0022-3565/90/2523-1043$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 252, No. 3
Printed in U.S.A.

Characterization of the Electrophysiological Effects of Metoprolol on Isolated Feline Ventricular Myocytes[1]

PEGGY L. BARRINGTON and ROBERT E. TEN EICK
Department of Pharmacology, Northwestern University, Chicago, Illinois
Accepted for publication November 20, 1989

ABSTRACT

Metoprolol is considered to be a class II antiarrhythmic agent that is highly specific for cardiac *beta*-1 adrenergic receptors, yet long-term administration can produce prolongation of the rate-corrected Q-T interval in humans. Action potentials and sodium (I_{Na}), "L"-type calcium (I_{Ca}) and transient outward (I_{to}) or inward rectifying potassium (I_{KI}) currents were recorded from isolated cat ventricular myocytes using the whole-cell-patch technique to determine if metoprolol can directly affect cellular electrophysiological activity. External and pipette solutions, holding potentials and voltage-clamp protocols appropriate to isolate and examine I_{Na}, I_{KI}, I_{to} and I_{Ca} were used. Metoprolol reversibly decreased both the duration and voltage of the action potential plateau but had no effect on upstroke velocity, the repolarization rate during phase 3 or the resting potential. Confirming previous reports suggesting that metoprolol appears to have little or no local anesthetic activity, I_{Na} was not affected by metoprolol at concentrations up to 100 μM during voltage-clamp pulses applied at less than 1 Hz when holding potential was negative to −110 mV. However, when trains of pulses to −10 mV from a holding potential of −110 mV were applied at 1 to 5 Hz, use-dependent inhibition of I_{Na} occurred, suggesting that 100 μM metoprolol may interact with inactivated Na channels to inhibit I_{Na}. Metoprolol (10 and 100 μM) also caused a concentration-dependent decrease in peak inward I_{KI} elicited in response to hyperpolarizing from −40 mV to potentials negative to the I_{KI} reversal potential. In addition, during strong hyperpolarizations (*i.e.*, $\leq$−150 mV) an inward (*i.e.*, downward) droop in current was observed during the inactivation phase ~20 to 30 msec after pulse onset. Metoprolol (10 μM) also reduced peak I_{to} and I_{Ca} without altering the time courses of inactivation of either current or the level of the steady-state outward current elicited positive to −40 mV; steady-state I_{Ca}, on the other hand, was reduced. The sensitivity to block by metoprolol was: $I_{KI} > I_{Ca} \geq I_{to} > I_{Na}$.

Metoprolol (Lopressor) is a relatively cardioselective third generation *beta*-1 adrenergic antagonist believed to have virtually no local anesthetic activity. The majority of published evidence seems to indicate that metoprolol should be regarded (using the antiarrhythmic drug classification scheme of Vaughan Williams, 1975) as a pure class 2 agent (Camm *et al.*, 1982; Cobbe *et al.*, 1983; Edvardsson *et al.*, 1984; Koch-Weser, 1979; Marchlinski *et al.*, 1984; Rizos *et al.*, 1984; Rizzon *et al.*, 1978). However, long-term metoprolol administration has been reported to prolong rate-corrected Q-T intervals in rabbits (Raine and Vaughan Williams, 1981) and humans (Edvardsson and Olsson, 1981, 1985). This suggests that metoprolol could have direct effects on the cardiac sarcolemmal membrane which can cause prolongation of the ventricular action potential and thus also confer class 3 antiarrhythmic properties on the compound. This study assessed the effects of metoprolol on the action potential and whole cell membrane current, including several of its components, by applying conventional whole-cell-patch and voltage-clamp techniques to enzymatically dispersed, isolated feline ventricular myocytes. The results indicate that metoprolol can exert direct effects on several species of sarcolemmal ionic channels; in contrast to an expectation that metoprolol would increase action potential duration, we found that it could decrease both action potential duration and depress membrane voltage during the plateau phase. Thus, this report does not support the notion that metoprolol has class 3 activity in cat myocardium. Instead, an inhibition of L-type I_{Ca} may be an important feature of the drug's direct effects on cellular electrical activity.

Received for publication February 27, 1989.
[1] This work was supported by a generous gift from the Ciba-Geigy Pharmaceutical Corporation and by Grants HL-38909, HL-38041 and HL-27026 from the United States Public Health Service, National Institutes of Health.

Methods

Preparation of myocytes. The method used to isolate adult cat ventricular myocytes is a modification of that described by Silver *et al.*

ABBREVIATIONS: I_{Ca}, calcium current; KHB, Krebs-Henseleit buffer solution; I_{Na}, sodium current; I_{KI}, inward rectifying potassium current; I_{to}, transient outward current; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I_K, delayed, outwardly rectifying outward K current; V_h, holding potential during voltage-clamp; V_m, membrane potential; V_t, membrane potential during test voltage clamping steps; V_{KI}, reversal potential for I_{KI}; E_K, equilibrium (Nernst) potential for the transmembrane K^+ gradient; V_c, membrane potential during conditioning voltage clamping steps.

(1983), and has been described in detail elsewhere (Follmer *et al.*, 1987). Briefly, cat hearts were rapidly excised, the aorta cannulated and coronary vessels perfused with a nominally Ca^{++}-free KHB. After 2 to 3 min, the perfusate was switched to KHB containing 0.15% type II collagenase (Worthington Biomedical, Freehold, NJ). After ~35 min of exposure to collagenase the perfusion was stopped, the heart was removed from the perfusion setup and the atria and right ventricular freewall were separated from the left ventricle including the ventricular septum. The left ventricular and septal tissues were minced and incubated with the solution containing collagenase in a shaker bath for ~5 min. Residual tissue pieces were removed by filtering the dispersed cells through a 200-μ mesh. The cells were washed free of collagenase and harvested by gentle centrifugation in fresh KHB. Cell pellets were resuspended and allowed to settle first in KHB with 1% albumin added and then in KHB with 1% albumin and 1 mM $CaCl_2$ added. Isolated cells were stored in Nalgene containers with Hank's buffer (Medium 199, Gibco Laboratories, Grand Island, NY) at room temperature. Only quiescent rod-shaped cells with a nongranulated cytoplasm and distinct striations were selected for subsequent electrophysiological studies. All experiments were conducted within 36 hr of isolation, during which time there were no discernible changes in the ionic currents under study.

Voltage-clamp and recording techniques. Action potentials and whole-cell membrane currents from single isolated ventricular myocytes were obtained using the whole-cell-patch technique originally described by Hamill *et al.* (1981) and used previously by other workers in our laboratory (*e.g.*, Follmer *et al.*, 1987; Harvey and Ten Eick, 1988, 1989a,b; Mogul *et al.*, 1989a,b). In some cases both action potentials and whole-cell currents were recorded from the same cell. Action potentials were elicited in response to intracellularly applied depolarizing cathodal stimuli (1–4 nA, 1 msec) delivered from an Axoclamp 2A device (Axon Instruments Co., Burlingame, CA) *via* the patch pipette. Suction pipettes were made using borosilicate glass capillary tubing (0.8–1.1 mm inside diameter; Kimble Glass) and a Narashigi two-stage vertical pipette puller (Narashigi, New York, U.S.A.). Pipettes were fabricated so that when filled with the appropriate internal solution, tip resistances ranged between 0.2 to 0.6 megohms for recording I_{Na} and between 0.8 and 2.0 megohms for electrodes used to record action potentials, whole-cell composite membrane current, I_{Kl}, I_{Ca} and I_{to}.

Whole-cell current was recorded using a whole-cell-patch voltage-clamp circuit and computer-controlled data acquisition system described previously (Narahashi *et al.*, 1986; Follmer *et al.*, 1987; Harvey and Ten Eick, 1988). Briefly, the clamp circuitry applied voltage-clamp pulses to the bath that were inverted relative to the cell interior. The membrane current was taken as the current required to clamp the patch pipette at 0 mV with a virtual ground. In instances when recordings of both action potentials and membrane currents were obtained from the same cell, they were recorded using the appropriate output signal of the Axoclamp 2A device. Membrane current and voltage were monitored and recorded digitally using a PDP 11/73 computer (Digital Equipment, Pittsfield, MA). Series resistance was compensated using a feedback circuit before initiating data acquisition.

Solutions. All action potentials and currents with the exception of I_{Na} were recorded using a HEPES-buffered modified Tyrode's solution with the following composition (millimolar): NaCl, 140; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 0.5; HEPES, 5.5 and sucrose, 5.0, pH adjusted to 7.4 with NaOH. The pipette or internal solution contained (millimolar): K-aspartate, 130; KCl, 20; KH_2PO_4, 1.0; ATP-K_2, 5.0; $MgCl_2$, 1.0; ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid, 5.0 and HEPES 5.0, pH adjusted to 7.2 with KOH. All experiments were performed at 32 to 36°C using a Peltier device to warm the superfusing solution.

