Voltage Dependence of \( \beta \)-Adrenergic Modulation of Conduction in the Canine Purkinje Fiber

Thomas M. Munger, Susan B. Johnson, Douglas L. Packer

Abstract

Although recent voltage-clamp and microelectrode studies have demonstrated \( \beta \)-adrenergic modulation of Na\(^{+}\) current \((I_{\text{Na}})\) the modulation of conduction by catecholamines and the voltage dependence of that process have not been elucidated. To determine whether voltage-dependent modulation of conduction occurs in the presence of a \( \beta \)-adrenergic agonist, the effect of 1 \( \mu \)mol/L isoproterenol on impulse propagation in canine Purkinje fibers was examined by using a dual-microelectrode technique. At physiological membrane potentials \((\left[\text{K}\right]_{o}=5.4\ \text{mmol/L})\), isoproterenol increased squared conduction velocity \((\theta^{2}, 0.39\pm0.25\ (\text{m/s})^{2}\) (mean\(\pm\)SD)) from 3.46\(\pm\)0.86 to 3.85\(\pm\)0.98 \((\text{m/s})^{2}\) \((P<0.01)\), an 11% change, without altering the maximum first derivative of the upslope of phase 0 of the action potential \((V_{\text{max}}, 641\pm50\) versus 657\(\pm\)47 \(\text{V/s}, P=\text{NS})\). At transmembrane potential of \(-65\ \text{mV},\) produced by 12 mmol/L \([\text{K}\] \(_{o}\), titration, \(\theta^{2}\) declined 79% to 0.73\(\pm\)0.44 \((\text{m/s})^{2}\) as \(V_{\text{max}}\) decreased 85% to 95\(\pm\)43 \(\text{V/s}\) \((P<0.02)\). The addition of isoproterenol further decreased \(\theta^{2}\) to 0.49\(\pm\)0.33 \((\text{m/s})^{2}\) \((P=0.02)\) in parallel with a further decline in \(V_{\text{max}}\) to 51\(\pm\)25 \(\text{V/s}\) \((P<0.05)\). Isoproterenol produced a 3-mV hyperpolarizing shift of apparent Na\(^{+}\) channel availability curves generated from both \(\theta^{2}\) and \(V_{\text{max}}\), used as indexes of the fast inward \(I_{\text{Na}}\), without changing the slopes of the relation. The relation between normalized \(\theta^{2}\) and \(V_{\text{max}}\) over a range of depolarized potentials was linear and was not appreciably altered by isoproterenol. These data suggest that \(\beta\)-adrenergic modulation of conduction is voltage dependent and follows comparable changes in \(I_{\text{Na}}\) to the extent reflected by \(V_{\text{max}}\). In partially depolarized canine Purkinje fibers, isoproterenol slows conduction and reduces \(V_{\text{max}}\), which may contribute to arrhythmogenesis during myocardial ischemia. (Circ Res. 1994;75:511-519.)

Key Words • Na\(^{+}\) currents • isoproterenol • conduction

Isoproterenol enhances several ion channel currents that govern plateau duration of the cardiac action potential, including the delayed rectifier K\(^{+}\) current \((I_{K})\), the L-type Ca\(^{2+}\) current \((I_{\text{Ca-L}})\), the transient outward K\(^{+}\) current \((I_{\text{to}})\), and the inward chloride current \((I_{\text{Cl-in}})\). Recent studies have shown that the Na\(^{+}\) current \((I_{\text{Na}})\) is also modulated by \(\beta\)-adrenergic stimulation. However, unlike the consistent augmentation of \(I_{K}\) and \(I_{\text{to}}\) after catecholamine exposure, several investigators have suggested that \(I_{\text{Na}}\) may be reduced by isoproterenol. Ono et al\(^{10}\) observed a reduction in \(I_{\text{Na}}\) in guinea pig ventricular myocytes due to 1 \(\mu\)mol/L isoproterenol applied at depolarized membrane potentials. Schubert et al\(^{8,9}\) made similar observations in a whole-cell examination of neonatal rat ventricular myocytes. These workers and others\(^{10-16}\) have documented a hyperpolarizing shift of Na\(^{+}\) inactivation curves by using voltage-clamp or microelectrode techniques.

In contrast, other investigators have demonstrated an augmentation of \(I_{\text{Na}}\) by \(\beta\)-adrenergic stimulation under patch-clamp examination. Both Matsuda et al\(^{17}\) and Murray et al\(^{18}\) have shown an increase in \(I_{\text{Na}}\) in rabbit or guinea pig ventricular myocytes challenged with isoproterenol. Others failed to detect any isoproterenol-induced alteration of \(I_{\text{Na}}\) under perforated-patch examination. Ono et al\(^{15}\) also showed an increase in \(I_{\text{Na}}\) at certain test potentials in a cell-attached examination of canine, rabbit, and guinea pig myocytes. The reason for these directionally different observations is not clear but may be related to the species studied, differences in study conditions, or the specific voltage-clamp techniques used to examine \(I_{\text{Na}}\).

