Electrophysiological and Beta-Receptor
Blocking Effects of MJ 1999 on Dog
and Rabbit Cardiac Tissue

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ABSTRACT

Effects of MJ 1999 (1 X 10^-6 M to 1 X 10^-2 M) on rabbit right atrial and
canine Purkinje fiber preparations were studied. MJ 1999 had a pA2 of 6.1
against the chronotropic effect of isoproterenol on rabbit SA node. Significant
slowing first occurred with MJ 1999 at 1 X 10^-6 M. The spontaneous firing rate
of Purkinje fibers in vitro did not change significantly after exposure to MJ
1999 1 X 10^-6 M. Idioventricular rate was slowed and idioventricular escape
time was prolonged in dogs with atrioventricular block after MJ 1999, 2.0
mg/kg. MJ 1999, 1 X 10^-7 M to 5 X 10^-7 M, had no effect on resting potential,
overshoot and amplitude of phase 0 of transmembrane potentials (TMP)
recorded from canine ventricular muscle, canine Purkinje fibers or rabbit atrial
fibers nor did it affect phase 0 Vmax of TMP recorded from the Purkinje or
atrial fibers. Similar concentrations of MJ 1999 had no effect on membrane
responsiveness in Purkinje fibers. In Purkinje fibers, control action potential
duration (APD) was 325 msec and the effective refractory period (ERP) 277
msec. MJ 1999, 1 X 10^-6 M, increased the APD to 470 msec and ERP to 404
msec. In ventricular muscle fibers, APD was 230 msec and the ERP was 223
msec, under control conditions. MJ 1999, 1 X 10^-6 M, increased the APD to 281
msec and ERP to 267 msec. Changes in APD and ERP induced by MJ 1999
were magnified at slower rates of stimulation. The effects of MJ 1999 on both
APD and ERP were more marked in Purkinje fibers than in ventricular muscle
fibers. The effects of MJ 1999 on phase 0 of the TMP and on repolarization and
refractoriness of the TMP differ markedly from those seen with quinidine,
procainamide, and lidocaine.

ADDITIONAL KEY WORDS transmembrane potentials automaticity
isolated rabbit atria isoproterenol action potential duration
papillary muscle-Purkinje fiber preparations effective refractory period
membrane responsiveness

■ Recent interest in the use of β-receptor
blocking drugs for treatment of angina
pectoris and cardiac arrhythmias has stimu-
lated a search for more selective β-receptor
blocking agents without undesirable side
effects. MJ 1999 (Sotalol) has recently been
introduced and is claimed to possess β-

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receptor blocking properties in the absence of local anesthetic effects or major myocardial depressant effects (1-12). Recent studies on the dextro- and levo-isomers of pronethalol and propranolol have suggested that their protection against ouabain-induced arrhythmias and arrhythmias induced by hydrocarbon-epinephrine is not solely dependent on their β-receptor blocking properties (13, 14). However, it is not clear to what extent the β-receptor blocking effects of these drugs contribute to their antiarrhythmic action. Thus, electrophysiologic studies utilizing an agent without local anesthetic properties might provide further information on whether the antiarrhythmic properties of other β-receptor blocking agents are due solely to the effects of β-receptor blockade or due to a summation of β-receptor blockade and “local anesthetic-membrane properties.”

Study of a β-receptor blocking agent lacking “local anesthetic-membrane properties” could provide the basis for a further understanding of the β-receptor effects of catecholamines on the electrophysiological properties of cardiac tissue.

Although β-receptor blockers share a common property of inhibiting certain effects of catecholamines, they may have different electrophysiological effects, as has been shown for pronethalol and propranolol (15, 16), and different effective therapeutic ratios (17). These differences necessitate an examination of the properties of each β-receptor blocking agent so that its effects as a β-receptor blocker and antiarrhythmic agent can be meaningfully compared. In the present study the strength of β-receptor blockade by MJ 1999 was assessed to permit comparison of the electrophysiological actions of this agent with other β-receptor antagonists at concentrations causing comparable β-receptor blockade.