For recording I_{Na} the external solution was modified to block other ion channels that could contribute to the whole-cell membrane current and to reduce peak I_{Na}. The external solution had the following composition (millimolar): NaCl, 12.5; tetramethylammonium chloride, 127.5; CsCl, 5; $MgCl_2$, 1.2; $CaCl_2$, 1.8; HEPES, 20; glucose, 11; pH 7.3 at 22°C. Pipettes were also filled with a solution specific for recording I_{Na} that contained (in millimolars): CsF, 142.8; NaF, 5.0; and HEPES 5.0, pH adjusted to 7.2 at room temperature with CsOH. The Cs^+ in external and pipette solutions blocked K channels, whereas F^- removed I_{Ca}. The size of I_{Na} was reduced by limiting the driving force for Na^+ to a 7.5 mM gradient across the membrane. In addition to limiting the size of the peak current, the time course of I_{Na} activation was slowed by reducing bath temperature to 12 to 15°C. These conditions enabled voltage clamp to be obtained rapidly (typically in 150–200 μsec) and produced currents of 5.4 ± 1.1 nA (N=8) which appeared to be adequately controlled by the clamp circuitry despite the minor voltage error of no more than 4 mV at peak current flow caused by the i-r voltage drop across the cellular series resistance.

Results

Metoprolol alters the action potential and composite membrane current. Figure 1A shows the effect of metoprolol (10 μM) on the cellular action potential (whole-cell-patch configuration) elicited in response to 3.8 nA, 1 msec stimulus pulses at 1 Hz. It indicates that the voltage time course of the action potential can be altered during exposure of the myocytes to metoprolol, and that the alteration was reversible upon return to drug-free solution. Action potential duration was decreased and plateau (phase 2) voltage was depressed; eventually the notch between phases 1 and 2 disappeared. No effects were detected on upstroke velocity, the repolarization rate during phase 3 or the resting potential. These changes in the action potential suggest that several components of the membrane current flowing during the action potential plateau (*e.g.*, the intracellular Ca^{++} activity independent components of I_{to} and I_{Ca}) had been modified by metoprolol, whereas those flowing during the upstroke and during the late repolarization and resting phases (*e.g.*, I_{Na}, I_K and I_{Kl}) were not affected. Because the effect of metoprolol to alter the plateau phase could be at least partially reversed by removing the drug, it is not reasonable to suggest that the decrease in plateau duration and voltage could have resulted solely from the well known "run down" of I_{Ca}.

These changes in the action potential were reflected in the whole-cell current elicited when the holding potential (V_h) was −40 mV and membrane voltage (V_m) was stepped for 100 msec to test potentials (V_t) ranging from −100 to +60 mV in 10 mV steps. Metoprolol (100 μM) reduced the inward directed peak elicited at potentials negative to −90 mV. There was little effect on the whole-cell current between −80 and −40 mV; positive to −40 mV the inward directed peak current observed when V_m was stepped to levels between −30 and +30 mV was reduced. In the example cell depicted in figure 1B, the outward directed peak associated with I_{to} was absent, as was the case in about one-half of all isolated cat ventricular myocytes. Similar results were obtained in 2 of 4 cells. The 2 nonconforming cells were different only with respect to the presence of an early outward directed peak within 2 to 4 msec which decayed over the next 20 to 30 msec when stepped from −40 mV to V_t positive to +30 mV. This peak associated with I_{to} also was reduced in amplitude by exposure to 100 μM metoprolol. Because whole-cell membrane current flowing during depolarizing steps from a V_h of −40 mV reflects the sum of I_{Ca} plus I_{to} plus several unspecified steady-state leak current(s), it is difficult to assess which component of whole-cell current depicted in these recordings was effected by metoprolol. Therefore, experiments assessing the effects of metoprolol on the isolated I_{Na}, I_{Kl}, I_{Ca} and I_{to} were done.

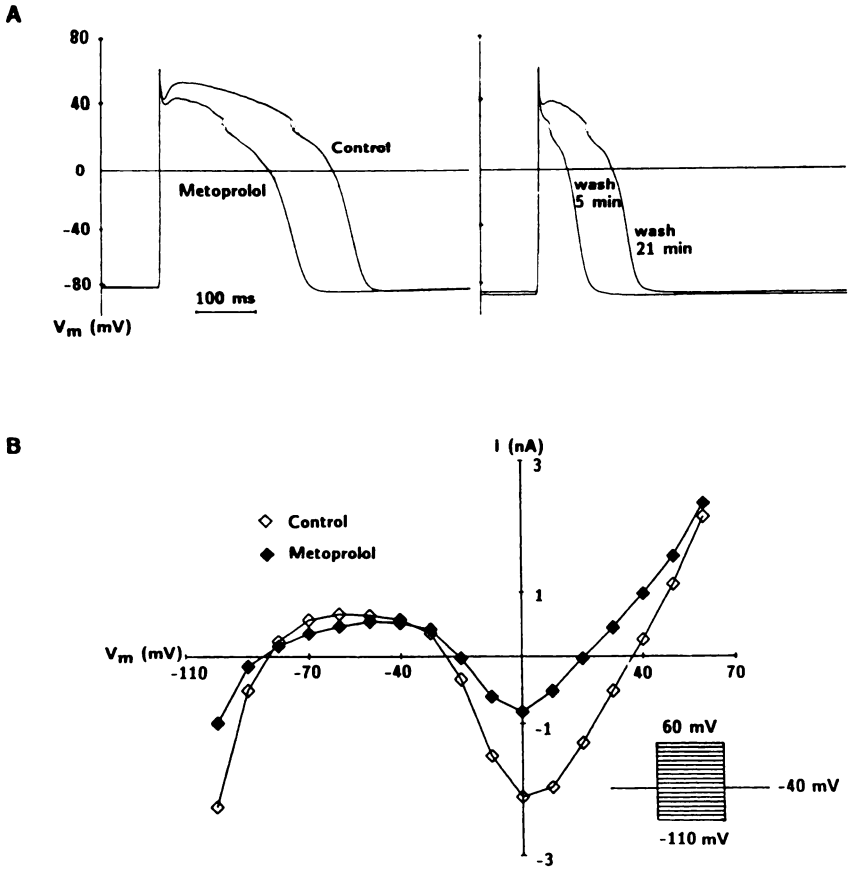

Fig. 1. Effects of metoprolol of the action potential and whole-cell composite membrane current of cat ventricular myocytes. A: typical effect on the action potential of 10 μM metoprolol for 10 min (left panel). Plateau duration and voltage were reduced. Plateau duration and voltage continued to decline for 5 min despite return to a drug-free Tyrode's solution but substantially returned toward the control values after 21 min in drug-free solution (right panel). The partial washout of the drug effect after 21 min suggests that the change in the action potential observed during drug exposure cannot be attributed exclusively to I_{Ca} run down (t=37±0.3°C; N=6). B: typical effect of 100 μM metoprolol on whole-cell composite peak current. the peak I-V relationship during exposure to metoprolol (♦) was reduced relative to control (◇) at potentials negative to −80 mV and between −20 and +50 mV. V_h was −40 mV; families of currents elicited by 100 msec voltage clamping pulses from −130 to +60 mV in 10 mV increments (t=34±0.3°C; N=5).

Effect of metoprolol on I_{Na} and its holding potential dependence. I_{Na} was isolated using Cs^+ in both the internal and external solutions to block K^+ currents and F^- was applied internally to inactivate Ca channels (Follmer *et al.*, 1987; Kostyuk *et al.*, 1977). Using a V_h ranging from −110 to −140 mV and 200 msec 0.2 Hz depolarizing pulse steps ranging from −70 to +60 mV, families of sodium currents were generated. Families generated when V_h was −130 mV produced the expected progressively developing Na currents whose peak inward level was maximal at ~−30 mV. Families generated when V_h was −130 mV could be superimposed on scaled families of I_{Na} recorded from the same cell at V_h of −110 mV. These data, obtained at cool experimental temperatures and reduced external [Na^+], suggest that control of voltage clamp was adequate to determine the nature of the effect(s) of metoprolol on I_{Na} and its voltage dependence and time course. During exposure to metoprolol (10 or 100 μM), peak I_{Na}, its time course and current-voltage relationship were unchanged from control (fig. 2) when defined using a pulsing frequency of 0.2 Hz and V_h levels ranging from −110 or −140 mV. Using these conditions, even 100 μM metoprolol had no detectable effect on I_{Na}.

The above data are in agreement with the finding of no change in the depolarization rate of the action potential upstroke (fig. 1A) and supports earlier findings by others that metoprolol did not alter the maximal depolarization rate of the action potential upstroke in canine and rabbit Purkinje fibers and ventricular strips. Thus, metoprolol does not appear to exhibit local anesthetic-like properties. If this were in fact the case, metoprolol would be somewhat unique because many *beta* antagonists have been reported to exhibit local anesthetic properties (Koch-Weser and Frishman, 1981). Therefore, we further evaluated the possibility that metoprolol might possess such properties by addressing the question of whether metoprolol (100 μM) could exhibit a frequency-dependent blocking action on I_{Na}.