Since prior investigations have noted a relation between \(I_{\text{Na}}\) and conduction velocity \((\theta^{2})\) as well as a linear relation between the normalized maximum first derivative of the upslope of phase 0 of the action potential \((V_{\text{max}})\) and \(\theta^{2}\), it follows that parallel modulation of conduction might accompany any voltage-dependent change in \(I_{\text{Na}}\) produced by adrenergic stimulation. Although adrenergic modulation of conduction has been previously examined both in vitro and in vivo, the dependence of \(\beta\)-adrenergic modulation of conduction on membrane potential and the effect of isoproterenol on \(\theta^{2}\) and \(V_{\text{max}}\) relation under such circumstances have not been completely elucidated. Given the relevance of such observations to a clarification of catecholamine effect under pathological conditions in vivo, the present study was undertaken to test the hypothesis that under conditions of membrane depolarization, any observed decrease in \(V_{\text{max}}\) after isoproterenol exposure should be accompanied by conduction slowing.

Materials and Methods

Tissue Preparation

Canine Purkinje fibers were retrieved from 15- to 25-kg mongrel dogs after pentobarbital (30 to 50 mg/kg IV) anesthesia, as previously described. After rapid excision, the intact hearts were immediately submersed in chilled (1°C to 2°C) high-K\(^{+}\) cardioplegia solution (17 mmol/L K\(^{+}\) L-glutamate added to Tyrode’s solution). Free-running unbranched...
Purkinje fibers (≥1.0 cm) were dissected free, fixed in a fast-flow Lucite chamber, and superfused with Tyrode's solution containing (mmol/L) NaCl 129, NaHCO3 20, Na2HPO4, 1 H2O 0.9, dextrose 5.5, MgCl2, 1 H2O 0.5, CaCl2 2.5, and KCl 5.4 delivered at a flow rate of 8 to 12 mL/min. Temperature was maintained at 36±0.5°C, and pH was maintained at 7.40±0.05 by equilibration with a 95% O2/5% CO2 gas mixture.

**Signal Recording and Data Analysis**

Transmembrane potential (Vm) recordings from the mid and distal regions of the fiber were made with borosilicate glass microelectrodes (outside/inside diameters, 1.0/0.5 mm) bevelled to a tip resistance of 8 to 12 MΩ (WPI beveler, model 1300M). Electrodes were filled with 3.0 mol/L KCl and coupled via an Ag/AgCl wire to a high-impedance WPI electrometer (model Duo 773). The bath was held at ground with a 3-mol/L K+ salt agar bridge. Analog action potential signals were filtered with a continuous 1- to 30-kHz low-pass roll-off filter (40 dB per decade), differentially electronically, and held for 90 milliseconds by using a sample-hold peak detection circuit. The analog differentiator output was linear from 20 to 800 V/s. The interelectrode distance (d), measured with a calibrated eyepiece (average d, 3.1±1.2 mm), and conduction time (CD), determined using Vmmax from each electrode as fiducial points, were used to calculate θ (d/CD). The minimum detectable time interval of the circuit was 0.01 millisecond. Recordings were digitized at a sampling frequency of 1 to 4 kHz by use of an A/D converter with 15-bit accuracy (IDA 15100, Indec Basic Fastlab System) and stored on a Compaq 386/33 personal computer for off-line analysis. Action potential characteristics were determined with customized software developed in our laboratory.

**Stimulation Protocol**

Purkinje fibers were stimulated with 1.0-millisecond constant current pulses using a pair of Teflon-coated silver wires positioned at least 4 mm proximal to the first electrode. Stimulus intensity was maintained at 1.5 to 3.0 times diastolic threshold as assessed at the beginning of each stimulation protocol. During K+ titration, stimulus intensity was adjusted to maintain fiber stimulation capture with consistent latency during each serial intervention. All fibers showed <5% variability in conduction time under control conditions. Only experiments in which single impalements were maintained and conduction remained continuous during the entire experiment were analyzed.

**Experimental Protocol**

I: **β-Adrenergic Modulation of Conduction at Control Resting Membrane Potentials**

In an initial series of 14 fibers, β-adrenergic modulation of CD, θ, and θ2 as measures of conduction and Vmmax as a reflection of Ih, were examined at control Vm and 1 hour after 10−4 mol/L isoproterenol was added to the superfusate. Isoproterenol was chosen as the test agent because of its reasonably pure β-adrenergic profile. Its cellular electrophysiological effects have also been extensively characterized in multiple preparations at a wide range of membrane potentials.7-19 Micromolar concentrations were used because of their documented effect on the voltage-dependent modulation of Ih, in vitro.7-19

II: **β-Adrenergic Modulation of Conduction in Partially Depolarized Fibers**

The isoproterenol effect on apparent Na+ channel inactivation kinetics, as expressed by measurements of conduction and Vmmax, was examined in a second series of eight fibers by use of a K+ titration technique described by Chen and Gettes.27 After control studies at [K+]o of 5.4 mmol/L, Vmmax, Vm, and CD were recorded continuously during 12 mmol/L [K+]o titration over a 15-minute period while stimulating at an interstimulus interval (ISI) of 0.7 second to favor preferential expression of depressed fast channel–driven conduction. After [K+]o was decreased to 5.4 mmol/L and all electrophysiological parameters returned to baseline, the fibers were superfused with 1 mmol/L isoproterenol (15 to 20 minutes to steady-state effect), and the K+ titration sequence was repeated. The relations of θ2 and Vmmax to Vm were generated and with and without isoproterenol were described by Boltzmann functions of the following form: θ2 = θ2e/[1+exp((Vm−h0)/S)], where θ2, h0, and S denote the maximum projected θ2, the Vm at which 50% inactivation occurred, and the slope factor, respectively. An additional four fibers were examined under light-free conditions after pretreatment with the Ca2+ channel antagonist nisoldipine. In three of these experiments, 10−4 mol/L tetrodotoxin (TTX) was added at peak K+ depolarization in the presence of isoproterenol to confirm the contribution of residual Ih to excitability and conduction.