In this study MJ 1999 is shown to: (1) be an effective β-receptor antagonist in our biological test system and (2) markedly alter transmembrane action potentials recorded from canine Purkinje and ventricular muscle fibers. At concentrations producing effective β-receptor blockade, MJ 1999 shows striking qualitative differences from propranolol in its effect on the action potentials recorded from these two cell types. In addition, by closely examining the influences of MJ 1999 on the relationship between the effective refractory period and the duration of action potential and comparing these effects with those caused by lidocaine and diphenylhydantoin (18-20), we have attempted to provide further insight into the mechanism of antiarrhythmic action of other cardioactive agents.

Methods

IN-VITRO STUDIES

Mongrel dogs (10 to 15 kg) were anesthetized with pentobarbital sodium (25 to 35 mg/kg iv) and rabbits (1.8 to 2.5 kg) were stunned by a blow to the head. Their hearts were quickly excised and dissected in cool modified Tyrode’s solution, the composition of which has been previously described (21). Preparations of canine false tendon (Purkinje fiber) and ventricular muscle, and rabbit right atrial preparations, were then pinned to the wax-lined bottom of a 30-ml lucite tissue chamber. Oxygenation and control of pH was achieved by bubbling the Tyrode solution in the reservoir bottles and in the tissue bath with a mixture of 95% O2 — 5% CO2. The false tendon and ventricular muscle preparations were usually maintained at 37.0°C ± 0.5°C and the rabbit right atrial preparations were maintained at 35.5°C ± 0.5°C, unless otherwise specified.

Stimuli were provided by a series of waveform and pulse generators (Tektronix), as previously described (21). The membrane responsiveness of the Purkinje fiber and the effective refractory period of both Purkinje and ventricular muscle fibers (21) were determined by stimulating the tissue with a drive pulse (S1) at a constant rate and placing the test pulses (S2) at desired S1-S2 intervals in every eighth cycle, as shown in Figure 8. Membrane responsiveness, effective refractory period, and action-potential duration which have been previously defined (21) were determined before and after exposure to the drugs and during sustained impalement of a single fiber.

Transmembrane voltage was recorded through glass microelectrodes filled with 3M KCl and having resistances of 15 to 35 megohms. Transmembrane voltage was amplified with high input impedance, capacity neutralizing amplifiers (Bioelectric Instruments, Inc., NF-1) and displayed on a dual-beam cathode ray oscilloscope (Tektronix, RM-565). Surface electrograms were recorded through Teflon-coated silver wire electrode pairs and displayed on the dual-beam oscilloscope. The peak Vmax of phase 0 of the
transmembrane action potential was obtained by electronic differentiation as previously described (21). Time marks provided by a time mark generator (Tektronix type 184) were also displayed on the oscilloscope trace. The oscilloscope trace was photographed on 35 mm film, the film was enlarged and measurements made from the magnified images.

For analysis of the rate of sinoatrial (SA) node, bipolar surface electrograms were recorded from the crista terminalis, and transmembrane potentials obtained with microelectrodes were recorded from the SA node of spontaneously beating rabbit right atrial preparations. The average duration of 200 consecutive spontaneous cycles was obtained and expressed to the nearest 0.1 msec as previously described (21). Results were sampled under control conditions and following exposure to isoproterenol and MJ 1999. The effects of isoproterenol were tested in the absence and in the presence of graded concentrations of MJ 1999 to provide a measure of drug antagonism; the ability of MJ 1999 to antagonize isoproterenol was expressed as its pA2 value. The pA2 is defined as the negative base 10 logarithm of the molar concentration of antagonist (MJ 1999) which doubles the dose of agonist (isoproterenol) required to produce a given effect (22, 23).

Drugs were added to the Tyrode solution in reservoir bottles to provide the desired final concentrations. Records were obtained from the tissues under control conditions and after effects produced by the test solution(s) had stabilized. Drugs used were MJ 1999 [4-(2-isopropylamino-1-hydroxyethyl) methane sulfonamid] (1 × 10^-8M to 1 × 10^-2M) and isoproterenol HCl (1 × 10^-10M to 1 × 10^-3M).

