Rapid Communications

β-Adrenergic Modulation of Fast Inward Sodium Current in Canine Myocardium Syncytial Preparations Versus Isolated Myocytes

Gary A. Gintant and Da-Wei Liu

Reports have suggested that the fast inward sodium current ($I_{Na}$) in cardiac tissues may be modulated by β-adrenergic stimulation and that such modulation may affect conduction in the setting of myocardial ischemia and infarction. However, many of these studies have used dissociated myocytes or broken cell preparations, whose responses need not necessarily reflect those of syncytial preparations. To investigate further the possibility that β-adrenergic stimulation of $I_{Na}$ may differ in various preparations, we compared the effects of the β-agonist isoproterenol (ISO) on syncytial canine Purkinje fibers and ventricular muscle preparations, as well as isolated ventricular myocytes. Alterations of the maximum rate of rise of the action potential upstroke ($V_{max}$) were used as an index of changes of $I_{Na}$. ISO (1 μM) had no effect on $V_{max}$ of upstrokes of normally polarized (fast responses) or partially depolarized (elevated $[K^+]_o$, depressed fast responses) syncytial ventricular muscle preparations or Purkinje fibers. In contrast, lower concentrations of ISO (0.5–1.0 μM) modulated $V_{max}$ of isolated ventricular myocytes, depending on the technique used to monitor transmembrane potential. When 2.7 M KCl-filled microelectrodes were used, ISO reduced $V_{max}$ of partially depolarized myocytes without affecting $V_{max}$ of normally polarized myocytes. However, when myocytes were dialyzed using patch pipettes, ISO reduced $V_{max}$ of partially depolarized myocytes and increased $V_{max}$ of normally polarized myocytes, effecting a hyperpolarized shift of the normalized inactivation curve relating $V_{max}$ to resting membrane potential. The different β-adrenergic responses of syncytial preparations and nondialyzed and dialyzed myocytes suggest that differences in the ionic or metabolic condition of the preparations likely alter cAMP-dependent responses and channel phosphorylation. These results suggest that β-adrenergic modulation of $I_{Na}$ can occur under some experimental conditions but that extrapolation of data obtained using isolated myocytes to syncytial preparations in vitro or in vivo requires further evaluation. (Circulation Research 1992;70:844–850)

Key Words • β-adrenergic agonist • ventricular myocytes • sodium current • maximum upstroke velocity

Numerous reports have suggested that β-adrenergic stimulation may modulate fast inward sodium current ($I_{Na}$) in the heart through cAMP-dependent pathways and direct G protein–channel interactions (see Reference 1 for a recent review). Some investigators have reported that β-adrenergic stimulation increases $I_{Na}$ or the maximum rate of rise of the action potential upstroke ($V_{max}$) in various cardiac preparations2–5; others have observed a decrease.6–8 In cases where the β-agonist isoproterenol (ISO) reduced $I_{Na}$, the reduction was greatest when sodium channel availability was low, implying a drug-induced shift of the normalized inactivation ($h_a$) curve. Based on these observations, it has been proposed that such modulation may play an important role in affecting cardiac conduction of depolarized tissues in the settings of ischemia and infarction. Although it is conceivable that these disparate effects of ISO represent unique species differences, it is also possible they arise from age differences, tissue origin, the preparation used (syncytial tissue, isolated myocytes, or isolated membrane fragment), or experimental techniques. For example, the techniques used to measure $I_{Na}$ (such as lowered temperature, intracellular dialysis during whole-cell patch clamping, membrane patch formation, and patch excision) modify various characteristics of $I_{Na}$ (see Reference 9). Although such studies provide insight into the mechanisms by which $I_{Na}$ may be modulated, they do not necessarily reflect how $I_{Na}$ is modulated under physiological (or pathophysiological) conditions.