I_{Na} was recorded during trains of repetitive 5-msec steps to −10 mV from two levels of V_h (−140 and −110 mV) at frequencies of 1, 2 and 5 Hz. Before exposure to metoprolol when holding potential was −140 mV (fig. 3A), a slight frequency-dependent decrease in I_{Na} was observed during pulse trains at 5 Hz. Metoprolol appeared to enhance the decrease but the effect was statistically insignificant. When V_h was reduced to −110 mV (fig. 3B), a small frequency-dependent decrease in I_{Na} became readily apparent in the control records; furthermore, at this level of V_h in the presence of metoprolol (100 μM) frequency (*i.e.*, use-)-dependent inhibition of I_{Na} was clearly evident, the degree of block being enhanced by increasing pulse frequency.

Inspection of the 5 Hz curves obtained in the presence of metoprolol when V_h was −110 mV indicates that it takes more than 10 pulses (5-msec duration at −10 mV) to achieve a steady-state use-dependent block. The rates of onset of use-dependent block in the presence of metoprolol was 1.8±0.46 pulses^{-1} at 5 Hz and 2.3±0.82 pulses^{-1} at 1 Hz. These rates of onset were slightly faster than that found by Clarkson *et al.* (1988) for lidocaine at 1 and 5 Hz using 20-msec pulses to −20 mV. The implication of this comparison is that, as with lidocaine, the onset of metoprolol-induced use-dependent block of I_{Na} should be regarded as rapid relative to that reported for class 1A or 1C antiarrhythmic agents.

A determination of the time course for the recovery from metoprolol-induced use-dependent block was beyond the scope of the present study. The recovery process may be composed of several components which may be either beat dependent (*i.e.*,

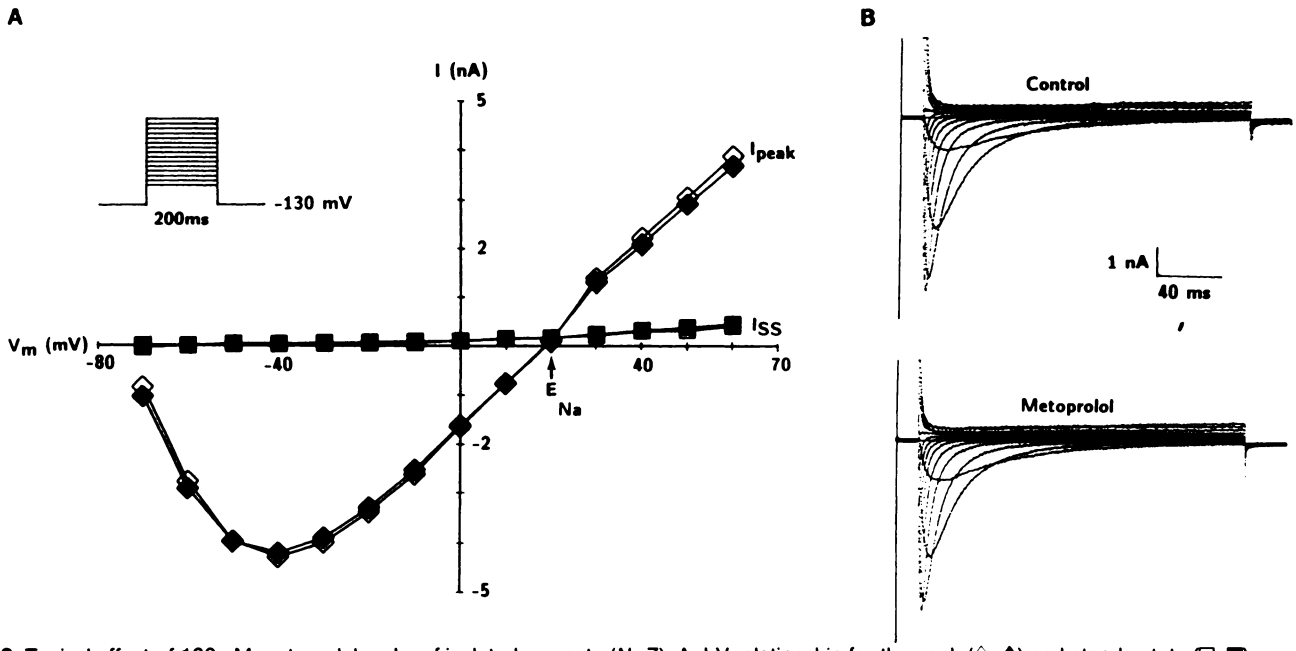

Fig. 2. Typical effect of 100 μM metoprolol on I_{Na} of isolated myocyte (N=7). A: I-V relationship for the peak (◇, ◆) and steady-state (□, ■) currents are not altered by exposure to metoprolol (filled symbols) relative to control (unfilled symbols). B: current families plotted during control superfusion and after addition of metoprolol show the same size and progression of currents as seen with the controls.

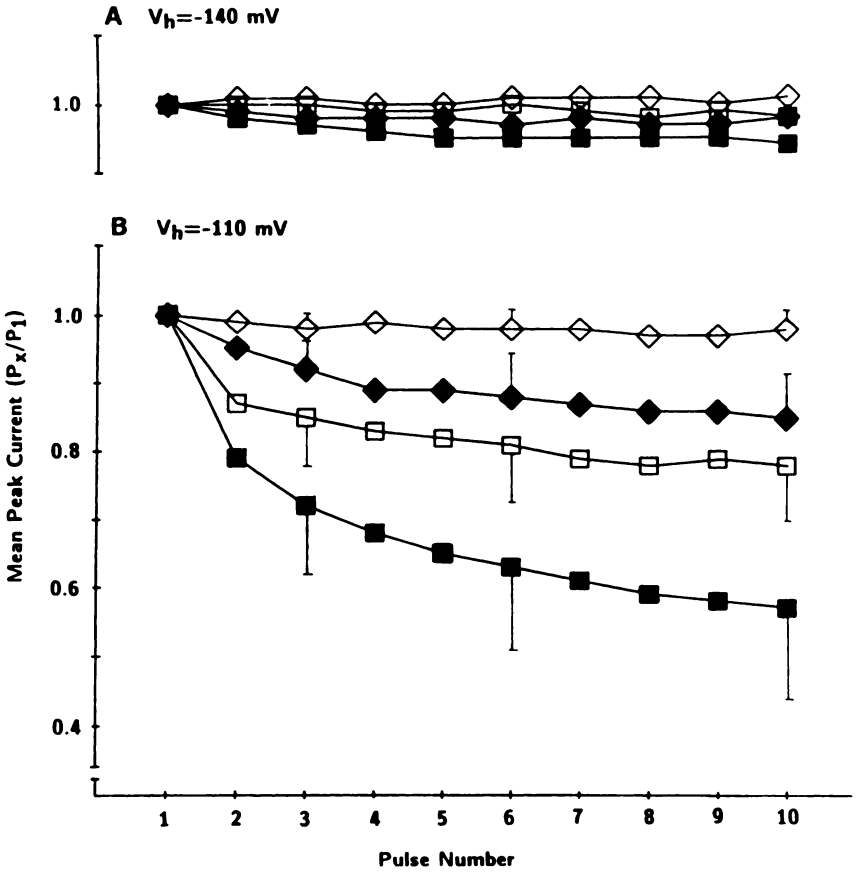

Fig. 3. The frequency dependence of I_{Na} during control (unfilled symbols) and exposure to 100 μM metoprolol (filled symbols) at 1 Hz (◇, ◆) and 5 Hz (□, ■). A: frequency dependence of I_{Na} at a V_h of −140 mV shows only minimal reduction in peak current even at 5 Hz (N = 6). B: at a V_h of −110 mV the frequency dependence of I_{Na} was readily apparent. Metoprolol produced block at 1 Hz and a greater degree of block at 5 Hz (N = 5). Points are means ± S.E.

channels must open for drug to escape *via* an aqueous pathway) or simply time dependent (*i.e.*, drug leaves channel blocking site by diffusion into the lipid phase of the perichannel membrane) or both. These possibilities can make the design of protocols, which are truly appropriate to define the recovery time course, a difficult problem to solve. An experimentally obtained recovery time course may be protocol-specific (Yeh and Ten Eick, 1987) and have an uncertain relationship to the rate at which drug can leave the channel blocking site during a series of action potentials occurring under more physiologically relevant conditions.