**Statistical Analysis**

All data are reported as mean±1 SD. Where statistical treatment was warranted, significance of differences between means was determined by the nonparametric Wilcoxon signed-rank test, with P<.05 taken as significant. This approach provided a more rigorous assessment of the significance of differences than found using parametric testing methods.

**Results**

I: **β-Adrenergic Modulation of Conduction at Control Resting Membrane Potentials**

Under control conditions, 1 μmol/L isoproterenol produced small but significant resting membrane hyperpolarization (2.5±1.7 mV, P<.002) in 14 fibers (Table 1), without appreciable change in the overshoot (Vm) or amplitude of the action potential (APA). Although the action potential duration (APD) at 50% repolarization was not altered, phase 3 repolarization was shortened, as indicated by changes in APD at 95% repolarization (APD95) (Table 1). Isoproterenol accelerated conduction modestly: CD decreased 5% (P<.005), and θ2 increased 11% (P<.002). Vmmax was not significantly altered. Isoproterenol-induced changes in conduction appeared after 1 to 3 minutes, were 50% complete after 8 to 10 minutes, and reached steady state within 15 to 20 minutes.

II: **β-Adrenergic Modulation of Conduction in Partially Depolarized Fibers**

β-Adrenergic modulation of action potential characteristics and conduction by isoproterenol at depolarized potentials was examined in eight fibers. External K+ (12 mmol/L) depolarized Vm from −88.3±1.1 to −65.1±2.6 mV (P<.02); APA, Vmmax, and APD95 also declined (P<.05, Table 1). After washout to control conditions and the subsequent addition of isoproterenol, the effect of repeat K+ titration produced no additional effect on Vm, Vmmax, APA, and APD95. As expected, conduction time prolonged with K+–induced membrane depolarization (1.62±0.24 versus 3.89±1.00 milliseconds, P<.02) (Table 1). Repeat K+ titration to an equivalent Vm in the presence of isoproterenol produced greater conduction slowing (to 4.96±1.64 milliseconds, P<.02) than generated by 12 mmol/L K+ alone. Analogously, θ2 and Vmmax also decreased with isoproterenol (Table 1). A second slower component of Vmmax due to Ica, which could be readily distinguished from the rapid Na+–carried component, was
only infrequently observed with membrane depolarization in the presence of isoproterenol.

Isoproterenol produced a hyperpolarizing shift in the Boltzmann curves generated from the $\theta^2$-$V_m$ relation, as shown for a representative fiber in Fig. 1. Comparable results obtained from unnormalized $V_{\text{max}}$ and $\theta^2$ data in eight fibers are also shown in Fig. 2. The midpoint of the apparent inactivation curves, $h_{\text{o},5}$, derived from normalized $\theta^2$ shifted an average of 3 mV, from $-71 \pm 2$ to $-74 \pm 2$ mV ($P<.05$) and from $-69 \pm 3$ to $-72 \pm 4$ mV ($P<.05$), when determined from the $V_{\text{max}}$-$V_m$ relation (Fig. 2). The slopes of the Boltzmann relations remained unchanged. The isoproterenol-induced increase in $\theta^2$

from $3.55 \pm 1.08$ to $4.05 \pm 1.32$ (ms)$^2$ before membrane depolarization and the 35% isoproterenol-induced decrease in $\theta^2$ with 12 mmol/L K$^+$ produced a crossover in the apparent inactivation curves at $-77$ mV. A similar crossover in the curves derived from $V_{\text{max}}$ was not observed, since $V_{\text{max}}$ was not altered appreciably by isoproterenol under control conditions ($652 \pm 60$ versus $663 \pm 53$ V/s, $P=\text{NS}$).

The effect of isoproterenol on the $\theta^2$-$V_{\text{max}}$ relation over a range of membrane potentials generated by 5.4 to 12 mmol/L K$^+$ was also examined. A plot of $\theta^2$ versus $V_{\text{max}}$ (values normalized to those at [K$^+$], of 5.4 mmol/L) is shown for a representative fiber in Fig 3A. Findings were similar for the composite of the eight fibers. With initial K$^+$ augmentation, enhanced excitability and conduction were present at a time of decreasing $V_{\text{max}}$, producing an upward shift in the ratio of $\theta^2$ to $V_{\text{max}}$. Subsequently, at $\theta^2$ and $V_{\text{max}}$, values $<0.8$, the normalized $\theta^2$-$V_{\text{max}}$ relation was linear, both before and after isoproterenol addition (Fig 3B). Since both $V_{\text{max}}$ and $\theta^2$ decreased proportionally in the presence of isoproterenol, the ratio of $\theta^2$ to $V_{\text{max}}$ at a given $V_m$ shifted downward and to the left along a nearly identical to that relating $\theta^2$ and $V_{\text{max}}$ without isoproterenol. Because of the initial increase in $\theta^2$, however, isoproterenol addition produced a slight steepening of the total $\theta^2$-$V_{\text{max}}$ relation (slope, 0.96 to 1.04), without changing the intercept (0.11 before isoproterenol and 0.12 after isoproterenol).

