), 65 μM lidocaine (*closed circles*), and 65 μM lidocaine plus 1 μM isoproterenol (*open inverted triangles*). Data are expressed as mean±SEM and symbols without error bars indicate error bars are smaller than symbol size. The data are fitted to a conventional Boltzmann distribution equation, where $V_{1/2}$ represents the half-inactivation value and k represents the slope factor. The difference in $V_{1/2}$ between control and lidocaine and lidocaine plus isoproterenol are significant, $P < 0.05$. The differences in k are not statistically significant.

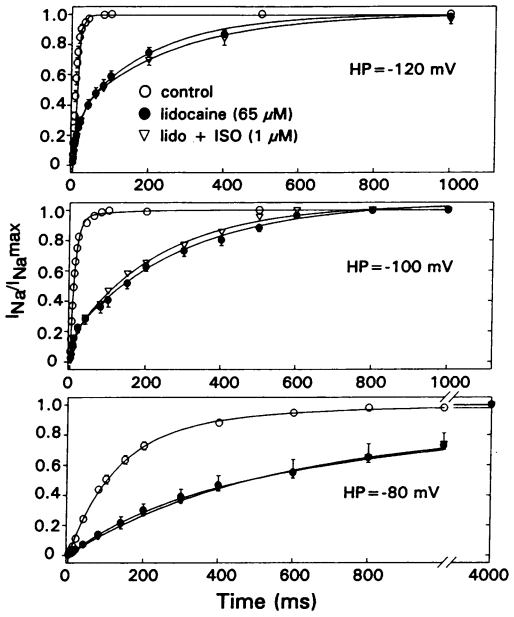

Figure 5. Effect of lidocaine (65 μM) and isoproterenol (1 μM) on recovery from inactivation of I_{Na} using a two-pulse paradigm. Three holding potentials are studied: −120 (*top*, n = 4), −100 (*middle*, n = 4), and −80 mV (*bottom*, n = 6). Conditioning pulses at 0 mV of 500-ms duration are followed by testing pulses at −35 mV of 40-ms duration with an intertrain interval of 5 s. For holding potentials of −120 and −100 mV, the interpulse intervals are between 1 and 1,000 ms. For holding potential of −80 mV, the interpulse intervals are between 1 and 4,000 ms. The results are expressed as mean±SEM for control (*open circles*), 65 μM lidocaine (*closed circles*), and 65 μM lidocaine plus 1 μM isoproterenol (*open inverted triangles*). Symbols without error bars indicate error bars are smaller than the symbol size.

Table I. Parameters of Sodium Channel Recovery from Inactivation

	Amp 1	τ_1	Amp 2	τ_2
		ms		*ms*
HP = −120 mV (n = 4)				
Control	1.08±0.02	8.7±1.0	—	—
Lidocaine	0.34±0.09*	16.1±5.9	0.66±0.09	269.9±80.3
Lido + ISO	0.33±0.03*	15.8±4.3	0.70±0.02	301.2±72.3
HP = −100 mV (n = 4)				
Control	0.95±0.02‡	11.1±0.2	0.05±0.02	99.2±9.0
Lidocaine	0.16±0.03*	9.8±1.8	0.84±0.03*	297.8±27.8*
Lido + ISO	0.15±0.02*‡	13.1±1.7	0.85±0.02*	238.7±9.7*
HP = −80 mV (n = 6)				
Control	0.61±0.03‡	111.3±12.3‡	0.39±0.03	249.3±35.1
Lidocaine	0.27±0.05*	192.0±33.5*‡	0.75±0.04*	925.5±71.5*‡
Lido + ISO	0.26±0.05*	195.8±60.9*‡	0.74±0.05*	766.9±82.1*‡

Data from Fig. 5 are analyzed using a two-exponential fit with an equation of the following form: f = Amp $1(1 - e^{-t/\tau_1})$ + Amp $2(1 - e^{-t/\tau_2})$. Amp 1 and Amp 2 represent the relative contribution (in ratio) and τ_1 and τ_2 represent the time constants (in ms) of the fast and the slow components of I_{Na} recovery, respectively. * Represents $P < 0.05$ compared with control values at each holding potential. ‡ Represents $P < 0.05$ compared with corresponding values at holding potential of −120 mV.