Because of the importance of $I_{Na}$ in cardiac conduction and the proposed role that β-agonists may play in influencing conduction, we compared the effects of ISO on $I_{Na}$ in canine syncytial as well as isolated ventricular myocyte preparations. Because it is not possible to directly measure $I_{Na}$ in syncytial preparations under physiological conditions, changes in $V_{max}$ were used as an indirect measure of $I_{Na}$. Results were compared with those obtained with isolated myocytes using standard
microelectrode techniques (to minimize changes in the intracellular milieu) and patch pipettes (with resulting internal dialysis). We observed that ISO 1) had no effect on \( V_{\text{m}} \) of syncytial canine Purkinje or ventricular preparations from any resting membrane potential, 2) reduced \( V_{\text{m}} \) of partially depolarized myocytes using either microelectrodes or patch pipettes, 3) increased \( V_{\text{m}} \) of normally polarized, dialyzed myocytes, and 4) effected a hyperpolarized shift of the sodium channel apparent \( h_{\text{s}} \) curve of dialyzed myocytes. Our results suggest that caution is necessary when comparing hormonal responses in preparations derived from the same species or when comparing responses using similar experimental conditions but different experimental techniques and that inferences regarding \( \beta \)-adrenergic modulation of \( I_{\text{Na}} \) in vivo require further direct study.

**Materials and Methods**

**Syncytial Preparations**

Free-running Purkinje fibers were excised from canine right or left ventricle. Endocardial preparations were obtained from shavings of canine right ventricular endocardium or papillary muscle; syncytial midmyocardial preparations were obtained from “cores” of left ventricular free wall that were drilled using a hollow (4-mm-diameter) tube. Tissues were placed in a bath that was temperature-controlled at 37–38°C (±0.5°C) and superfused with Tyrode’s solution containing (mM) NaCl 118.5, KCl 2.8, NaHCO3 14.5, KH2PO4 1.2, MgSO4 1.2, and glucose 11.1, along with 1 mg/mL bovine serum albumin (fatty acid free), followed by recirculation with solution supplemented with 0.5 mg/mL collagenase (Type II, Worthington Biochemical Corp., Freehold, N.J.) and 1 mg/mL bovine serum albumin. After 30 minutes of perfusion, the wedge was removed, and tissues were superfused at 37°C superfusion chamber for experiments. Additional samples were placed in a second enzyme solution supplemented with 0.3 mM CaCl2 and 3 mg/mL bovine serum albumin for 15–20 minutes. From this digest, cells were filtered, centrifuged, and stored in a HEPES-buffered solution (below) containing 0.5 mM Ca2+ at room temperature for later use. No recovery solutions were necessary. In later experiments, myocytes were prepared according to Barrington et al.14 Only quiescent (when not stimulated) rod-shaped cells with uniform sarcomeres were used. Superfusion solution (aerated with 100% O2) contained (mM) NaCl 132, HEPES 20, MgSO4 1.2, glucose 11.1, and CaCl2 2, with KCl (from 1 M stock) added as indicated; pH was adjusted with 5N HCl to pH 7.35. Isolated myocytes were either 1) impaled with 2.7 M KCl–filled microelectrodes (20–50 MΩ resistance) or 2) “dialyzed” using whole-cell patch pipette recording techniques (see reference 15), with pipette resistance ranging from 2 to 8 MΩ in the superfusate. Patch electrodes contained (mM) potassium aspartate 125, KCl 20, EGTA 10, ATP (magnesium salt) 5, MgCl2 1, and HEPES (free acid) 5, adjusted to pH 7.3 with 5N KOH. Stimulation (basic cycle length range, 800–1,000 msec) was accomplished using either intracellular current pulses (≤2 msec in duration, typically <4 nA) or (occasionally) bipolar stimulation via platinum wires. In experiments in which constant current was used to influence resting membrane potential, bridge balance was monitored (on fast time base) by following the voltage deflection during the 2-msec depolarizing current pulse used to stimulate each action potential. Cells were used within 12 hours of isolation; results obtained within 1 hour after dissociation were not noticeably different from those obtained after 10–12 hours ex vivo.

To assess possible differences in the sensitivity of \( V_{\text{m}} \) to the reduction of maximum conductance (\( g_{\text{Na}} \)) in the different experimental preparations, cumulative dose–response curves for tetrodotoxin (TTX) reduction of \( V_{\text{m}} \) were constructed for syncytial endocardial preparations and isolated myocytes. To minimize possible use-dependent block by TTX, preparations were stimulated at 0.5 Hz (2-second basic cycle length); to minimize voltage-dependent reduction of \( V_{\text{m}} \) observed in partially depolarized fibers,12 preparations were superfused at [K+]o of 4 mM and had resting membrane potentials of \( >-80 \) mV. TTX (Calbiochem, La Jolla, Calif., or Sigma) was prepared as a 1 mg/5 mL stock solution and added to the modified Tyrode’s solution as required.