No appreciable preparation rundown was noted during these experiments. K$^+$ titration with and without isoproterenol produced similar levels of membrane depolarization ($-65.4 \pm 3.8$ versus $-65.1 \pm 2.6$ mV, $P=\text{NS}$). In seven fibers, washout to 5.4 mmol/L K$^+$ at the end of the experiment restored $V_m$ and CD to initial values (for $V_n$, $-87.9 \pm 4$ versus $-88.6 \pm 3.4$ mV, $P=\text{NS}$; for CD, $1.53 \pm 0.22$ versus $1.55 \pm 0.25$ milliseconds, $P=\text{NS}$).

### Table 1. Effect of $10^{-6}$ mol/L Isoproterenol on the Electrical Properties of Canine Purkinje Fibers at Normal and Depolarized Resting Membrane Potentials (K$^+$ Titration After Isoproterenol Addition)

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>$10^{-6}$ ISO</th>
<th>Control 2</th>
<th>$12$ mmol/L K$^+$</th>
<th>$10^{-6}$ M ISO/K$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
<td>14</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>$V_m$, mV</td>
<td>$-86.6 \pm 3.4$</td>
<td>$-89.1 \pm 3.3^*$</td>
<td>$-88.3 \pm 1.1$</td>
<td>$-65.1 \pm 2.6^*$†</td>
<td>$-65.4 \pm 3.8$</td>
</tr>
<tr>
<td>APA, mV</td>
<td>123±4</td>
<td>122±5</td>
<td>123±3</td>
<td>75±7†</td>
<td>80±6</td>
</tr>
<tr>
<td>$V_{\text{ax}}$, mV</td>
<td>36±3</td>
<td>33±5</td>
<td>35±2</td>
<td>10±6†</td>
<td>14±6</td>
</tr>
<tr>
<td>APD$_{90}$, ms</td>
<td>202±40</td>
<td>196±43</td>
<td>195±31</td>
<td>93±7†</td>
<td>94±13</td>
</tr>
<tr>
<td>APD$_{95}$, ms</td>
<td>280±48</td>
<td>264±58§</td>
<td>278±40</td>
<td>125±10†</td>
<td>123±15</td>
</tr>
<tr>
<td>APD$_{99}$, ms</td>
<td>331±49</td>
<td>309±62§</td>
<td>319±32</td>
<td>153±16‡</td>
<td>147±15</td>
</tr>
<tr>
<td>$\theta^2$, (m/s)$^2$</td>
<td>3.46±0.86</td>
<td>3.85±0.98*</td>
<td>3.44±1.02</td>
<td>0.73±0.44†</td>
<td>0.49±0.33</td>
</tr>
<tr>
<td>$\theta$, m/s</td>
<td>1.85±0.24</td>
<td>1.95±0.26*</td>
<td>1.83±0.30</td>
<td>0.81±0.28†</td>
<td>0.66±0.26</td>
</tr>
<tr>
<td>CD, ms</td>
<td>2.04±0.83</td>
<td>1.93±0.81*</td>
<td>1.62±0.24</td>
<td>3.89±1.00†</td>
<td>4.96±1.64‖</td>
</tr>
<tr>
<td>$V_{\text{max}}, V/s$</td>
<td>641±50</td>
<td>657±47</td>
<td>652±60</td>
<td>95±43†</td>
<td>51±25§</td>
</tr>
</tbody>
</table>

Control 1 indicates the set of 14 experiments demonstrating the effects of isoproterenol (ISO) on the electrical properties of normally polarized fibers; control 2, the set of experiments demonstrating the electrical effects of initial K$^+$ titration to 12 mmol/L (column 4) and then subsequent return to the control value, followed by $10^{-6}$ mol/L ISO addition and repeat K$^+$ titration to 12 mmol/L (column 5) for eight separate fibers; $V_m$, resting membrane potential; APA, action potential amplitude; $V_{\text{ax}}$, action potential overshoot amplitude; APD$_{90}$, APD$_{95}$, and APD$_{99}$, action potential durations at 50%, 75%, and 95% repolarization, respectively; $\theta$, conduction velocity; CD, conduction delay; and $V_{\text{max}}$, maximum first derivative of the upslope of phase 0 of the action potential. Values are mean±1 SD.

*P<.01 vs control 1 (n=14); †P<.02 vs control 2 (n=8); ‡P<.05 vs control 2 (n=8); §P<.05 vs control 1 (n=14); ‖P<.02 vs 12 mmol/L K$^+$ (n=8); and ‡P<.05 vs 12 mmol/L K$^+$ (n=8).
The dependence of the observed conduction slowing on \( I_{Ca} \) was examined in four additional fibers after nisoldipine pretreatment. Nisoldipine had minimal effect on conduction and apparent Na\(^+\) channel availability curves. In the presence of nisoldipine, isoproterenol produced a 2- to 3-mV hyperpolarizing shift in the midpoint of the Boltzmann-fit curves: \(-70\pm2\) to \(-72\pm4\) mV from \( \theta^2 \) and \(-70\pm3\) to \(-73\pm4\) mV from \( V_{max} \) (Fig 4). The slope factor of the normalized relation between \( \theta^2 \) and \( V_{max} \) decreased insignificantly (from 4.2\(\pm3.1\) to 3.3\(\pm1.7\) mV\(^{-1}\)). In three fibers, exposure to 1 \( \mu \)mol/L TTX after 12 mmol/L K\(^+\) depolarization in the presence of isoproterenol resulted in a beat-by-beat decrease in \( V_{max} \) and \( \theta^2 \) until membrane excitability was lost (at maximal stimulus intensity of 4.5 mA) without altering \( V_{m} \) (Fig 5). This indicated that conduction was still driven by depressed fast inward currents at [K\(^+\)], of 12 mmol/L in the presence of isoproterenol.