Because of the uncertainties inherent in the more classical

(and usual) approach, we have taken another tack. Some information about the rate of recovery from use-dependent block (in terms of whether it can be regarded as fast, slow or very slow) can be obtained from the onset curves shown in figure 3. Comparison of the curve obtained with the 5 Hz pulse train (when V_h was −110 mV) with that obtained at 1 Hz (V_h = −110 mV; fig. 3) reveals that the second pulse in a train delivered 200 msec later (*i.e.*, the second pulse of the 5 Hz train) elicits about one-half the peak current elicited by a second pulse delivered 1 sec later (*i.e.*, the second pulse of the 1 Hz train). This finding suggests that, when recovery occurs at −110 mV, about one-half of the use-dependent block developed during the first 5 msec pulse to −10 mV can dissipate within ~1 sec. The implication of this simple analysis is that the rate of recovery at 15°C from metoprolol-induced use-dependent block is similar to that for lidocaine-induced use-dependent block at 16°C described by Clarkson *et al.* (1988, fig. 2C), the half-time of unblock for both drugs being about 1 sec and regarded as fast compared to quinidine.

The above results indicate that metoprolol can exhibit local anesthetic-like properties which may inhibit I_{Na} when used in concentrations substantially higher than those ordinarily employed to achieve *beta*-1 receptor blockade (100 μM *vs.* 0.1–0.3 μM) (Koch-Weser and Frishman, 1981; Marchlinski *et al.*, 1984; Rizos *et al.*, 1984). The results also indicate that, in contrast to many class 2 drugs (*e.g.*, propranolol), *beta*-1 blocking concentrations of metoprolol probably exert very little or no effect on I_{Na}. Therefore, the present results agree with the earlier findings showing no effect on the action potential upstroke despite the present demonstration that metoprolol can indeed cause Na channel block.

I_{Kl} blocked by metoprolol. I_{Kl} was elicited by 100 msec hyperpolarizing steps from a V_h of −40 mV to V_m ranging from −50 to −150 mV. During strong hyperpolarizing steps (*e.g.*, −150 mV), the time course of I_{Kl} was characterized by a rapid activation to a peak inward level within 2 to 4 msec, followed by a time- and voltage-dependent decay over the next 30 to 50 msec (see fig. 4B, V_t = −150 mV), which has been described as an "inactivation" and shown to be Na^+-dependent (Biermans *et al.*, 1988; Harvey and Ten Eick, 1988). Both the peak and steady-state (the current present at the end of the 100-msec test pulse) current-voltage relationships were reduced at hyperpolarizing V_m during exposure to 10 μM metoprolol (fig. 4A). When V_m was stepped to levels negative to the reversal potential for I_{Kl} (V_{Kl}), the inward current was reduced. At V_m positive to V_{Kl} and equal or negative to −40 mV, the outward directed current was essentially unaffected by the drug. The reduction in peak and steady-state I_{Kl} observed at V_m negative to V_{Kl} was concentration-dependent and was apparent in 2 of 5 cells exposed to 1 μM metoprolol and in all cells exposed to 10 μM metoprolol. The decrease in the slope conductance derived from the peak I_{Kl} *vs.* V_m curve for V_m negative to V_{Kl} suggests metoprolol can decrease the whole-cell conductance for inward flowing I_{Kl}. This effect is not expected to have much impact on the resting or action potential because membrane voltage is normally always positive to E_K. Indeed, the recordings of action potentials shown in figure 1A support this notion.

Inspection of the current traces recorded during exposure to metoprolol reveals that the time course of the inactivation process occurring during clamp steps negative to −130 mV was altered, developing a "droop" (see fig. 4B, V_t = −150 mV). The droop appeared as an increase in inward directed current that became noticeable within ~20 msec of pulse onset and continued to develop slowly during the remainder of the 100 msec pulse. This late increase in inward current appeared to be time-dependent, therefore the duration of the hyperpolarizing clamp step to −150 mV was increased to 200 (N=4), 500 (N=2) and 1000 (N=3) msec to determine whether steady-state I_{Kl} could return completely to control or if it could become even more inward than the control level. With longer strongly hyperpolarized clamp steps the steady-state I_{Kl} recovered virtually to the control level but never exceeded it, not even when clamp steps of 500 or 1000 msec duration were applied. These data suggest that the droop results from voltage- and time-dependent relief of metoprolol block and not from an effect of the drug to modify the normal inactivation process(es).

Relief of metoprolol-induced block of I_{Kl}, as reflected in the droop, was evident during strong hyperpolarizing pulses (*e.g.*, at $V_m \geq -130$ mV), suggesting that unblock exhibits voltage dependence. This finding raises the question of whether block of peak current might also exhibit voltage dependence. Therefore, the effect of V_m on drug-induced blockade of peak I_{Kl} was examined using the voltage-clamp protocol depicted in figure 5. Conditioning pulses from V_h (*i.e.*, −40 mV) to V_c ranging from −50 to −150 mV for 200 msec that were followed by return to −40 mV for 10 msec were applied before single 100 msec test pulses to a V_t of −160 mV. The rationale of this pulse sequence was the following. The conditioning pulses were designed to set a level of metoprolol-induced block that presumably would be different from that in force at V_h. However, the conditioning pulses, in addition to setting the level of block, are also expected to cause voltage-dependent inactivation of I_{Kl}. The purpose of the short 10 msec step from V_c back to the −40 mV was to take advantage of the rapid recovery of I_{Kl} from inactivation (~90% in 10 msec; Harvey and Ten Eick, 1988) so that any decrease from control levels of peak I_{Kl} evoked by test pulses would principally reflect the extent of metoprolol-induced block produced during conditioning pulses. The success of this approach will depend upon the rate of blocking at −40 mV being sufficiently slow that only a fraction of the steady-state block at −40 mV can be achieved during the 10 msec pulse preceding the test pulse. We have no direct evidence on this point. However, the slowness of the rate of the "unblocking" process reflected in the droop during 200 msec conditioning pulses to V_c = −150 mV (see fig. 5, upper left panel) gave hope that the blocking rate at −40 mV would be slow enough for the clamp protocol to demonstrate that the level of block associated with the conditioning pulses can exhibit voltage dependence.

The above described voltage-clamp protocol, depicted in figure 5, was used to examine the voltage dependence of the metoprolol-induced block of I_{Kl}. The results show that 10 μM metoprolol decreased peak I_{Kl} with the maximal reduction (71± 12%) occurring when V_c was ~V_{Kl} (fig. 5, right upper panel, V_c = −90 mV). The fractional block was less than maximal when V_c was either negative or positive to V_{Kl}. When plotted as the ratio of peak I_{Kl} during drug exposure to peak I_{Kl} during control as a function of the voltage of the conditioning pulses (fig. 5, lower panel), the mean results (N=6) yield a shallow curve with maximal block occurring at ~V_{Kl}. This curve is better fit by equations for either the sum of a decaying exponential plus a rising exponential or a parabola than by a straight line. The sums of the residual squares were 10-fold smaller for the sum of 2 exponentials and 4-fold smaller for the parabolic function than for the straight zero sloped line. This suggests that block