To confirm the physiological relevance of the observations in isolated cardiac myocytes, we studied the effects of isoproterenol on lidocaine suppression of conduction velocities. These studies were performed in isolated rabbit hearts using an epicardial mapping system as described in Methods. Using such a setup, the control conduction velocities were 0.585±0.001 m/s (n = 7) in the longitudinal direction (θ_L) and 0.257±0.014 m/s in the transverse direction (θ_T) (n = 8) (Fig. 6). These results are in agreement with reported values from other laboratories with different species (30, 31). Lidocaine (10 μM) significantly depressed both longitudinal and transverse conduction velocities, with θ_L of 0.430±0.024 m/s and θ_T of 0.206±0.012 m/s (P = 0.001 compared with control values). The lidocaine effects were significantly reversed by isoproterenol (0.05 μM) with θ_L of 0.503±0.027 m/s and θ_T of 0.234±0.015 m/s (P = 0.014 and 0.004 compared with the respective lidocaine measurements). These results are consistent with the findings of I_{Na} in isolated cardiac myocytes. However, the effect of lidocaine on conduction velocities is anisotropic; the depression of θ_L by lidocaine is greater than that of θ_T. The ratio of θ_L/θ_T was significantly lower in the presence of lidocaine (from 2.39±0.20 at baseline vs. 2.15±0.20 with lidocaine, n = 6, $P < 0.05$). These results were consistent with previous observations in canine myocardium (32). On the other hand, the effects of isoproterenol were not anisotropic (θ_L/θ_T = 2.28±0.24, n = 6, P = NS compared with lidocaine alone), suggesting that the site of isoproterenol action on the sodium channel was different from that of lidocaine.

Discussion

The major new finding of the present study is that β-adrenergic stimulation reverses lidocaine suppression of cardiac I_{Na}. Lidocaine was chosen because it is a prototype sodium channel blocking antiarrhythmic drug and its effects in heart and heart cells have been well characterized (26, 32–36). Sodium channel blockade is thought to result from binding of lidocaine to specific receptors in the sodium channel in both activated and inactivated states (35). Single sodium channel studies in mammalian cardiac myocytes showed that lidocaine reduces both the number of open channel events and the average duration of opening for each event (36). Furthermore, the effects of lidocaine on conduction velocities in the intact heart has been characterized (32). Our study shows that reversal of the lidocaine effects on the rabbit I_{Na} by isoproterenol is observed both in isolated cardiac myocytes and isolated hearts.

In contrast to the effects of isoproterenol on the cardiac calcium and potassium channels, which have been well characterized, much less information is available regarding β-adrenergic effects on the cardiac I_{Na}. Conflicting results have been