The possibility that the expression of the \( \beta \)-adrenergic effect was dependent on the order of isoproterenol/K\(^+\) addition was examined in 10 additional fibers. Conduction and \( V_{max} \) were examined in the presence of 5.4 mmol/L K\(^+\), subsequently with 10 or 12 mmol/L K\(^+\), and, after a 15- to 20-minute equilibration period, with 1 \( \mu \)mol/L isoproterenol. Isoproterenol-provoked slowing of conduction beyond that produced by K\(^+\) alone was again confirmed at [K\(^+\)], of 12 mmol/L (\( V_{m}, -65 \) mV) but not with 10 mmol/L K\(^+\). After 10 mmol/L K\(^+\)-induced membrane depolarization to \(-70 \) mV, CD increased 48\% (P<.05) but was unchanged after isoproterenol addition (Table 2), although a small accompanying decrease in \( V_{max} \) was observed (P<.05). These findings were not altered by 10\(^{-6}\) mol/L nisoldipine pretreatment in four additional fibers. Isoproterenol produced significant additional conduction slowing in fibers depolarized to \(-65 \) mV by 12 mmol/L K\(^+\), as shown in Fig 6. CD increased 114\% and \( \theta^2 \) decreased 77\% with 12 mmol/L K\(^+\) (P<.05, Table 2; Fig 6). After isoproterenol addition, CD increased 34\% to 4.16\(\pm1.81\) milliseconds (P<.05), and \( \theta^2 \) decreased an additional 35\% from 0.63\(\pm0.08\) to 0.41\(\pm0.16\) (m/s)\(^2\) (P<.05). These changes were not frequency dependent over a range of interstimulus intervals from 1.5 to 0.5 seconds. In three other depolarized fibers pretreated with 10\(^{-6}\) mol/L nisoldipine, isoproterenol produced directionally similar changes in conduction as observed without Ca\(^{2+}\) channel blockade: CD increased 11\% (P=NS) and \( \theta^2 \) decreased 15\% (Table 2). In each case, these changes were less pronounced, however, than those observed when isoproterenol was added before membrane depolarization.

**Discussion**

In this examination of \( \beta \)-adrenergic modulation of conduction at physiological temperatures in isolated Purkinje fibers, we found that the effect of isoproterenol on
Fig 3. Graphs showing effect of isoproterenol on the relation between normalized squared conduction velocity ($\theta^2_{vn}$) and normalized action potential ($V_{max}$) during K$^+$ titration in a single Purkinje fiber. A. $\theta^2_{vn}$ vs. $V_{max}$ relation without (control) and with isoproterenol. The data are normalized to those $\theta^2_{vn}$ and $V_{max}$ values obtained at $[K^+]_o$ of 5.4 mmol/L. With initial K$^+$ titration under control conditions at which $V_{max}$ decreased from 1.0 to 0.8, $\theta^2_{vn}$ increased, reflecting an area of supernormal excitability and conduction. With isoproterenol, this increase in $\theta^2_{vn}$ was exaggerated. With further K$^+$ titration, $V_{max}$ and $\theta^2_{vn}$ decreased linearly with and without isoproterenol. B. Linear relation between $\theta^2_{vn}$ and $V_{max}$ in eight fibers with subsequent membrane depolarization produced by titration to 12 mmol/L K$^+$ both before (control) and after isoproterenol treatment. The data are normalized $\theta^2_{vn}$ and $V_{max}$ values, where membrane voltage is $-75$ mV and $[K^+]_o$ is 8 mmol/L. A modest change in the slope of this relation from 0.96 to 1.04 was seen with isoproterenol addition.

Impulse propagation shows marked voltage dependence. Although at normal resting membrane potentials isoproterenol shortened conduction time, $\beta$-adrenergic stimulation of depolarized fibers produced additional conduction slowing and decrease in $V_{max}$ beyond that seen with high K$^+$ alone. These findings were accompanied by a significant shift in apparent Na$^+$ channel availability curves generated from both $\theta^2$ and the $V_{max}$ of propagated action potentials. The linear relation between $V_{max}$ and $\theta^2$ during progressive K$^+$ depolarization was also maintained in the presence of isoproterenol. These findings extend prior investigations and have more completely explored the voltage dependence of catecholamine-induced conduction modulation in a controlled setting. Mendez et al$^{24}$ previously demonstrated that epinephrine had little direct dromotropic effect on ventricular conduction in the denervated intact heart but indirectly accelerated intraventricular conduction via hepatic K$^+$ release. This effect was prevented by ligation of the hepatic artery and $\alpha$-but not $\beta$-adrenergic blockade. The study was limited by the complexity of the intact preparation, the inability to precisely regulate $[K^+]_o$, and the effect of the mixed adrenergic profile of epinephrine. Kassebaum and Van Dyke$^{25}$ saw no direct effect of $10^{-7}$ mol/L isoproterenol on conduction in ovine Purkinje fibers at normal membrane potentials. The voltage dependence of the $\beta$-adrenergic effect on $\theta$ was not examined, although isoproterenol produced no shift in Na$^+$ activation curves in two preparations. Other studies have documented the restoration of propagating slow response action potentials by catecholamine addition in depolarized preparations.$^{26-31}$ A more detailed review of the effect of catecholamines on propagation under normal conditions is available elsewhere.$^{26}$ In contrast, the present data elucidate the voltage dependence of isoproterenol modulation of conduction in a simple propagating system.