IN-VIVO STUDIES

Mongrel dogs (15 to 25 kg) anesthetized with pentobarbital sodium had atrioventricular block created in a manner previously described (24). Immediately following this operative procedure or several weeks later, a bipolar catheter was passed into the right ventricle for endocardial stimulation at a rate of 100 beats/min, using a battery-powered pacemaker (Medtronix, Inc.). Following right ventricular stimulation for 120 seconds, the escape time, i.e., the interval between the last driven beat and the first ventricular escape beat, was determined. Idioventricular rates and escape times following pacing for 120 seconds were

![Figure 1](image_url)

**Figure 1**

*Effect of MJ 1999 on the cumulative concentration effect curves of isoproterenol on isolated SA node of rabbit. Change in heart rate is plotted as percent of maximal effect on the ordinate. The negative common logarithm of the molar concentration of isoproterenol is plotted on the abscissa. Solid circles represent values during first exposure of SA node to increasing concentrations of isoproterenol. Values during a second exposure to isoproterenol are shown in the presence of different concentrations of MJ 1999. Each of the plotted points in the first curve represents the mean of values from 20 different experiments; and each plotted point in each subsequent curve represents the mean of values from five different experiments. After initial application of isoproterenol, the tissue was perfused with drug free Tyrode’s solution for 45 minutes and then exposed to one selected concentration of MJ 1999 for 45 minutes before the second isoproterenol exposure.*
determined under control conditions and following exposure to increasing doses of MJ 1999 (0.02 to 2.0 mg/kg).

**Results**

**ADRENERGIC BLOCKING ACTION**

The effect of MJ 1999 (1 x 10^-5 M to 1 x 10^-3 M) on the cumulative concentration-effect curve for isoproterenol was evaluated in 20 rabbit sinoatrial preparations (Fig. 1). In each experiment, a control concentration-effect curve for the chronotropic action of isoproterenol was obtained. Then, during exposure to a fixed concentration of MJ 1999, the preparation was reexposed to isoproterenol. Only one concentration of MJ 1999 was used in each experiment. During exposure to MJ 1999, the curves were shifted to the right in a nearly parallel fashion and there was no depression of the maximum response indicating that the antagonism of MJ 1999 for isoproterenol is wholly surmountable (entirely competitive). The pA2 was obtained by calculating the ratio of concentrations of isoproterenol required to produce a half maximal effect in the presence and absence of MJ 1999 (Fig. 2). The pA2 for MJ 1999, in terms of isoproterenol's chronotropic effect, was 6.1 for rabbit SA node at 35.5°C. When the log (concentration ratio -1) was plotted against the negative logarithm of the molar concentration of the antagonist, the slope of the line was close to 1, indicating that the antagonism is almost entirely of a simple competitive type (23, 25, 26).