**Isolated Myocyte Preparations**

Isolated myocytes were prepared in a manner similar to that described previously.13 Briefly, a wedge of left ventricular myocardium was perfused for 2 minutes with nominally calcium-free Tyrode’s solution containing (mM) NaCl 118.5, KCl 2.8, NaHCO3 14.5, KH2PO4 1.2, MgSO4 1.2, and glucose 11.1, along with 1 mg/mL bovine serum albumin (fatty acid free), followed by recirculation with solution supplemented with 0.5 mg/mL collagenase (Type II, Worthington Biochemical Corp., Freehold, N.J.) and 1 mg/mL bovine serum albumin. After 30 minutes of perfusion, the wedge was removed, and samples of midmyocardial cells were removed and placed on a temperature-controlled (36.5–37°C) superfusion chamber for experiments. Additional samples were placed in a second enzyme solution supplemented with 0.3 mM CaCl2 and 3 mg/mL bovine serum albumin for 15–20 minutes. From this digest, cells were filtered, centrifuged, and stored in a HEPES-buffered solution (below) containing 0.5 mM Ca2+ at room temperature for later use. No recovery solutions were necessary. In later experiments, myocytes were prepared according to Barrington et al.14 Only quiescent (when not stimulated) rod-shaped cells with uniform sarcomeres were used. Superfusion solution (aerated with 100% O2) contained (mM) NaCl 132, HEPES 20, MgSO4 1.2, glucose 11.1, and CaCl2 2, with KCl (from 1 M stock) added as indicated; pH was adjusted with 5N HCl to pH 7.35. Isolated myocytes were either 1) impaled with 2.7 M KCl–filled microelectrodes (20–50 MΩ resistance) or 2) “dialyzed” using whole-cell patch pipette recording techniques (see reference 15), with pipette resistance ranging from 2 to 8 MΩ in the superfusate. Patch electrodes contained (mM) potassium aspartate 125, KCl 20, EGTA 10, ATP (magnesium salt) 5, MgCl2 1, and HEPES (free acid) 5, adjusted to pH 7.3 with 5N KOH. Stimulation (basic cycle length range, 800–1,000 msec) was accomplished using either intracellular current pulses (≤2 msec in duration, typically <4 nA) or (occasionally) bipolar stimulation via platinum wires. In experiments in which constant current was used to influence resting membrane potential, bridge balance was monitored (on fast time base) by following the voltage deflection during the 2-msec depolarizing current pulse used to stimulate each action potential. Cells were used within 12 hours of isolation; results obtained within 1 hour after dissociation were not noticeably different from those obtained after 10–12 hours ex vivo.
As with syncytial preparations, sodium channel availability was altered by varying $[K^+]_o$. Similar to the changes reported for rabbit ventricular myocytes, modest changes in latency between stimulus and time of $V_{\text{max}}$ ($\leq 1$ msec) produced no effects on $V_{\text{max}}$, $V_{\text{max}}$ values from normally polarized myocytes (300–600 V/sec) were typically higher than those from syncytial ventricular preparations (200–300 V/sec), as has been reported by others. Consequently, $V_{\text{max}}$ values were normalized when comparing $V_{\text{max}}$ from the different experimental preparations.

Data using whole-cell patch techniques was obtained a minimum of 15 minutes after cell break-in to allow for equilibration of intracellular space with the pipette solution. No noticeable drift was observed in the shape or position of apparent $h_c$ curves constructed before or after washout of ISO, indicating minimal time-dependent alterations of this measure during this time. Curves were fit using nonlinear least-squares regression techniques. Where possible, data are presented as mean±SD. Means were compared using paired $t$ tests or analyses of variance followed by the Bonferroni procedure, with statistical significance set at $p<0.05$.