Mechanism of Isoproterenol-Induced Conduction Slowing
Contribution of Active Property Alteration
At least three candidate mechanisms could account for the isoproterenol-induced conduction slowing observed at depolarized membrane potentials. First, the
Fig 5. Tracings showing use-dependent change in action potential morphology on addition of 1 μmol/L tetrodotoxin to fibers pretreated with 1 μmol/L disoildipine and 1 μmol/L isoproterenol ([K+]o, 12 mmol/L) (only every fifth pulse is shown for clarity). The first action potential, after a 40-second pause, reflects the effect of isoproterenol at transmembrane potential of −65 mV. With repetitive stimulation at an interstimulus interval of 0.7 second, loss of the Na+-carried contribution to the upstroke of the action potential was observed. With tetrodotoxin, the maximum first derivative of the upstroke phase of the action potential and action potential amplitude fell progressively until membrane excitability was lost. This indicates that the conductance slowing caused by isoproterenol was driven by the depressed fast inward current.

Preservation of conduction time could be related to an isoproterenol-induced decrease in \( I_{\text{Na}} \). This interpretation is consistent with several microelectrode investigations of guinea pig papillary muscle \(^{11-14} \) showing significant declines in \( V_{\text{max}} \) with 100 to 1000 isoproterenol when membranes were depolarized by 8 to 15 mmol/L.

**TABLE 2. Effect of 10⁻⁶ mol/L Isoproterenol on Electrical Characteristics of 10 and 12 mmol/L K⁺ Depolarized Canine Purkinje Fibers (K⁺ Titration Before Isoproterenol Addition)**

<table>
<thead>
<tr>
<th>10 mmol/L K⁺ Study (n=5)</th>
<th>12 mmol/L K⁺ Study (n=5)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>( V_{\text{m}} ), mV</td>
<td>-87.3±1.1</td>
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<tr>
<td>( V_{\text{m}} ), mV</td>
<td>120±2</td>
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<tr>
<td>( V_{\text{m}} ), mV</td>
<td>32±1</td>
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<td>( V_{\text{m}} ), mV</td>
<td>331±16</td>
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<td>( V_{\text{m}} ), mV</td>
<td>353±18</td>
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<tr>
<td>( \theta^2 ), (m/s)²</td>
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<td>( \theta ), m/s</td>
<td>1.63±0.15</td>
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<td>( V_{\text{max}} ), V/s</td>
<td>662±64</td>
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<td>( V_{\text{max}} ), V/s</td>
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</tbody>
</table>

\( V_{\text{m}} \) indicates resting membrane potential; \( APA \), action potential amplitude; \( V_{\text{m}} \), action potential overshoot amplitude; \( AP_{\text{D0}} \), \( AP_{\text{D50}} \), and \( AP_{\text{D90}} \), action potential durations at 50%, 75%, and 95% repolarization, respectively; \( \theta \), conduction velocity; \( \theta \), conduction delay; and \( V_{\text{max}} \), maximum first derivative of the upstroke phase of 0 of the action potential.

\(*P<.05 vs control value in 10 mmol/L K⁺ study; \( P<.05 vs control value in 12 mmol/L K⁺ study; \( P<.05 vs K⁺ alone in 12 mmol/L K⁺ study; \( P<.05 vs K⁺ alone in 10 mmol/L K⁺ study; \( P<.05 vs K⁺ alone in 10 mmol/L K⁺ study.

K⁺. An accompanying hyperpolarizing shift in inactivation curves relating \( V_{\text{max}} \) and \( V_{\text{m}} \) has also been described.\( ^{11-14} \) More recently, others have shown a pronounced reduction of \( I_{\text{Na}} \) by 1 μmol/L isoproterenol in guinea pig and neonatal rat\( ^{6,9} \) ventricular myocytes at inactivated holding potentials, findings that were mimicked by both dibutyryl cAMP (a membrane-permeable analogue of cAMP) and forskolin and blocked by atenolol. The accompanying shifts in Na⁺ channel inactivation curves to more negative membrane potentials were felt to be greater than could be accounted for by spontaneous drift of inactivation kinetics occurring under prolonged voltage clamp.\(^{15,32-33} \) A similar decline in \( I_{\text{Na}} \) without change in unitary Na⁺ conductance, mean channel open times, or open-time distribution in a cell-attached patch examination of neonatal rat myo-
cytcher has also been reported.\textsuperscript{10} Comparable changes in $I_{Na}$ in guinea pig ventricular myocytes with the addition of the catalytic subunit of cAMP-dependent protein kinase A\textsuperscript{34} or the addition of extracellular cAMP\textsuperscript{35} have been reported. Taken together, these data support an "inhibitory" effect of \textbeta-adrenergic stimulation on the Na\textsuperscript{+} channel. That this remains controversial, however, is underscored by other observations of a 10\% to 50\% increase in $I_{Na}$ with isoproterenol in rabbit,\textsuperscript{17} guinea pig,\textsuperscript{18} and canine\textsuperscript{15} ventricular myocytes studied by using cell-attached patch methods. Also, Wendt et al\textsuperscript{19} saw no appreciable change in $I_{Na}$ with isoproterenol when rabbit atrial myocytes were studied with perforated-patch techniques.