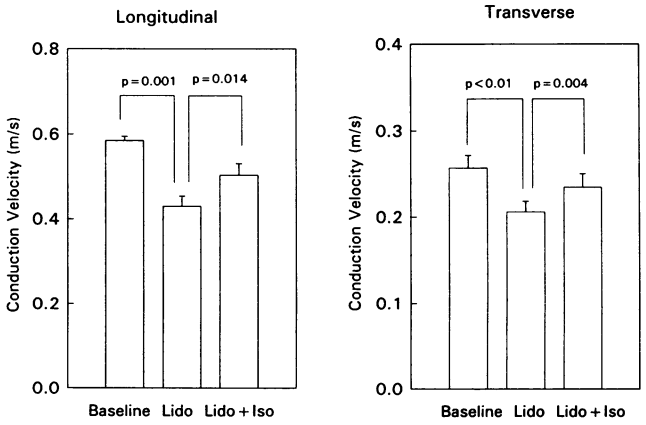

Figure 6. Epicardial impulse conduction in isolated rabbit hearts. (*Left*) Epicardial conduction velocities in m/s in the longitudinal direction at baseline, in the presence of 10 μM lidocaine and 10 μM lidocaine plus 0.05 μM isoproterenol. Values represent mean±SEM in seven experiments. (*Right*) Epicardial conduction velocities in m/s in the transverse direction at baseline, in the presence of 10 μM lidocaine and 10 μM lidocaine plus 0.05 μM isoproterenol. Values represent mean±SEM in eight experiments. P values are obtained using paired t tests.

reported on the modulation of the cardiac I_{Na} by isoproterenol. Ono et al. (37) showed reduction of I_{Na} by isoproterenol in guinea pig cardiac myocytes. Such isoproterenol effects were most prominent at depolarized membrane potentials. Schubert et al. (38) also reported inhibitory effects of isoproterenol on I_{Na} in neonatal rat cardiac myocytes. Here, the isoproterenol effects were associated with a shift of the steady state inactivation curve to a more negative potential. However, other investigators observed that isoproterenol enhances I_{Na} in cardiac myocytes (39, 40). The discrepancy between results from different laboratories is not immediately clear, but species-dependent differences in cardiac I_{Na} regulation is a possible explanation. Recently, we found enhancement of the cardiac I_{Na} in rabbit cardiac myocytes by β-adrenergic stimulation through dual G-protein regulatory pathways (20). The existence of dual regulatory pathways is an indication that β-adrenergic stimulation plays an important physiological role in the modulation of sodium channel activities. In the present study, we demonstrate that the stimulatory effects of isoproterenol on I_{Na} can significantly reverse the suppressive effects of lidocaine. These results have important clinical implications.

Reversal of lidocaine effects on I_{Na} in isolated myocytes. Our study shows that in rabbit ventricular myocytes, isoproterenol reverses lidocaine blockade of I_{Na} without changing the steady state activation or inactivation properties of the sodium channel. The steady state activation and inactivation variables for I_{Na} are similar to those reported previously in similar cells (20), in bullfrog atrial myocytes (41), and in rabbit cardiac Purkinje fibers (42). The effect of isoproterenol reversal of lidocaine I_{Na} block is voltage dependent. Normal resting membrane potentials (−85 to −95 mV) are within the range where the effect of isoproterenol on I_{Na} is the steepest and their interaction the most dynamic. Reversal of tonic I_{Na} block of lidocaine and the degree of reversal by isoproterenol are dependent on lidocaine concentration as well as membrane potential. The increase in tonic I_{Na} block by lidocaine at partially depolarized potentials (−80 mV) is explained by the shift of steady state inactivation curve in the hyperpolarized direction (Fig. 4). The voltage dependence of isoproterenol reversal of lidocaine suppression of I_{Na} is similar to the voltage range through which isoproterenol enhancement of cardiac I_{Na} was observed (20). Isoproterenol does not appear to have significant effects on use-dependent lidocaine block. Although isoproterenol significantly enhances I_{Na} in the presence of lidocaine, isoproterenol appears to produce a parallel shift of the use-dependent reduction of I_{Na} by lidocaine without changing the profile of use dependence. This observation is in agreement with our findings that isoproterenol does not alter sodium channel recovery from blockade by lidocaine (Fig. 5). Use-dependent reduction of I_{Na} can also be demonstrated in the absence of sodium channel blockers at partially depolarized potentials (−80 mV). It illustrates that use-dependent reduction of I_{Na} is due to incomplete channel recovery from inactivation, resulting in accumulation of channel blockade with sequential depolarizations until a new steady state is reached (28).