**Results**

**Syncytial Preparations**

From earlier reports, it was anticipated that ISO would reduce $I_N$ more prominently in partially depolarized fibers, when sodium channel inactivation is enhanced. However, we observed no consistent (if any) effect of ISO on $V_{\text{max}}$ of depressed fast responses in syncytial ventricular muscle or Purkinje fiber preparations. Figure 1A illustrates the typical lack of effect of ISO on a depressed fast response upstream from canine endocardium. It is apparent that $V_{\text{max}}$ was not reduced even though ISO altered the overshoot and plateau height (inset), confirming drug activity and consistent with its well-known enhancement of $L$-type calcium current.

Figure 1B illustrates the lack of effect of ISO on the steady-state $h_c$ curve from canine endocardial tissues. Data was obtained by superfusing tissues with Tyrode’s solution containing either 4, 7, 10, or 12 mM $[K^+]_o$, in the absence or presence of ISO (1 $\mu$M). Although ISO did tend to hyperpolarize the resting membrane potential in solutions containing lower $[K^+]_o$, there was no statistically significant difference in either $V_{\text{max}}$ or resting membrane potential in the absence versus presence of ISO ($n=7$, paired $t$ tests); a single Boltzmann curve was fit to both sets of data. For example, with 4 mM $[K^+]_o$, $V_{\text{max}}$ was $249±67$ V/sec without ISO (control) versus $235±51$ V/sec with ISO (corresponding resting membrane potentials, $−87.4±2.7$ versus $−89.5±2.4$ mV); with 12 mM $[K^+]_o$, $V_{\text{max}}$ was $71±36$ V/sec without ISO (control) versus $75±33$ V/sec with ISO (corresponding potentials, $−61.6±4.4$ and $−61.8±4.1$ mV). No abnormal automaticity (early or delayed afterdepolarizations) or triggered activity was observed in syncytial preparations during exposure to ISO.

Figure 1C illustrates the lack of effect of ISO on the apparent $h_c$ curve of a Purkinje fiber using a different experimental protocol. In these experiments, resting membrane potential and corresponding $V_{\text{max}}$ values were first obtained while increasing $[K^+]_o$ from 4 to 8 mM, taking ~3 minutes. ISO (1 $\mu$M) was then added, and $[K^+]_o$ was then reduced to 4 mM in the continued presence of ISO. There was no difference in $V_{\text{max}}$ values obtained in the absence or presence of ISO for any resting potential. Similar results were obtained in each of three experiments.

**Isolated Myocytes**

To determine whether results obtained in syncytial preparations differed from those of isolated myocytes from the same species, we first studied the effects of ISO on $V_{\text{max}}$ of ventricular myocytes impaled with standard microelectrodes (to minimize alterations in the intracellular milieu). ISO (either 0.5 or 1 $\mu$M) decreased $V_{\text{max}}$ of partially depolarized myocytes ($[K^+]_o=12$ mM) with no significant changes in resting membrane potential. With 0.5 $\mu$M ISO, $V_{\text{max}}$ was decreased (from $125±25.6$ to $94.3±20.2$ V/sec, $n=3$, $p<0.05$), whereas resting membrane potential remained unchanged (from $−69.6±2.24$ to $−70.4±2.45$ mV). With a greater ISO concentration of 1.0 $\mu$M, $V_{\text{max}}$ was reduced (from $94.6±21.3$ to $65.0±21.2$ V/sec, $n=4$, $p<0.05$) with no change in resting membrane potential ($−69.1±2.7$ versus $−68.6±2.3$ mV).

In normally polarized myocytes superfused with 4 mM $[K^+]_o$, ISO (1 $\mu$M) typically induced abnormal automaticity (early and delayed afterdepolarizations) and triggered activity, preventing a reliable assessment of changes of $V_{\text{max}}$ for a given resting membrane potential and stimulation rate. To circumvent this problem, constant hyperpolarizing current (typically 0.3–0.4 nA) was applied (via a balanced bridge circuit) to prevent triggered activity. Under such conditions, ISO (0.75–1.0 $\mu$M) had no effect on $V_{\text{max}}$. In each of three myocytes tested, $V_{\text{max}}$ was altered by <5% (458±76 V/sec [control] versus $451±77$ V/sec [ISO]), while an increased plateau height and prolonged action potential duration were noted. That ISO decreased $V_{\text{max}}$ of partially depolarized myocytes while not affecting $V_{\text{max}}$ of normally polarized myocytes implies either a hypopolarizing shift of the apparent $h_c$ curve and/or a change in the slope of the curve under these experimental conditions.