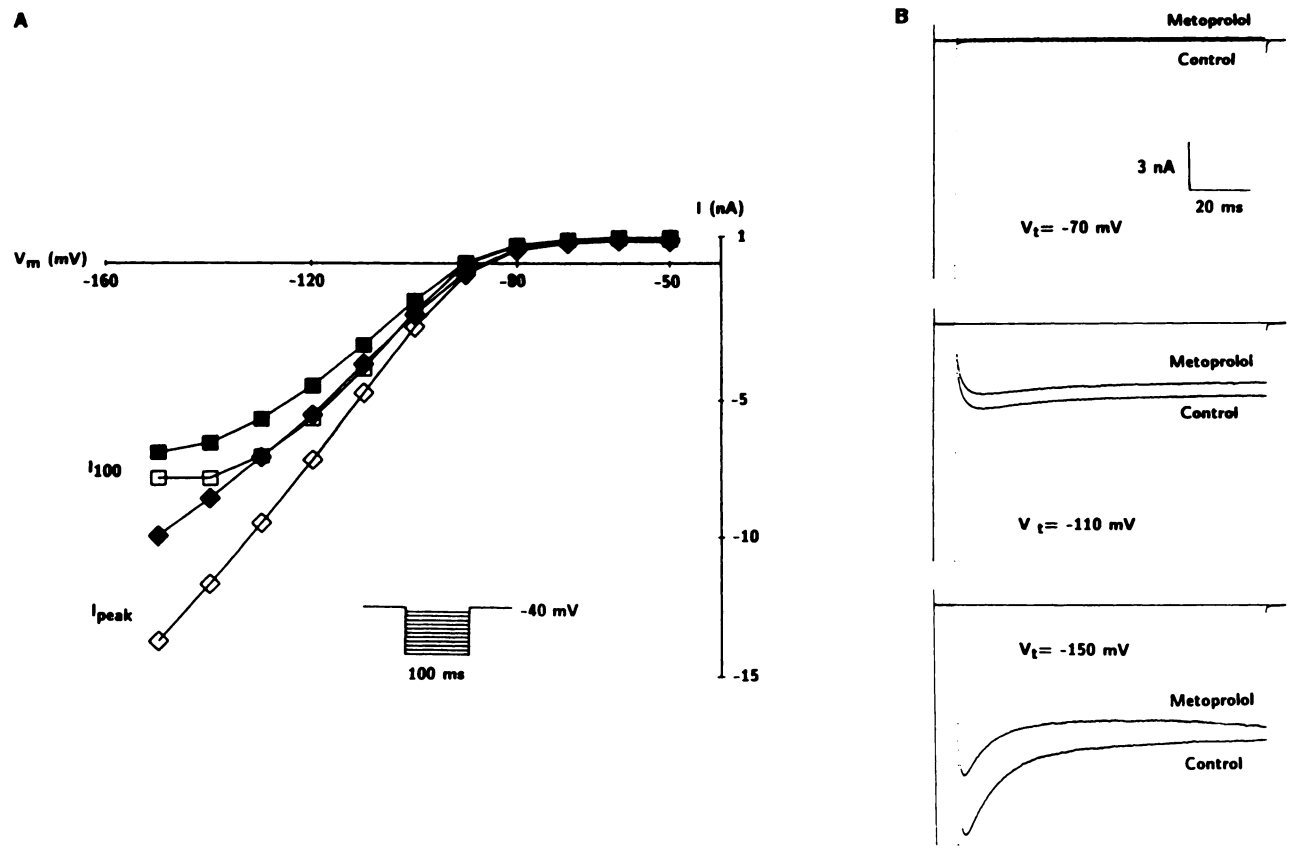

Fig. 4. Effects of 10 μM metoprolol on I_{Kl}. A: I-V relationship for both peak (◇, ◆) and steady-state (□, ■) currents are reduced at hyperpolarizing potentials during exposure to metoprolol (filled symbols) relative to the currents during control (unfilled symbols). B: currents paired at the same test potentials during control and exposure to metoprolol. There is a 75% reduction in peak current. Currents evoked by strong hyperpolarizing test pulses (*e.g.*, −150 mV) in the presence of metoprolol produced a time-dependent inward droop in the current rather than approaching a steady state. Data are representative of 7 of 7 cells.

of I_{Kl} by metoprolol has a modest voltage-dependence. Both functions fitting the data defining the voltage-dependence of the block of I_{Kl} were symmetrical about V_{Kl}. The symmetrical relationship contrasts with the large currents apparent at hyperpolarizing potentials negative to E_K (calculated to be ~−90.3 mV with the Nernst equation for these conditions) due to the strong inward rectification of I_{Kl}. This observation suggests that metoprolol-induced block primarily exhibits voltage-dependence rather than current-dependence. However, these data cannot be taken to indicate anything quantitative about the voltage-dependence block because, as stated earlier, we have no clues concerning the rate of block onset at −40 mV and therefore cannot reconstruct the extent of block in force at the end of the conditioning pulses.

I_{to} reduced by metoprolol. I_{to} was defined as cadmium ion (0.5 mM)-insensitive current elicited by applying 100 msec test pulses in 10 mV steps positive to the V_h of −40 mV. I_{to} is an outward K^+ current that activates and peaks in 1 to 3 msec (at 35–37°C) and then decays to a background outward level over the next 30 to 50 msec (see fig. 6B, middle panel). This current, present in only about 50% of cat ventricular cells (R. E. Ten Eick, unpublished observation), was activated at potentials positive to ~0 mV and became larger with more positive depolarizations. In figure 6, metoprolol (10 μM) modestly decreased peak I_{to} but had no effect on the time course of I_{to} decay (see fig. 6B) or on the steady-state current remaining at the end of 100 msec pulses except at very positive potentials (*i.e.*, at +70 or +80 mV). Lower concentrations of metoprolol (0.1 and 1 μM) did not appear to cause any block of I_{to}. At very positive potentials normally out of the physiologically relevant range of V_m (*e.g.*, +70 to +80 mV), there is suggestive evidence that, after the complete decay of I_{to}, a slowly developing time-dependent outward current began to flow (see fig. 6B, middle and lower panels); this component, which probably reflects the feline ventricular cell's version of the delayed outwardly rectifying K current, I_K, was not altered by 10 μM metoprolol, a concentration which could block 25 to 30% of peak I_{Kl}.

Peak I_{Ca} reduced by metoprolol. I_{Ca} (L-type) is defined as the Cd^{++}-sensitive current elicited by 100 msec duration clamp steps from a V_h of −40 mV to test potentials ranging from −30 to +80 mV in 10 mV steps. I_{Ca} was obtained by subtracting the whole-cell current recorded in the presence of 0.5 mM Cd^{++} from the whole-cell current recorded at comparable V_m in the absence of Cd^{++}. The subtraction revealed an inward current that activated to a peak in ~2 to 3 msec and then decayed as a double exponential process (analysis not shown) to a steady state that was net inward at V_m negative to the reversal potential (*i.e.*, ~+70 mV). Peak inward I_{Ca} was maximal between 0 and +10 mV. Qualitatively, the peak and steady-state levels of the subtraction currents decreased in the presence of metoprolol (10 μM), but I_{Ca} time course appeared to be unchanged (fig. 7). Similar effects were noted without subtraction in cells with no discernable I_{to}, which therefore did not require use of Cd^{++} to isolate I_{Ca}. These data suggest that

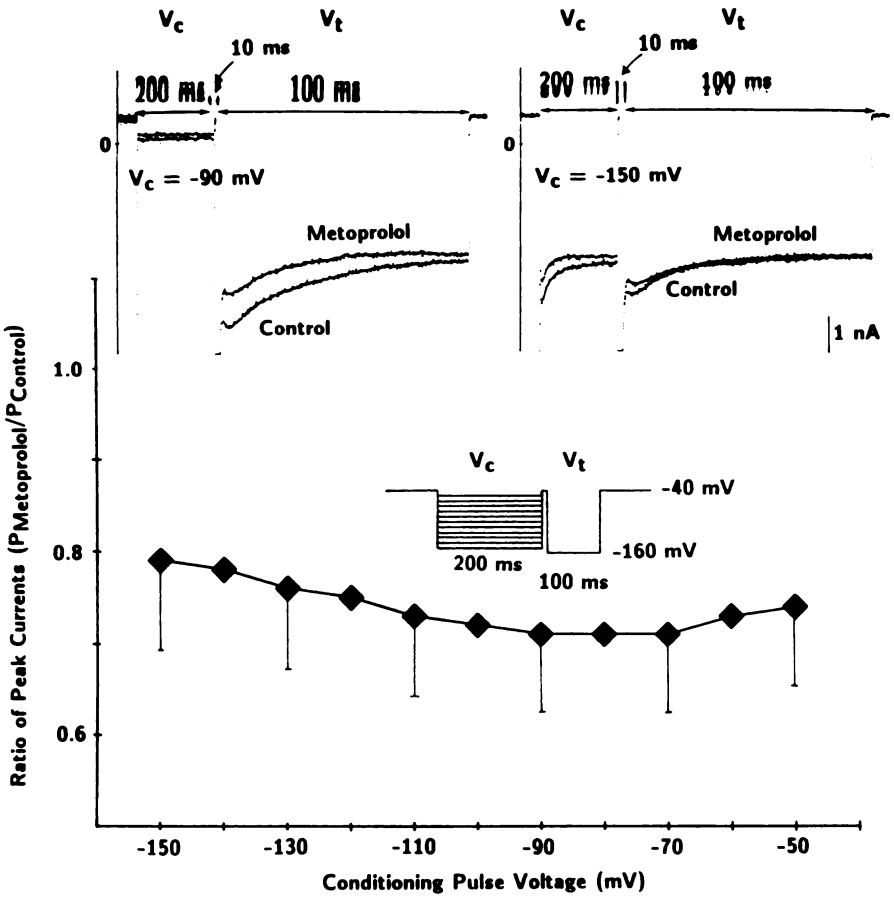

Fig. 5. Effect of conditioning pulse voltage on the block of peak I_{Kl} current by 10 μM metoprolol. The mean values for the ratio of metoprolol to control of peak currents elicited at −160 mV *vs.* voltage of a 200 msec conditioning pulse (V_c) (see text). The currents depicted in the inserts (at top) show the metoprolol and control currents elicited at V_t = −160 mV when V_c was −90 mV (left) and −150 mV (right). Less blockade of the peak current was seen with the more negative V_c. Be aware that the discontinuity in current time course arises from different sampling rates during data acquisition. Points are means ± S.E.; N = 6; V_t = −160 for both currents.

metoprolol reduced I_{Ca} by decreasing the whole cell conductance for I_{Ca}. The problem of I_{Ca} run down in isolated cells and the difficulty of attaining complete records for control and metoprolol with and without Cd^{++} within a reasonably short time period (*i.e.*, <20 min) make a truly quantitative assessment of I_{Ca} blockade impossible with the present data. However, partial recovery of I_{Ca} during washout of metoprolol in 2 cells lacking I_{to} (data not shown) confirms a true blocking effect which cannot be attributed mistakenly to I_{Ca} run down. This conclusion receives further support from the recordings of action potentials shown in figure 1A. As described earlier, the changes in the duration and voltage of the action potential plateau were at least partially reversed by returning to metoprolol-free (control) perfusate.