We found that the two components of sodium channel recovery from inactivation are similar to those described by Bean et al. (26). At normal or hyperpolarized potentials (−120 and −100 mV), lidocaine does not change the time constant of the fast recovery component (τ_1) but decreases its contribution. On the other hand, lidocaine increases both the contribution of the slow recovery component and prolongs its time constant. At HPs of −80 mV, lidocaine prolongs τ_1, suggesting the kinetics of I_{Na} recovery in partially depolarized tissue are more complex and may be better described with more than two exponential components. However, detailed analysis of I_{Na} recovery kinetics is beyond the scope of the present study. Isoproterenol exhibits no significant effects on the fast or the slow component of I_{Na} recovery in the presence of lidocaine. Lidocaine shifts the I_{Na} inactivation curve in the hyperpolarizing direction and isoproterenol has no effect on the normalized steady state inactivation curve. These results suggest that isoproterenol does not affect lidocaine binding and unbinding to the sodium channel receptor site. Among the various potential mechanisms for isoproterenol to reverse the suppressive effects of lidocaine on the sodium channel, an increase in channel recruitment is a distinct possibility as sodium channel availability is increased (Fig. 4 *A*). Detailed mechanisms involved in the reversal of the lidocaine blockade of I_{Na} by isoproterenol, however, will require further studies using single sodium channel recordings.

Reversal of lidocaine effects on conduction velocities in isolated hearts. The epicardial conduction experiments were performed to further investigate the physiological significance of the whole-cell I_{Na} studies. These studies were performed under physiological electrolyte compositions and temperature. Suppression of I_{Na} by lidocaine and reversal of the lidocaine effect by isoproterenol should be reflected by changes in impulse conduction velocities. Wallace and Sarnoff (43) reported an 8% enhancement of intraventricular conduction velocities by sympathetic nerve stimulation in dogs. Dhingra et al. (44) reported shortening of H-V intervals by isoproterenol, suggesting facilitation of His-Purkinje conduction in humans. However, other investigators detected catecholamine-induced increase in conduction velocities only in ischemic or partially depolarized tissue but not in normal tissue (45). The effect of isoproterenol on conduction in human myocardium has not been directly measured. Our results suggest that the epicardial conduction velocity findings are consistent with our single-cell I_{Na} measurements. Isoproterenol significantly reverses the suppression of conduction by lidocaine. The effect of lidocaine on epicardial impulse conduction velocities in rabbit hearts is anisotropic with greater depression of θ_L than θ_T, which is consistent with previous observations in canine hearts (32). Such anisotropic effects are thought to be due to directional differences in lidocaine binding. The total open time of the sodium channels is thought to be greater in longitudinal propagation, with greater access to receptor sites for sodium channel blockers (46). Also, repolarization intervals are longer in longitudinal propagation, leading to greater binding of drug to the inactivated channel (47). Since lidocaine blocks both the activated and inactivated sodium channels (48), both of these mechanisms contribute to the anisotropic effects of lidocaine. Isoproterenol does not reverse the anisotropic effects of lidocaine, again suggesting that isoproterenol does not directly inhibit the interaction of lidocaine with the sodium channel but reverses the effects of lidocaine through other mechanisms to enhance I_{Na}. Our observations do not entirely exclude the possibility that isoproterenol facilitates impulse conduction via other mechanisms, such as effects on gap junctions. The strong agreement between single-cell and isolated-heart experiments, however, suggests that modulation of the cardiac sodium channel is an important mechanism by which β-adrenergic stimulation reverses the effects of class I antiarrhythmic drugs. The conduction velocity results also confirm that our single cell I_{Na} observations bear important physiological relevance. However, quantitative comparison between isoproterenol reversal of single-cell I_{Na} and

whole-heart conduction velocities cannot be made because of differences in lidocaine concentrations and temperature.