**AUTOMATICITY**

**Sinoatrial Node.**—The effect of MJ 1999 on the rate of isolated SA node of rabbit was evaluated in six experiments (Fig. 3). In these experiments the concentration of MJ 1999 was increased in steps from 1 x 10^-5 M to 1 x 10^-3 M and rate of SA node was determined 40 minutes after exposure to each concentration. Under control conditions rate of SA node was 139 ± 11 beats/min (mean ± SE). No significant change in rate occurred
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FIGURE 4
Effects of MJ 1999 (10^{-5}M and 10^{-3}M) on transmembrane potentials recorded from a single canine Purkinje fiber stimulated every 800 msec. Time marks are repeated every 100 msec in the upper trace of each panel. The middle trace of each panel shows the transmembrane potentials and the bottom trace shows the calibrating sawtooth pulses and its first time derivative, and the first time derivative (peak V_{max}) of phase 0 of the action potential (17). The amplitude of the V of the calibrating pulse represents 500 mV/sec. The resting potential, peak V_{max} of phase 0, duration and amplitude of the action potential were assessed under control conditions (A); 40 minutes after exposure to MJ 1999, 10^{-5}M (B); 40 minutes after exposure to MJ 1999, 10^{-4}M (C); and 40 minutes after addition of isoproterenol, 3 \times 10^{-7}M, to the MJ 1999, 10^{-5}M (D). Exposure to MJ 1999 alone or MJ 1999 and isoproterenol did not alter the resting potential, amplitude and peak V_{max} of phase 0 of the action potential recorded from Purkinje fiber. Action potential duration was prolonged from 305 msec (control) to 321 msec (10^{-5}M) and to 368 msec (10^{-4}M). This change resulted from prolongation of phase 2 and decrease in the slope of phase 3, particularly the terminal part of phase 3 (C). Once a steady state was achieved with MJ 1999 (10^{-5}M) the addition of isoproterenol (3 \times 10^{-7}M) caused only a further prolongation of the action potential from 368 msec to 400 msec, primarily due to a decrease in the slope of phase 3 of the action potential. P.F. = Purkinje fibers.

until concentrations greater than 1 \times 10^{-5}M were used. The maximum decrease in rate was seen at 1 \times 10^{-5}M, where the rate was reduced by 32% to 95 \pm 4 beats/min (P < 0.005). In two experiments MJ 1999 (1 \times 10^{-5}M) was added to the perfusate immediately after the control rate of SA node had been recorded; the slowing was the same as that described above, indicating that the decrease in rate at this concentration was drug induced and not related to deterioration of the preparation with time. The decrease in rate seen at a concentration of 1 \times 10^{-4}M was stable for 2 hours. In four experiments carried out at 32.5 \pm 0.5°C and within a concentration range of 1 \times 10^{-5}M to 1 \times 10^{-3}M, sinus arrest was not demonstrated. In fact, the percent change in rate was similar to the rate change seen at the same concentration studied at 35.5 \pm 0.5°C.

Canine Purkinje Fiber.—The effect of MJ 1999 on automaticity of seven isolated preparations of Purkinje fiber was quite variable. The control rate was 39 \pm 6 beats/min (mean \pm se) and was essentially unchanged during exposure to MJ 1999 at a concentration of 1 \times 10^{-4}M (34 \pm 9 beats/min, P < 0.5). This concentration of MJ 1999 blocked the positive chronotropic effect of isoproterenol (3 \times 10^{-7}M), a concentration of isoproterenol that has been shown to reliably produce a positive chronotropic effect in isolated Purkinje fibers (27).

The effects of MJ 1999 on automaticity in vivo were assessed in four dogs with complete atrioventricular block. Both the idioventricular rate and escape time following stimulation of the ventricles at a rate of 100 beats/min were measured under control conditions. These measurements were repeated following administration of 0.02, 0.2, and 2.0 mg/kg doses of MJ 1999. Slight slowing of the idioventricular rate was seen at a dose of 2.0 mg/kg (from 44 \pm 7 beats/min to 33 \pm 6 beats/min, P < 0.025). At this 2.0 mg/kg dose the escape time increased from 12.6 \pm 5.4 seconds to 17.3 \pm 4.8 seconds. The smaller doses of MJ 1999 caused no change in the idioventricular rate or escape time.

EFFECTS OF MJ 1999 ON TRANSMEMBRANE ACTION POTENTIALS

Canine Purkinje Fiber.—In 11 experiments on Purkinje fibers, under control conditions at a cycle length of 800 msec resting potential was
92 ± 0.9 mv (mean ± sr.), amplitude of phase 0 was 126 ± 2.0 mv, overshoot was 34 ± 1.5 mv, and peak $V_{\text{max}}$ of phase 0 was 679 ± 53.8 v/sec. No significant change in these values occurred with concentrations of MJ 1999 between $1 \times 10^{-7}$M and $5 \times 10^{-4}$M (Figs. 4 and 5).