Discussion

The findings indicate metoprolol can inhibit I_{Kl}, I_{Ca}, I_{to} and I_{Na}. In our cat myocytes, inhibition of I_{Na} results from the development of use-dependent block occurring in the absence of any tonic block. It became evident only at rapid pulsing rates during exposure to concentrations much higher (*i.e.*, 100 μM) than those required for *beta*-1 receptor blockade (*i.e.*, <1 μM; R. Decker, W. Clark and R. E. Ten Eick, unpublished observation). In contrast, inhibition of I_{Kl} was seen in 2 of 5 cells exposed to metoprolol concentrations as low as 1 μM. In sum, the effects of metoprolol on I_{Kl}, I_{Ca}, I_{to} and I_{Na} suggest the compound can exert direct effects on cardiac cells that can produce block of sarcolemmal ion channels. These inhibitory effects on membrane channels appear to translate into shortening of the action potential duration and depression of plateau voltage. The nature of these direct effects suggests that, at concentrations which are 1 to 3 orders of magnitude greater than that required to achieve *beta* receptor blockade, metoprolol possesses properties which resemble those of both class 1 antiarrhythmic agents and Ca channel blockers, in addition to its well known cardio-selective *beta* adrenergic antagonist activity.

Comparison of the families of I_{Na} recorded in the absence of and in the presence of 100 μM metoprolol indicates that, when virtually all Na channels are available (*i.e.*, the steady-state inactivation parameter, h_∞, is equal to 1.0 due to V_h being −140 mV), metoprolol has no effect on I_{Na} even when pulsing rates as high as 5 Hz were used; neither peak or steady-state I_{Na}, time course of the current or its voltage-dependence were changed by the drug. When some portions of the channels were inactivated (*i.e.*, h_∞ was somewhat less than 1.0 because V_h was −110 mV), although metoprolol did not alter the voltage-dependence or inactivation of I_{Na} using slow pulsing rates (*e.g.*, 0.2 Hz), during use of more rapid pulsing rates (1, 2 or 5 Hz), metoprolol could cause use-dependent block of I_{Na}. However, even when V_h was −110 mV, the findings suggest metoprolol does not cause tonic block of I_{Na} nor did it alter the time course of I_{Na} evoked at V_m between −60 and ~+50 mV. The role of the V_h in the development of use-dependent block of I_{Na}, in the rate-dependence of its development and in the apparent time-dependence of the recovery from block, suggests that metoprolol-induced use-dependent block may be mediated through an affinity of the drug for an inactivated state of the Na channel. The results also imply that metoprolol, like many *beta* blocking agents (Koch-Weser and Frishman, 1981), at sufficiently high concentrations, can exhibit at least weak local anesthetic properties reminiscent of those of lidocaine (Clarkson *et al.*, 1988).

In cat ventricular myocytes I_{Kl} appears to be the component

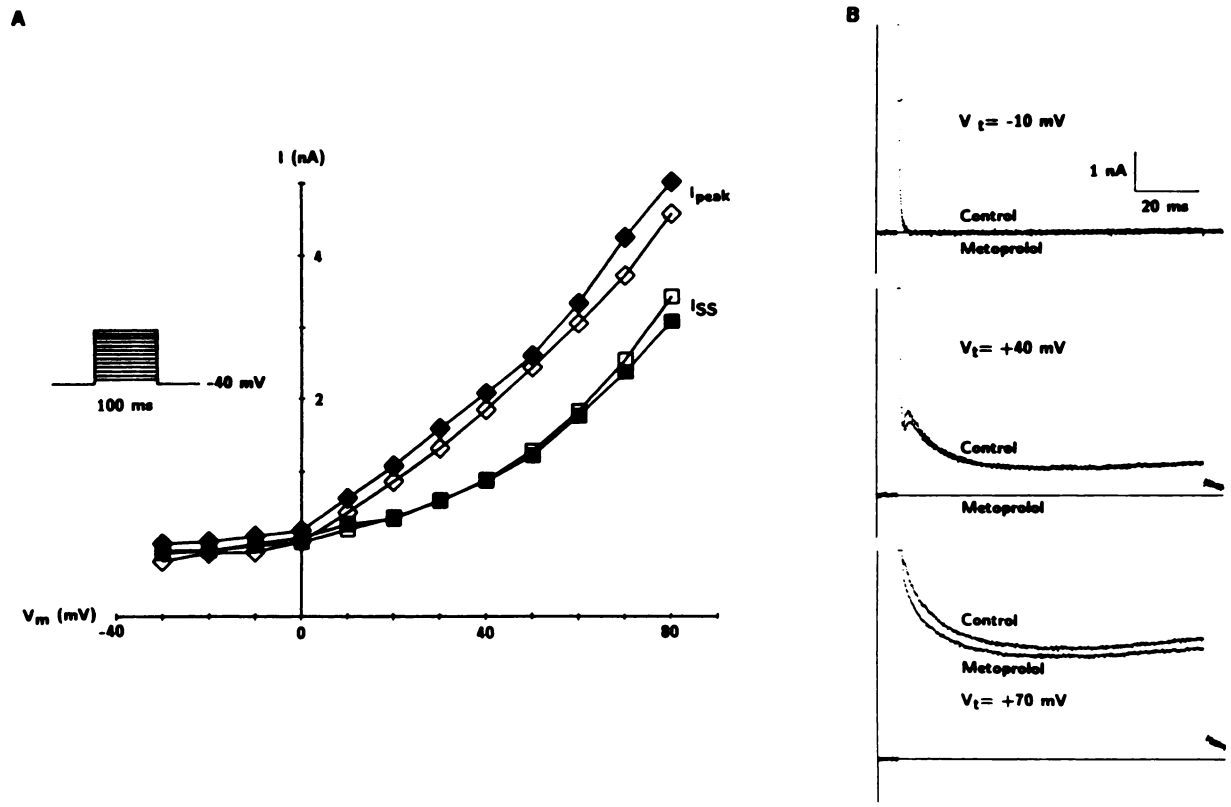

Fig. 6. Effect of 10 μM metoprolol on I_{to}. A: I-V relationship for the peak current (◇, ◆) is reduced at test potentials positive to 30 mV during exposure to metoprolol (filled symbols) relative to the currents during control (unfilled symbols). The steady-state current (□, ■) remained unchanged from −30 to +50 mV. B: currents paired at the same test potentials during control and exposure to metoprolol. The effects of metoprolol are most apparent during strong depolarizing test pulses. Plot is representative of 5 of 5 cells.

of the whole-cell membrane current most sensitive to metoprolol. This membrane effect was significant at a concentration of 10 μM, a level that is 30 to 60 times the plasma level for effecting *beta* blockade in humans (50–100 ng/ml; Marchlinski *et al.*, 1984; Rizos *et al.*, 1984; Escoubet *et al.*, 1986). Both block and unblock of I_{K1} appear to be voltage-dependent. Block of peak I_{K1} was maximal at ~E_K with less block at more positive and negative potentials. Because of the strongly inwardly rectifying nature of I_{K1} with very little outward current flow at V_m positive to E_K, the consequences of metoprolol-induced I_{K1} blockade on physiologically relevant, normal cellular electrophysiological activity in cat myocytes will be slight and appreciated only, if at all, in well polarized tissue. This prediction is verified by the fact that metoprolol had virtually no effect on the resting potential while at the same time causing a marked change in the plateau of the action potential (fig. 1A). If, on the other hand, metoprolol were applied to a myocyte (obtained from another species) capable of generating significant outward I_{K1} at V_m positive to E_K, the I_{K1} blocking action of the drug might then alter resting potential and the time course of late repolarization. In contrast to the effect on I_{K1} seen at V_m negative to V_{K1}, no evidence was obtained from either action potentials (fig. 1A) or from voltage-clamp data (fig. 4A), suggesting that the very small outward background current seen at V_m positive to the I_{K1} reversal potential can be blocked by metoprolol.