We also studied the effects of ISO on $V_{\text{max}}$ of isolated myocytes using patch pipettes to record transmembrane potentials. When these dialyzed myocytes were superfused with solutions containing either 12 or 14 mM $[K^+]_o$, ISO (1 $\mu$M) consistently decreased $V_{\text{max}}$ (77±18.9 versus 39.25±6.6 V/sec, $n=4$, $p<0.05$) without altering resting membrane potential ($−62.3±4.5$ versus $−62.1±4.5$ mV). In contrast, in normally polarized myocytes ($[K^+]_o=4$ mM), ISO increased $V_{\text{max}}$ in all five preparations tested (431.8±100.0 versus 476.2±119.0 V/sec, $p<0.05$) without altering resting membrane potential ($−87.5±5.8$ versus $−87.9±6.0$ mV). ISO did not evoke abnormal automaticity and triggered activity when cells were accessed with patch pipettes.

To assess how ISO altered the apparent $h_c$ curve, the resting membrane potential was altered by varying $[K^+]_o$, from 4 to 14 mM in the absence and presence of ISO. In the typical example illustrated in Figure 2A, ISO (0.5 $\mu$M) increased $V_{\text{max}}$ of normally polarized myocytes and decreased $V_{\text{max}}$ of depressed fast responses. When the curves were plotted on a normalized scale, it was apparent that ISO induced a hyperpolarizing shift of the
midpoint of the apparent hₐ curve (Figure 2B), without appreciably altering the slope of the curve. In each of three experiments, the midpoint of the hₐ curve was shifted in a hyperpolarized direction (−63.2±2.6 versus −66.1±3.0 mV, n = 3).

It was possible that the lack of effect of ISO on Vₘₐₓ in syncytial preparations versus those observed in isolated myocytes resulted from a lower sensitivity of Vₘₐₓ of syncytial preparations to a reduction of gsₙ. To address this possibility, we compared the effects of the specific sodium channel blocker TTX, known to act by reducing gsₙ, from an extracellular site. Cumulative dose–response curves were constructed for the reduction of Vₘₐₓ by selected TTX concentrations for our experimental preparations under comparable conditions ([K⁺]₀ = 4 mM, 36–37°C, basic cycle length of 2 seconds). Figure 3 illustrates that for any TTX concentration tested, syncytial (endocardial) preparations were more sensitive to the reduction of Vₘₐₓ by TTX than were isolated myocytes studied using microelectrodes, whereas dialyzed myocytes were the least sensitive. Statistical significance was obtained when comparing syncytial and dialyzed myocytes at TTX concentrations ≥4×10⁻⁶ M. These results suggest that the ISO-induced modulation of Vₘₐₓ absent in syncytial preparations but present in isolated myocytes cannot be ascribed to a lesser sensitivity of Vₘₐₓ of syncytial preparations to reduction of gsₙ.

Discussion

Our results indicate that ISO has no effect on Vₘₐₓ of upstrokes of various syncytial canine ventricular preparations throughout the range of resting membrane potentials known to alter sodium channel inactivation. In contrast, lower concentrations of ISO altered Vₘₐₓ of isolated canine ventricular myocytes, reducing Vₘₐₓ of partially depolarized myocytes studied using either microelectrodes or patch pipettes. ISO also increased Vₘₐₓ of normally polarized, dialyzed myocytes using patch pipettes and effected a hyperpolarized shift of the apparent hₐ curve. We are unaware of any report in which ISO increased and decreased cardiac Iₙₐ in the same preparation dependent on the resting membrane potential.

We could not attribute the disparate responses of partially depolarized syncytial versus dissociated myocyte preparations to different experimental conditions or protocols. Recognizing the possibility that the pharmacological responsiveness of syncytial endocardial and midmyocardial preparations could differ, we performed