In contrast to its lack of effect on resting potential and phase 0, MJ 1999 increased duration of the action potential in concentrations of $1 \times 10^{-5}$M to $1 \times 10^{-3}$M (Figs. 4, 5 and 7). Under control conditions, duration of action potential was 325 ± 12.2 msec and increased to a maximum of 470 ± 14.3 msec during exposure to MJ 1999 $1 \times 10^{-3}$M. Prolongation of the action potential by MJ 1999 was due to prolongation of phases 2 and 3, and particularly the terminal part of repolarization (Figs. 5 and 12). MJ 1999's effects on the duration of action potential (at a constant cycle length) is dependent on concentration as shown in Figure 7. The effect of MJ 1999 on the duration of action potential of fibers driven at cycle lengths from 200 to 2000 msec is shown in Figure 6A; this figure

Effects of MJ 1999 on transmembrane potentials recorded from canine ventricular muscle and Purkinje fibers stimulated at a constant-drive cycle length of 850 msec. Time markers are seen every 100 msec (top traces); records of transmembrane potentials from canine Purkinje fibers and ventricular muscle fibers (second and third traces); V of the calibrating sawtooth pulse and the phase 0 of the Purkinje fiber action potential (bottom trace). Under control conditions ventricular muscle and Purkinje fiber action potentials were 220 msec and 310 msec, respectively. With increasing concentrations of MJ 1999, action potentials were prolonged; at a concentration of $5 \times 10^{-4}$M, the ventricular muscle and Purkinje fiber action potentials were 250 msec and 416 msec, respectively. The effects of this $\beta$-receptor blocker on duration of action potential were thus more pronounced in Purkinje fiber than in ventricular muscle fiber. MJ 1999 had no effect on resting potential, amplitude and peak $V_{\text{max}}$ of phase 0 of the Purkinje fiber action potential, from $1 \times 10^{-6}$M to $5 \times 10^{-4}$M. In addition, MJ 1999 ($5 \times 10^{-4}$M) in the voltage range of 55 to 70 mv decreased the slope of phase 3 of the action potential to about half the control value.
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FIGURE 6
Effect of MJ 1999 (1 × 10⁻⁷M to 5 × 10⁻³M) on rate-related changes in duration of action potential. Duration of action potential is plotted on the ordinate and the driven cycle length is shown on the abscissa. In A the relationship is plotted for Purkinje fiber and in B for ventricular muscle fiber. The data shown in this figure was obtained from one experiment and show that: (1) duration of action potential had a direct relationship with the concentration of MJ 1999; (2) the prolongation of the duration of action potential was more apparent the longer the drive cycle length, and (3) Purkinje fibers were more sensitive to the effects of MJ 1999 on duration of action potential than ventricular muscle fibers. Similar results were seen in five other experiments.

demonstrates that the absolute increase in duration of action potential is greater at slower rates of stimulation. In addition, when the effects of MJ 1999 became steady for a concentration of 1 × 10⁻⁴M, the addition of isoproterenol (3 × 10⁻³M) had no effect on resting potential and phase 0, but caused further prolongation of the action potential (Fig. 4).

Canine Ventricular Muscle Fiber.—Under control conditions in five experiments resting potential was 90 ± 1.2 mV, amplitude of phase 0 was 122 ± 3.0 mV, and overshoot 32 ± 2.4 mV. These variables were not significantly changed by concentrations of MJ 1999 between 1 × 10⁻⁷M and 5 × 10⁻³M (Fig. 5). Duration of action potential was increased to a lesser extent than in Purkinje fibers (Figs. 5-7). For example, at a cycle length of 1000 msec and a concentration of 5 × 10⁻³M, duration of the action potential of ventricular muscle increased by 15% while the Purkinje fiber duration of the action potential increased by 37%. Although the increase in duration of action potential is more apparent at slower rates of stimulation (Fig. 6, B) the magnitude of the changes in the duration of action potential at all cycle lengths were less marked than those seen in Purkinje fiber (Fig. 6, A).