The voltage-dependence of unblock is reflected in the altered time course of I_{K1} during the latter portion of 100 msec duration hyperpolarizing pulses to V_m at which a prominent degree of I_{K1} inactivation can usually be observed. Normally, I_{K1} exhibits time- and voltage-dependent inactivation at voltages negative to −130 mV (Harvey and Ten Eick, 1988). In the presence of metoprolol the current appeared to inactivate, but about 20 to 30 msec after the onset of a pulse to −150 mV, the inward current slowly increased. With long test pulses the steady-state level in the presence of drug approached the steady-state level observed in the absence of metoprolol. This slowly developing recovery of current toward the control steady-state level could indicate either that unblock occurs during the hyperpolarizing pulse or that the drug reverses (or inhibits) the normal Na^+-dependent inactivation process (Biermans *et al.*, 1988; Harvey and Ten Eick, 1988). Because the steady-state current during hyperpolarizing pulses of 400 msec or longer could return *to* but *not* beyond the control steady-state level, the best explanation for the droop in current is that a time- and voltage-dependent relief of the metoprolol-induced block underlies the return of steady-state I_{K1} to control levels. Scholtysik *et al.* (1988) reported that I_{K1} in guinea pig ventricular myocytes exposed to the 5-hydroxytryptamine antagonist, ICS 205-930, developed an inward droop which began shortly after pulse onset even when V_t was to levels of V_m at which inactivation is not a feature of the current's time course. They concluded that the mechanism of their slowly developing outward current also involved hyperpolarization-induced unblock. The fact that they observed droop during hyperpolarizations to V_m as low as −80 mV, whereas we did not detect droop until hyperpolarizing to at least −130 mV (and at such V_m, the metoprolol-induced droop, which appeared after ~20 msec, was superimposed on the inactivation phase of I_{K1}), suggests that the nature of the

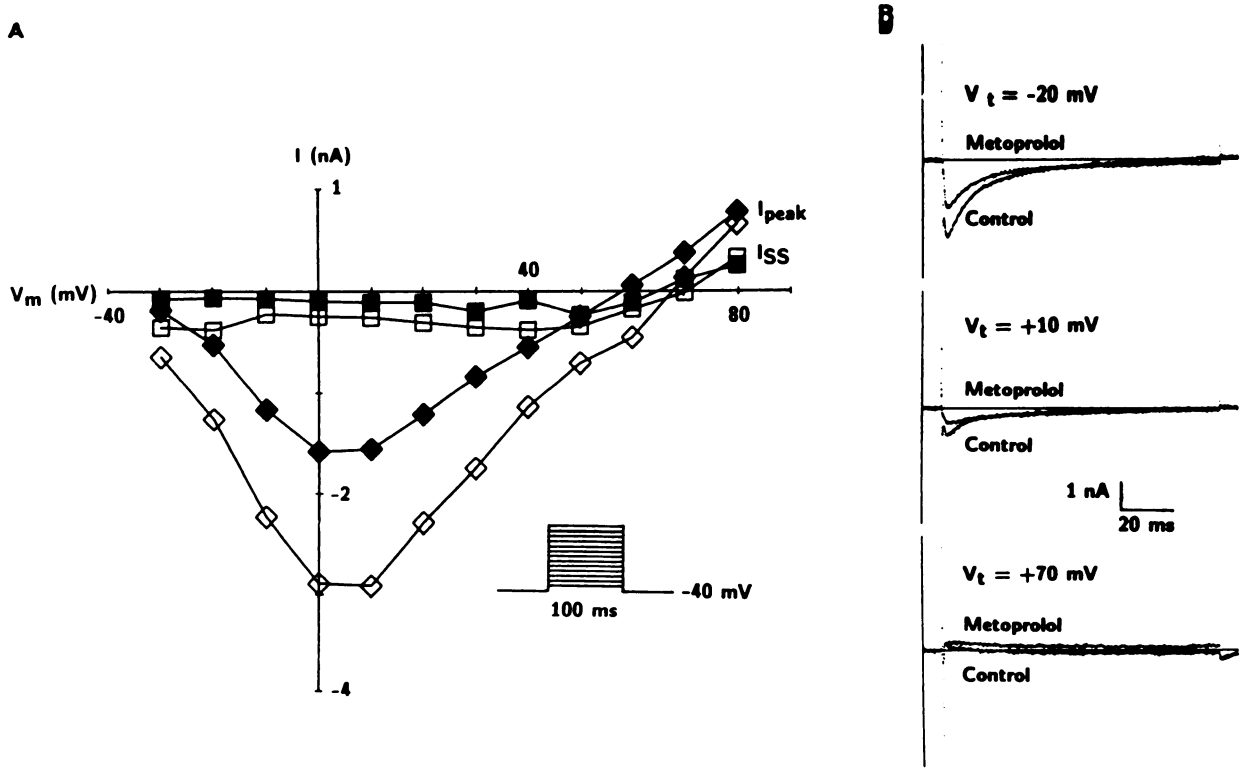

Fig. 7. Effect of 10 μM metoprolol on the Cd^{++}-sensitive current, I_{Ca}. A: I-V relationship for the peak current (◇, ◆) is reduced between −30 and +60 mV. In this cell the steady-state current (□, ■) does not appear to be effected by metoprolol (filled symbols) relative to the currents during control (unfilled symbols). I_{Ca} is determined by subtracting the Cd^{++}-resistant current (I_{to}) from the total membrane current. B: currents paired at the same test potentials during control and exposure to metoprolol. The effect of metoprolol is apparent over a range of test potentials between −30 and +70 mV. Plot is representative of 5 of 5 cells.

voltage dependency of unblock with metoprolol is quite different from that with ICS 205-930.

The whole-cell current evoked by depolarizing steps from a V_h of −40 mV was reduced at all voltages positive to −40 mV in the presence of 100 μM metoprolol (see fig. 1B). When the whole-cell currents were separated into their Cd^{++}-sensitive and -insensitive components, Cd^{++} (0.5 mM) being used to remove I_{Ca}, about one-half of the cat ventricular myocytes were found to lack measurable Ca^{++}-independent I_{to}. However, in cells exhibiting I_{to} in the form of Cd^{++}-insensitive current, 10 and 100 μM caused a concentration-dependent small but readily detectable decrease in the peak current but failed to alter the steady-state outward current observed during depolarizing pulses positive to −40 mV to any appreciable extent. This finding is interpreted to indicate that metoprolol can at least partially block the conductance for I_{to}, but does not alter the conductances of channels underlying the steady-state outward current. These unaffected channels presumably would include those contributing to the late time-dependent outwardly rectifying K^+ current (I_K), the time-independent voltage-dependent K^+ current (I_{Kp}; see Yue and Marban, 1988), and any nonspecific channel(s) contributing to the leak current flowing at V_m positive to −40 mV. With the present data we are unable to assess the efficacy of metoprolol to block I_{to}. We can only say that the threshold concentration for a blocking action on I_{to} was >1 μM and <10 μM and that the extent of the blockade in 100 μM was less than twice that in 10 μM, suggesting that metoprolol may be only a modestly efficacious blocker of I_{to}. This notion is supported by the finding that phase 1 of the myocyte action potential shown in figure 1A was altered only slightly by a concentration of metoprolol which caused a marked change in plateau duration and voltage, despite the fact that the threshold concentrations for the blocking effect on I_{to} and I_{Ca} appear to be in similar ranges (~1–10 μM). When I_{to} did not contribute appreciably to the composite whole-cell current, 10 to 100 μM metoprolol shifted the level of the steady-state current in the outward direction. This finding can be interpreted to mean that the drug inhibits noninactivating L channel I_{Ca} flowing at V_m positive to −40 mV.

All myocytes exhibited measurable I_{Ca}. The effect of metoprolol on the family of Cd^{++}-sensitive I_{Ca} used to assess the effect on the peak I_{Ca} *vs.* V_m curve suggests that, although metoprolol can block the whole-cell conductance for I_{Ca}, it has no significant effect on the voltage-dependence of I_{Ca} or on its time course. Single channel studies will be required to determine if the nature of the drug-channel interaction underlying the inhibition of I_{Ca} is a simple plugging of the channel or a modification of the predominant mode of channel operation from mode 1 to mode 0 (Hess *et al.*, 1983). Although we cannot account quantitatively for the extent to which I_{Ca} run down contributes to the decrease in I_{Ca} seen in the presence of metoprolol, it should be stated that with our experimental conditions, I_{Ca} was still measurable after 40 to 60 min, indicating that the rate of run down was relatively slow. This slow rate of run down enabled the I_{Ca} component of the whole-cell current evoked at +10 mV in a cell lacking Cd^{++}-insensitive I_{to} to recover at least partially from the blocking action of metoprolol on the current. The effects of the drug on the action potential plateau also were reversed by drug washout, a finding which also supports the conclusion that metoprolol can inhibit

I_{Ca} in cat ventricular myocytes. The possibility that the observed inhibition of I_{Ca} resulted solely from *beta* blockade and not from a direct effect on Ca channels cannot be ruled out. However, this possibility would seem remote because the experiments were done using solutions which were completely free of *beta* agonists. Even if *beta* agonist were present, its enhancing effect on I_{Ca} would have been abolished at drug concentrations almost two orders of magnitude less than were required to demonstrate the direct effect of metoprolol on Ca channels with voltage-clamp experiments.

The changes in the myocyte's evoked action potential suggest that an important, if not the most important, direct membrane effect of metoprolol, from the physiological point of view, may be the effect to inhibit I_{Ca}. The shortening of the action potential duration and the depression of the plateau voltage in the absence of any other detectable changes can be conveniently and easily explained only by postulating an inhibition of I_{Ca}. It is not unreasonable to think that a Ca channel blocking action may contribute importantly to metoprolol's reported ability to suppress several types of cardiac rhythm disturbances clinically, which does not appear to be explained on the basis of *beta* blockade.