Figure 1. Panel A: Lack of effect of isoproterenol on depressed fast response upstroke of canine endocardium. Upstrokes of transmembrane potentials and their first derivatives were recorded from canine endocardium ([K⁺]₀ = 12 mM) in the absence (closed circles) and presence (open circles) of 1×10⁻⁴ M isoproterenol. Resting membrane potential (−55 mV) and the maximum rate of rise of the action potential upstroke (Vₘₐₓ, 50 V/sec) remained essentially unaltered, while the overshoot increased. Inset: Alterations of depressed fast response action potential configuration with isoproterenol. Results are typical of six of seven experiments (in the seventh experiment, isoproterenol depolarized the resting membrane potential by 8 mV, preventing comparison of the Vₘₐₓ value with the drug-free control value). Panel B: Apparent steady-state inactivation curve from canine endocardium. Data are grouped by [K⁺]₀ (4, 7, 10, and 12 mM, left to right) in the absence (closed circles) and presence (open circles) of isoproterenol. No effect of isoproterenol on the curve was observed. The curve was fit by the least-squares method as fractional Vₘₐₓ = 1/[1 + e(Vₘₐₓ − Vₐₚ)/k], where Vₐₚ is the midpoint (−68 mV), and k is the slope factor (6.1); 100% Vₘₐₓ = 250 V/sec; n = 7. Panel C: Apparent inactivation curve (from Purkinje fiber) constructed by varying [K⁺]₀ from 4 to 8 mM in the absence (closed circles) and then presence (open circles) of isoproterenol (1 μM). Initial data were obtained by elevating [K⁺]₀ from 4 to 8 mM (K₄→K₈); subsequently, isoproterenol was added to 8 mM [K⁺]₀ solution, which was then switched to 4 mM [K⁺]₀ solution with isoproterenol (K₈→K₄). The curve was fit by the least-squares method to the Boltzmann equation as fractional Vₘₐₓ = 1/[1 + e(Vₘₐₓ − Vₐₚ)/k] where Vₐₚ = −71.5 mV and k is 3.4; 100% Vₘₐₓ = 580 V/sec.
two additional experiments in which the effects of ISO (1 μM) on $V_{\text{max}}$ of depressed fast responses ([K$^+$]e = 12 mM) were examined in midmyocardial transmural cores from the left ventricle: ISO had no effect on $V_{\text{max}}$ of these preparations. Recognizing further that different buffer systems were used in the superfuse of syncytial and myocyte preparations (bicarbonate/CO$_2$ versus HEPES, respectively), we examined the effects of ISO on syncytial preparations using HEPES-buffered Tyrode’s solution. ISO (1 μM) did not affect $V_{\text{max}}$ of either normally polarized ([K$^+$]e = 4 mM, one experiment; [K$^+$]e = 6 mM, one experiment) or partially depolarized ([K$^+$]e = 12 mM, two experiments) syncytial preparations.

One could further argue that the lack of effect of ISO in syncytial preparations is due to the postulated insensitivity of $V_{\text{max}}$ of propagating action potentials as an indicator of alterations of $I_{\text{Na}}$. Although the validity of changes in $V_{\text{max}}$ as an indicator of changes of $g_{\text{Na}}$ of “membrane” action potentials has been debated for some time (see Reference 18), the relation between $V_{\text{max}}$ and $I_{\text{Na}}$ at physiological temperatures may be more linear than earlier anticipated. Assuming that TTX reduces $g_{\text{Na}}$ equally in syncytial and isolated myocytes under our experimental conditions, $V_{\text{max}}$ of syncytial preparations was the most sensitive to reduction of $g_{\text{Na}}$ than were isolated myocytes, whereas dialyzed myocytes were the least sensitive (Figure 3). TTX sensitivity for myocytes (using microelectrodes) was comparable to that obtained previously for isolated canine myocytes. Further experiments are necessary to explain the basis for the differential TTX sensitivity of these experimental preparations.

We speculate that the different responses of $V_{\text{max}}$ to ISO arise from differences in the metabolic condition or intracellular ionic composition of the preparations. In further support of this hypothesis, ISO caused accentuated prolongation of the APD, abnormal automaticity, and triggered activity in isolated myocytes (using microelectrodes) but not in dialyzed myocytes or syncytial preparations. The increase in $V_{\text{max}}$ observed at normally polarized potentials using patch pipettes suggests that intracellular dialysis alters at least one component responsible for modulating $I_{\text{Na}}$. Evidence suggests that β-adrenergic modulation of $I_{\text{Na}}$ involves the β-receptor–G protein–adenylate cyclase–cAMP-dependent kinase cascade and channel phosphorylation. The multiple steps in this pathway are likely sites for alterations during the preparation of myocytes as well as for the use of different experimental techniques. The factor(s) responsible for modulation of this hormonal response in isolated myocytes (and not syncytial preparations) remains to be elucidated.