Rabbit Atrial Muscle Fibers.—Under control conditions and at a cycle length of 800 msec, in five experiments resting potential was 74 ± 1.6 mV, the amplitude of phase 0 was 93 ± 1.8 mV, overshoot 19 ± 1.7 mV, phase 0 peak V_max was 195 ± 30.6 V/sec and the duration of action potential was 105 ± 6.3 msec. Concentrations of MJ 1999 between 1 × 10⁻⁷M and 5 × 10⁻⁴M did not significantly change any of these values.

EFFECT OF MJ 1999 ON EFFECTIVE REFRACTORY PERIOD

Canine Purkinje Fiber.—The effect of MJ 1999 (1 × 10⁻⁴M to 1 × 10⁻³M) on the effective refractory period was determined for five different Purkinje fibers stimulated at a cycle length of 800 msec (Figs. 8-10). Under control conditions the effective refractory period was 277 ± 7.1 msec. The lowest concentration causing a significant increase in effective refractory period was 1 × 10⁻³M (289 ± 5.2 msec, P < 0.01). At higher concen-
Effect of various concentrations of MJ 1999 on the duration of action potential of both ventricular muscle and Purkinje fibers. The negative log_{10} of the molar concentration of MJ 1999 is plotted on the abscissa and the duration of action potential in milliseconds is plotted on the ordinate. Values shown for Purkinje fiber (solid triangles) and for ventricular muscle fiber (solid squares) are expressed as the mean ± SE. Values to the left of the double vertical line were obtained under control conditions at a cycle length of 800 msec. Plots of points to the right of the double vertical lines were obtained during exposure to cumulative concentrations of MJ 1999. The data were obtained from 11 different experiments and show that: (1) change in duration of action potential was related to the concentration of MJ 1999, and (2) duration of action potential in Purkinje fibers was far more sensitive to the effects of MJ 1999 at all concentrations studied than was ventricular muscle fiber, as shown by sharper upswing of upper set of plotted points.

trations, the curve relating the effective refractory period to the MJ 1999 concentration rose sharply to a maximum of 404 ± 10.0 msec at a concentration of 1 × 10^{-4}M (the highest concentration studied).

A plot of the increase in the duration of action potential as a function of the increase in the effective refractory period caused by MJ 1999 is seen in Figure 9. A straight line would be expected if there were no shift in the relationship of the effective refractory period to the duration of action potential (17). The onset of a significant shift of the plotted points to the left of the line of identity was seen at 1 × 10^{-4}M.

Canine Ventricular Muscle Fiber.—The effects of MJ 1999 (1 × 10^{-6}M to 1 × 10^{-3}M) on the effective refractory period was evaluated in five different experiments on ventricular muscle fibers, each stimulated at a cycle length of 800 msec (Figs. 8-10). Under control conditions the effective refractory period was 233 ± 5.1 msec. The onset of a significant change in the effective refractory period was seen at a concentration of 1 × 10^{-4}M (effective refractory period 236 ± 5.2 msec, P < 0.05). The maximum effective refractory period was 267 ± 9.2 msec at a concentration of 1 × 10^{-3}M. The magnitude of the change in effective refractory period in ventricular muscle was less marked than in Purkinje fiber at all concentrations studied. In the plot of the change in duration of action potential as a function of the change in effective refractory period (Fig. 10) a significant shift of the plotted points to the left of the line of identity was seen beginning at a concentration of 1 × 10^{-4}M. Observations made during the determination of the effective refractory period of Purkinje fiber before and after exposure to MJ 1999 showed that it had no effect on the propagation of graded responses to test stimuli applied during phase 3, i.e., early graded responses were not aborted after exposure to this drug (see Fig. 8). Failure to prevent propagation of early graded responses would be predicted by the lengthening of the action potential relative to the effective refractory period as shown by the shift of the plotted points to the left of the line of identity in Figure 10. In two experiments, after MJ 1999 prolonged the duration of action potential, test responses, initiated at low transmembrane voltages did slowly propagate the length of the fiber whereas, propagated responses initiated at similar low transmembrane voltages did not occur under control conditions.