The basis of these differences is unclear. It is possible that internal cell dialysis occurring with whole-cell voltage clamp or excised patch techniques\textsuperscript{5,9} alters the intracellular milieu and therefore $I_{Na}$. That this is an incomplete explanation is suggested by isoproterenol-induced decreases in $I_{Na}$ observed under cell-attached patch examination of single rat ventricular myocytes\textsuperscript{10} and in intact rat papillary muscles studied with a loose-patch technique.\textsuperscript{16}

Differences in study protocols, including holding and test potentials used in stimulation paradigms, may have contributed to variable findings. We observed an increase in $\theta$\textsuperscript{2} at potentials more negative than $-77$ mV, although both $\theta$ and $V_{max}$ decreased significantly with isoproterenol at $V_{m}$ of $-65$ mV. In contrast, in the synecytial Purkinje studies of Gintant and Liu\textsuperscript{14} showing no isoproterenol effect, cells were depolarized to $-70$ mV with 8 mmol/L K\textsuperscript{+}, which may have been insufficient to alter $I_{Na}$. The increase in $I_{Na}$ observed by Matsuda et al\textsuperscript{17} was also strongly voltage dependent; the largest isoproterenol-induced increase in $I_{Na}$ occurred after a $-120$-mV prepulse potential. A lesser effect of isoproterenol and forskolin on $I_{Na}$ was produced at holding potentials between $-80$ and $-70$ mV, and no change in $I_{Na}$ was seen when cells were held at $-70$ mV. The possibility of an isoproterenol-induced decrease in $I_{Na}$ at even less negative potentials was not examined. Kirsten et al\textsuperscript{16} also reported a 42\% decrease in rat papillary muscle $I_{Na}$ due to isoproterenol when studied from a holding potential of $-60$ mV, which is more consistent with our results. In contrast, $I_{Na}$ was increased by 68\% when the preparations were studied from holding potentials of $-130$ mV.

The recent macropatch, on-cell, voltage-clamp examination of canine, rabbit, and guinea pig myocytes by Ono et al\textsuperscript{20} provides additional support for this contention. These investigators demonstrated a $7$-mV hyperpolarizing shift in the midpoints of both voltage-dependent availability and conductance curves with the addition of 8-(4-chlorophenylthio)cAMP (8-CPT-cAMP). Whether $I_{Na}$ increased, remained unchanged, or decreased was dependent on the holding and test potentials used in the voltage-clamp protocol. Hyperpolarizing holding potentials of $-150$ mV and test potentials delivered in the steep slope region of the shifted conductance curve in the presence of conditions producing protein kinase A-dependent phosphorylation increased $I_{Na}$. In contrast, under conditions of less than full Na\textsuperscript{+} channel availability, 5 mmol/L isoproterenol produced a 50\% decrease in peak $I_{Na}$ elicited by more positive test potentials. Specifically, a decrease in $I_{Na}$ was observed on exposure to isoproterenol or 8-CPT-cAMP when cells were held at $-90$ mV and studied at test potentials to $+30$ mV.

Our finding of significant decreases in both $V_{max}$ and $\theta$\textsuperscript{2} in a propagating preparation is at variance with the findings of Gintant and Liu\textsuperscript{14} who noted an isoproterenol-induced reduction in $V_{max}$ or $I_{Na}$ in single dispersed myocytes but not in syncytial myocardium. The reason for this difference in similar syncytial preparations is unclear but could be related to the difference in resting membrane potentials during the study or might be related to the order of isoproterenol/K\textsuperscript{+} addition. In the present study, the decrease in $V_{max}$ and $\theta$\textsuperscript{2} was maximized by isoproterenol pretreatment and subsequent K\textsuperscript{+} depolarization. The reverse order of K\textsuperscript{+} depolarization followed by isoproterenol exposure as used by Gintant and Liu showed less effect. Although $\theta$\textsuperscript{2} decreased under these circumstances, $V_{max}$ was unchanged. This raises the possibility that channel conformational changes occurring with K\textsuperscript{+} inactivation under such circumstances alter isoproterenol-related phosphorylation of Na\textsuperscript{+} channels.