The present study indicates that metoprolol (10 μM) can decrease action potential duration while inhibiting I_{Kl}, I_{Ca} and the Ca^{++}-independent I_{to}. Although it is conceivable that such alterations in these three components of the whole-cell membrane current could contribute to the changes in action potential configuration and duration typical of class 3 antiarrhythmic agents, their combined effect in cat ventricular myocytes apparently does not cause plateau duration to prolong (see fig. 1A). Therefore, the present results are inconsistent with the notion that the rate-corrected Q-T interval is prolonged by metoprolol administered clinically because the drug exhibits class 3 antiarrhythmic drug activity. However, had a Ca^{++}-dependent I_{to} been permitted to flow (*i.e.*, had ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid, more commonly known as EGTA, been absent from the internal solution), it is conceivable that inhibition of such a current might have at least partially offset the impact of blocking I_{Ca} on the duration of the action potential, or even have caused the duration of the action potential to prolong. It is also conceivable that in cardiac myocytes from other species, metoprolol could cause the action potential duration to prolong, particularly if it were a species (*e.g.*, guinea pig) exhibiting substantial outward flowing I_{Kl} at V_m positive to V_{Kl}. It may be that expression of class 1 and/or class 3 antiarrhythmic drug properties by a given compound is as dependent on the "experimental model" as it is on the particular channel blocking properties of the drug.

At higher concentrations than required to obtain substantial block of I_{Kl} and even of I_{Ca} (*i.e.*, 100 *vs.* 10 μM), metoprolol produces a weak frequency-dependent blockade of I_{Na}, implying weak local anesthetic activity and class 1 effects. However, the greater sensitivity of I_{Kl} to concentrations which have only small inhibitory actions on either I_{to} or I_{Ca}, and which do not even slightly inhibit I_{Na} (even at high pulsing rates), suggests that any effects of metoprolol administered clinically which are manifest in the electrocardiogram of humans (or other mammals), probably arise as result of the effects of the drug to: 1) produce *beta* adrenergic receptor blockade and 2) inhibit I_{Kl}. Because the primary direct electrophysiologic effects of metoprolol on the heart are expected to be mediated *via* an effect on I_{Kl} rather than on I_{Na}, metoprolol is quite different from the original prototype for class 2 drugs, propranolol, in terms of its spectrum of activity when used in clinically relevant concentrations.

References

BIERMANS, G., VEREEKE, J. AND CARMELIET, E.: The mechanism of the inactivation of the inward rectifying K current during hyperpolarizing steps in guinea pig ventricular myocytes. Pfluegers Arch. **410:** 604–613, 1988.

CAMM, A. J., WARD, D. E. AND WHITMARSH, V. B.: The acute electrophysiological effects of intravenous metoprolol. Clin. Cardiol. **5:** 327–331, 1982.

CLARKSON, C. W., FOLLMER, C. H. YEH, J. Z., TEN EICK, R. E. AND HONDEGHEM, L. M.: Evidence for open channel block of cardiac Na current by lidocaine in cardiac myocytes. Circ. Res. **63:** 869–878, 1988.

COBBE, S. M., HOFFMAM, E., RITZENHOFF, A., BRACHMANN, J., KUBLER, W. AND SENGES, J.: Action of sotalol on potential reentrant pathways and ventricular tachyarrhythmias in conscious dogs in the late post myocardial infarction phase. Circulation **68:** 865–871, 1983.

EDVARDSSON, N., HIRSCH, I. AND OLSSON, S. B.: Acute effects of lignocaine, procainamide, metoprolol, digoxin and atropine on human myocardial refractoriness. Cardiovasc. Res. **18:** 463–470, 1984.

EDVARDSSON, N. AND OLSSON, S. B.: Effects of acute and chronic beta-receptor blockade on ventricular repolarization in man. Br. Heart J. **45:** 628–636, 1981.

EDVARDSSON, N. AND OLSSON, S. B.: Induction of delayed repolarization during chronic beta-receptor blockade. Eur. Heart J. **6:** (suppl.) 163–169, 1985.

ESCOUBET, B., LECLERCQ, J. F., MAISON-BLANCHE, P., POIRIER, J. M., GOURMEL, B., DELHOTAL-LANDES, B. AND COUMEL, P.: Comparison of four beta-blockers as assessed by 24-hour ECG recording. Clin. Pharmacol. Ther. **39:** 361–368, 1986.

FOLLMER, C. H., TEN EICK, R. E. AND YEH, J. Z.: Sodium current kinetics in cat atrial myocytes. J. Physiol. **384:** 196–197, 1987.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. AND SIGWORTH, F. J.: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfluegers Arch. **391:** 85–100, 1981.

HARVEY, R. D. AND TEN EICK, R. E.: Characterization of the inward-rectifying potassium current in cat ventricular myocytes. J. Gen. Physiol. **91:** 593–615, 1988.

HARVEY, R. D. AND TEN EICK, R. E.: On the role of sodium ions in the regulation of the inward rectifying potassium conductance in cat ventricular myocytes. J. Gen. Physiol. **94:** 329–348, 1989a.

HARVEY, R. D. AND TEN EICK, R. E.: Voltage-dependent block of inward rectifying K^+ current by monovalent cations in cat ventricular myocytes. J. Gen. Physiol. **94:** 349–361, 1989b.

HESS, P., LANSMAN, J. B. AND TSIEN, R. W.: Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. Nature (Lond.) **311:** 538–544, 1983.

KOCH-WESER, J.: Drug therapy. Metoprolol. N Engl. J. Med. **301:** 698–703, 1979.

KOCH-WESER, J. AND FRISHMAN, W. H.: Drug therapy. β-Adrenoceptor Antagonists: New drugs and new indications. N Engl. J. Med. **305:** 500–506, 1981.

KOSTYUK, P. G., KRISHTAL, O. A. AND PIDOPLICHKO, V. I.: Asymmetrical displacement currents in nerve cell membrane and effect of internal fluoride. Nature (Lond.) **267:** 70–72, 1977.

MARCHLINSKI, F. E., BUXTON, A. E., WAXMAN, H. L. AND JOSEPHSON, M. E.: Electrophysiologic effects of intravenous metoprolol. Am. Heart. J. **107:** 1125–1131, 1984.

MOGUL, D. J., RASMUSSEN, H. H., SINGER, D. H. AND TEN EICK, R. E.: Inhibition of Na, K-pump current in guinea pig ventricular myocytes by dihydro-ouabain occurs at high and low affinity sites. Circ. Res. **64:** 1063–1069, 1989a.

MOGUL, D. J., SINGER, D. H. AND TEN EICK, R. E.: Ionic diffusion in voltage-clamped isolated cardiac myocytes: Implications for Na,K-pump studies. Biophys. J. **56:** 565–577, 1989b.

NARAHASHI, T., TSUNOO, A. AND YOSHII, M.: Characterization of two types of calcium channels in neuroblastoma cells. J. Physiol. (Lond.) **383:** 231–249, 1986.

RAINE, A. E. G., VAUGHAN WILLIAMS, E. M.: Adaptation to prolonged β-blockade of rabbit atrial, Purkinje, and ventricular potentials, and of papillary muscle contraction. Time-course of development of and recovery from adaptation. Circ. Res. **48:** 804–812, 1981.

RIZOS, J., SENGES, J., JAUERNIG, R., LENGFELDER, W., CZYGAN, E., BRACHMANN, J. AND KUBLER, W.: Differential effects of sotalol and metoprolol on induction of paroxysmal supraventricular tachycardia. Am. J. Cardiol. **53:** 1022–1027, 1984.

RIZZON, P., DI BIASE, M., CHIDDO, A., MASTRANGELO, D. AND SORGENTE, L.: Electrophysiological properties of intravenous metoprolol in man. Br. Heart. J. **40:** 650–655, 1978.

SCHOLTYSIK, G., IMOTO, Y., YATANI, A. AND BROWN, A. M.: 5-Hydroxytryptamine antagonist ICS 205-930 blocks cardiac potassium, sodium and calcium currents. J. Pharmacol. Exper. Ther. **245:** 773–778, 1988.

SILVER, H. L., HEMWALL, E. L., MARINO, T. A. AND HOUSER, S. R.: Isolation and morphology of calcium-tolerant feline ventricular myocytes. Am. J. Physiol. **245:** H891–H896, 1983.

VAUGHAN WILLIAMS, E. M.: Classification of antiarrhythmic drugs. Pharmacol. Ther. **1:** 115–138, 1975.

YEH, J. Z. AND TEN EICK, R. E.: Molecular and structural basis of resting and use-dependent block of sodium current defined using disopyramide analogues. Biophys. J. **51:** 123–135, 1987.

YUE, D. T. AND MARBAN, E.: A novel cardiac potassium channel that is active and conductive at depolarized potentials. Pfluegers Arch. **413:** 127–133, 1988.

Send reprint requests to: Dr. Peggy L. Barrington, Department of Pharmacology, Northwestern University, 303 E. Chicago Ave., Chicago, IL 60611.