Our results with syncytial preparations differ from those reported in guinea pig papillary muscle and in which β-adrenergic stimulation reduced $V_{\text{max}}$ of depressed fast responses and effected a hyperpolarized shift of the apparent $h_{\text{a}}$ curve. Such differences may reflect a species difference between guinea pig papillary muscle and canine myocardium. Our results with depressed fast responses from isolated myocytes are similar to those reported in guinea pig myocytes and canine myocardium. An ISO-induced increase in $I_{\text{Na}}$ without a shift of the $h_{\text{a}}$ curve has been reported in guinea pig myocytes, suggesting that unbuffered increases in [Ca$^{2+}$]i were responsible for the $h_{\text{a}}$ curve shift reported by others. Our results do not support this hypothesis, because ISO effected a hyperpolarized shift of the apparent activation curve using patch pipettes that strongly buffered [Ca$^{2+}$]. That ISO may alter the apparent $h_{\text{a}}$ curve independent of affecting the maximal $V_{\text{max}}$ values (micropipette versus patch pipette techniques) suggests that multiple regulatory sites modulate $I_{\text{Na}}$.

It seems most likely that ISO-induced alterations of $V_{\text{max}}$ in dissociated myocytes reflect alterations of $I_{\text{Na}}$. For a membrane action potential (approximated by a single myocyte), all membrane current is applied to the membrane capacitance (i.e., no local circuit currents for propagation). Thus, any difference in $V_{\text{max}}$ reflects changes in net inward membrane current. Assuming $I_{\text{Na}}$ remains the predominant current during an upstroke, any changes in $I_{\text{Na}}$ are reflected in $V_{\text{max}}$. Although contaminating (nonsodium) currents evoked by ISO could alter $V_{\text{max}}$ and erro-
neously be interpreted as changes in $I_{Na}$, we believe this to be unlikely. For normally polarized myocytes, we know of no time-dependent currents of sufficient strength or rapid kinetics that could enhance upstrokes with $V_{max}$ values of 400–600 V/sec attained in <2 msec. A time-independent Cl$^-$ current activated by ISO has been reported in rabbit and guinea pig ventricular myocytes; this ISO-activated current might contribute inward current during the upstroke. If present in isolated myocytes, this current would not be expected to significantly increase $V_{max}$ of normally polarized myocytes because of 1) its small size relative to $I_{Na}$ and 2) an expected reversal potential in the range of −60 mV. Furthermore, this current would not be expected to produce the greater peak plateau values of $V_{max}$ seen in the $I_{Na}$ curves (Figure 2), because the inward Cl$^-$ current increases with hyperpolarization, whereas $V_{max}$ values plateau with more polarized resting potentials. Adrenergic modulation of the transient outward current in isolated canine Purkinje myocytes has also been described. This current would not likely reduce $V_{max}$ (even with a very depressed fast response), because activation of this component of the transient outward current in canine myocytes occurs at potentials positive to −30 mV. Stimulation of calcium currents by ISO (see Reference 25) would likely lead to an underestimation of reduced $I_{Na}$, especially in partially depolarized myocytes. Observations by others in which ISO was shown to modulate $I_{Na}$ measured directly support the hypothesis that the alterations of $V_{max}$ we observed are due to alterations of $I_{Na}$. Elucidation of the mechanisms responsible for altering $I_{Na}$ (altered channel kinetics, functional channel numbers, or channel selectivity) remain to be determined.

In summary, our results suggest that $\beta$-adrenergic modulation of $I_{Na}$ in vitro does not occur in syncytial preparations but does occur in isolated myocytes, depending on the resting membrane potential and experimental techniques used. It would be interesting to determine whether $\beta$-adrenergic modulation of $I_{Na}$ occurs under pathological conditions in vivo. If identified, those factors responsible for ISO-induced modulation of $I_{Na}$ could form the basis for a novel “metabolic” approach to antiarrhythmic drug therapy targeted to pathological tissues, enhancing $I_{Na}$ and conductance in “normal” myocardium, while depressing conduction in partially depolarized ischemic, injured, or diseased myocardium.

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