**Passive Property Modulation by \textbeta-Adrenergic Effect**

Modulation of passive properties by isoproterenol is an alternative mechanism for the observed slowing of conduction. With initial K\textsuperscript{+} titration, changes in conduction were directionally different from those of $V_{max}$, reflecting an area of enhanced excitability and accompanying conduction.\textsuperscript{12,30-38} The relation between $V_{max}$ and $\theta$\textsuperscript{2} with further K\textsuperscript{+} titration, however, remained linear (Fig 3), as predicted by the cable equation and previous studies.\textsuperscript{21-23} With isoproterenol, the divergence of $\theta$\textsuperscript{2} and $V_{max}$ in the range of $V_{max}$ values of 0.80 to 1.0 was exaggerated. This disproportionate increase in $\theta$\textsuperscript{2} in this region is consistent with recent studies showing increased junctional permeability of atrial muscle with intracellular augmentation by cAMP.\textsuperscript{39,40} Cell-to-cell coupling in canine Purkinje cells\textsuperscript{41} is also enhanced, and gap junctional conductance ($g_{ij}$) in paired neonatal rat myocytes is increased by $50\%$ to $100\%$ by cAMP, 8-bromo-cAMP, or, more important, isoproterenol exposure.\textsuperscript{12,42} It has been suggested that such a change in $g_{ij}$ could be responsible for a $10\%$ increase in $\theta$,\textsuperscript{44} which is consistent with our observations at normal resting membrane potentials. The remaining portion of the $\theta$-$V_{max}$ relation generated by increasing [K\textsuperscript{+}], to achieve $V_{max}$ values $<0.8$ was unchanged by isoproterenol, a finding in favor of $I_{Na}$ modulation rather than appreciable passive property alteration.

The failure of nisoldipine to eliminate isoproterenol-induced conduction slowing is also against changes in passive membrane properties related to $[Ca^{2+}]$-induced alteration of $g_{ij}$. Prior studies suggest that changes in $[Ca^{2+}]$, may be too small to alter $g_{ij}$ even in the presence of substantial membrane depolarization by 50 mmol/L K\textsuperscript{+}.\textsuperscript{45} More direct assessment of intracellular $Ca^{2+}$ activity will be required to define the relative and oppositely directed contributions of an isoproterenol-invoked indirect increase in $[Ca^{2+}]$, and the direct effect of isoproterenol on $g_{ij}$.

**Slow Channel-Mediated Propagation**

Finally, a shift in the inward current driving impulse propagation from depressed fast $I_{Na}$ to $I_{Cs}$ could also
decrease both $V_{max}$ and $\theta^2$ in Purkinje fibers.\textsuperscript{28-31} The resulting slow response action potentials have $V_{max}$ values of 2 to 20 V/s,\textsuperscript{28,30,49,50} and propagate at velocities of 0.02 to 0.15 m/s.\textsuperscript{28,30,49,50} That this was not the prevailing mechanism responsible for our findings is suggested by several factors: (1) Prominent deflections in the upstroke of the action potential, previously ascribed to the slow inward $Ca^{2+}$ current,\textsuperscript{28,30,51} were uncommon and were distinguishable from the fast component of the action potential upstroke from which $V_{max}$ was derived. (2) In the presence of nisoldipine, the hyperpolarizing shift in inactivation curves generated from $V_{max}$ and $\theta^2$ persisted. (3) Micromolar TTX decreased $V_{max}$ and abolished propagation, as expected with conduction driven by depressed fast responses.

**Limitations**

Several limitations should be considered in interpreting these results. The precise relation between $V_{max}$ and Na$^+$ conductance ($g_{Na}$) is unclear,\textsuperscript{22-34} although the potential overestimation of $g_{Na}$ by $V_{max}$ appears to be small in the range of $V_{max}$ change and temperatures observed in our studies.\textsuperscript{55} It is also unclear whether the presence of isoproterenol results in an improvement or further distortion in the relation between $V_{max}$ and $g_{Na}$. It is also possible that contaminating currents augmented by isoproterenol might have altered $V_{max}$ further altering its relation with $g_{Na}$, although it has been argued that this is unlikely.\textsuperscript{16} The present studies performed in the presence of nisoldipine limited the chance of sufficient $V_{max}$ contamination from inward $Ca^{2+}$ currents, and the addition of TTX further validated our contention that a Na$^+$-carried current was the chief contributor to propagation. In contrast, the microelectrode technique allowed an examination of isoproterenol modulation of conduction at physiological temperatures, in the absence of appreciable internal environment disruption.

**Clinical Implications**

This demonstration of a catecholamine-mediated depression of conduction at depolarized potentials may have important implications in explaining arrhythmogenesis under ischemic conditions. An increase in [K$^+$],\textsuperscript{10} to 12- to 16-mmol/L levels within the first 10 to 20 minutes of ischemia\textsuperscript{6,57} and an increase in local catecholamine release from sympathetic nerve terminals\textsuperscript{8,59} have been well documented. Given these pathophysiological changes, it is not unreasonable to speculate that the effect of both channel inactivation and sympathetic stimulation in ischemic tissue could produce even greater conduction slowing than that produced by membrane depolarization alone. The 35% slowing of conduction we observed under abnormal conditions is even more dramatic given the acceleration of conduction with isoproterenol in physiological solutions. This, in theory, could provide a mechanism for an increased dispersion of conduction in ischemic versus nonischemic tissue. Furthermore, the expected decline in APD and refractoriness with catecholamines in addition to conduction slowing could favor the generation of reentrant circuits within an ischemic zone. If the findings from relatively linear conduction in Purkinje fibers can be generalized to more complex conduction in ventricular myocardium where conduction is slower and complicated by anisotropy, analogous in vivo changes might be expected to lead to serious arrhythmias. Conversely, these findings suggest an additional rationale for $\beta$-blocker therapy in the ischemic or postinfarction setting.

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