Clinical implications. Our findings identify modulation of cardiac I_{Na} by β-adrenergic stimulation as a major factor by which the protective effects of antiarrhythmic drugs are antagonized by catecholamines. Our results provide mechanistic insight into the clinical observations regarding the β-adrenergic facilitation of arrhythmias in the electrophysiology laboratory (6, 7, 10, 11) as well as the protective effects of β-blockers in the prevention of sudden cardiac death (8, 9). Although we believe our findings are of major importance in depicting the β-adrenergic reversal of antiarrhythmic drug effects, we would like to acknowledge other potential mechanisms that may also contribute to such β-adrenergic effects. Beta-adrenergic stimulation is known to modulate a number of ionic channels (12) in the heart and has profound effects on the cardiac action potential (5). The delayed rectifier I_K in both mammalian and amphibian hearts (18, 49), the transient outward current I_{to} (17), and the chloride current (15, 16) are known to be activated by isoproterenol and by intracellular application of cAMP or the catalytic subunit of protein kinase A. Blockers of the inactivated sodium channels would become less effective as these channels are activated and the cardiac action potential duration becomes shorter. Activation of the Na^+/K^+ pump by β-adrenergic stimulation will lead to hyperpolarization of the membrane potential (50), which may diminish the amount of tonic and use-dependent I_{Na} block, as well as improve recovery of the sodium channel from blockade by antiarrhythmic drugs like lidocaine. These β-adrenergic effects on cardiac ion channels can therefore compromise antiarrhythmic drug action independent of the effects on the sodium channel. On the other hand, activation of the Na^+-dependent inward current (19) would cause depolarization of membrane potential with greater I_{Na} block by drug and may produce toxic side effects, including proarrhythmia. Beta-adrenergic stimulation is also known to facilitate certain types of cardiac arrhythmias, including delayed afterdepolarizations through intracellular Ca^{++} overload (51) and ventricular fibrillation through increase in cAMP (52, 53). The daily fluctuations of body catecholamine levels may therefore play an important role not only in the modulation of antiarrhythmic drug action but also in the pathogenesis of arrhythmias that may jeopardize the long-term survival of patients on class 1 antiarrhythmic drugs for treatment of life-threatening ventricular arrhythmias. In addition, chronic administration of antiarrhythmic drugs may result in parent drug–metabolite interaction that may diminish the efficacy of antiarrhythmic drugs. Glycylxylidide, a deethylated metabolite of lidocaine that accumulates in patients on lidocaine therapy, competes with lidocaine for the same sodium channel receptor but has different kinetics of recovery from block. Glycylxylidide has been shown to competitively displace its parent compound in vitro, producing a significant increase in I_{Na} (54).

Beta-adrenergic stimulation has been shown to restore conduction velocities in tissues where conduction is depressed, e.g., ischemic and partially depolarized myocardium (45). This is similar to our findings in isolated rabbit hearts where conduction depressed by lidocaine is restored by isoproterenol. Other than the effects on conduction velocity, β-adrenergic stimulation may augment development of ventricular arrhythmias, both spontaneous and induced, through shortening of refractoriness (55) so that premature depolarizations can be more closely coupled to allow better penetration of reentry circuits.

Our observation that isoproterenol reversal of lidocaine effects on I_{Na} is voltage dependent suggests that β-adrenergic stimulation is potentially arrhythmogenic in hearts with ischemic disease. Heterogeneity of impulse conduction in partially depolarized tissue may predispose the heart to development of conduction block and reentry. The spatial nonuniformity in excitability and recovery of excitability may also give rise to ventricular fibrillation. Indeed, rate-dependent conduction velocity depression and nonuniform activation are identified as important determinants for the genesis of ventricular arrhythmias (32). Isoproterenol with a drug like lidocaine may further exaggerate such nonuniformity and predispose the ischemic heart to lethal arrhythmias.

In summary, the β-adrenergic–mediated antagonistic effects to class 1 antiarrhythmic drugs are likely to involve multiple factors. Nevertheless, the present study provides the first direct evidence that I_{Na} modulation significantly contributes to such β-adrenergic effects.

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