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Figure 8

Effect of MJ 1999 on the effective refractory period of canine ventricular muscle and Purkinje fibers. In top traces in each panel are time marks every 100 msec. The bottom two traces are records of transmembrane potentials obtained from ventricular muscle and Purkinje fibers. A simultaneous recording of surface electrical activity, obtained from a distal site on the Purkinje fiber, is shown in the second traces. First action potential in each panel is the last of a series of eight potentials occurring at a regular interval of 800 msec. Top panels: Premature action potentials shown are the earliest that could be elicited in the ventricular muscle fiber (effective refractory period) and do not traverse the Purkinje fiber as indicated by the absence of the local Purkinje action potential and distant Purkinje electrogram. Bottom panels: Earliest premature action potential obtained in Purkinje fiber (effective refractory period). That these premature responses slowly traverse the Purkinje fiber to the distal recording site is indicated by the electrogram recordings (arrows). E.R.P. = effective refractory period; V.M. = ventricular muscle; P.F. = Purkinje fiber.

EFFECT OF MJ 1999 ON MEMBRANE RESPONSIVENESS

The effects of MJ 1999 on the membrane responsiveness of Purkinje fibers were studied in six experiments. Membrane responsiveness may be defined as the relationship between transmembrane voltage at the time of activation and the Vmax of phase 0 of the resultant action potential (28). This relationship is usually characterized by a S-shaped curve (Fig. 11). MJ 1999, in concentrations up to 5 x 10^-4 M, did not depress membrane responsiveness. In some experiments, responses were obtained at lower transmembrane voltages during exposure to MJ 1999, so that the lower portion of the S-shaped curves were obtainable after but not before MJ 1999.

TOXIC EFFECTS

Toxic effects of MJ 1999 were seen at concentrations of 1 x 10^-5 M (Fig. 12). Short exposure to 1 x 10^-3 M suppressed phase 4 depolarization of the Purkinje fiber whose action potentials are shown in Figure 12, which caused the spontaneous cycle length to increase from 815 msec to 4,670 msec.

Transmembrane voltage at the time of spontaneous activation increased from 52 mv (control) to 71 mv resulting in an increased amplitude and Vmax of phase 0 of the action potential. At this time phases 2 and 3 were markedly prolonged, and particularly the terminal part of phase 3 which became a plateau. This plateau, which lasted 185 msec, started at 72 mv and gradually polarized to 82 mv, and finally membrane voltage quickly polarized to the maximum diastolic transmembrane voltage of 95 mv (Fig. 12, B). Later, repetitive depolarizations originated from this plateau before complete repolarization (at 71 to 73 mv). Repetitive firing occurred at a time when phase 4 depolarization remained suppressed. These repetitive depolarizations were not sustained indefinitely but terminated by repolarization (see Fig. 12, C and D). When toxicity became more advanced, small depolarizations were even superimposed on phases 2 and 3 of the repetitive depolarizations (see Fig. 12, D).

In two experiments, preparations depressed
by exposure to quinidine were washed in drug-free Tyrode's solution and allowed to recover for a period of 1½ hours. Subsequent exposure to MJ 1999 (1 x 10⁻⁴M) resulted in a rapid, marked deterioration of the fiber.

**Discussion**

The present study defined the strength and nature of the β-receptor blockade caused by MJ 1999. Using this information we then studied the effects of various β-receptor blocking concentrations of MJ 1999 on transmembrane potentials recorded from canine ventricular muscle and Purkinje fibers. These effects were compared both with those of other β-receptor blocking agents and with previously studied antiarrhythmic agents.

The strength and nature of MJ 1999 as a β-receptor antagonist was determined from the ability of MJ 1999 to antagonize the chronotropic effects of isoproterenol. Increasing concentrations of MJ 1999 had a progressive antagonistic effect on the chronotropic responses of the rabbit sinoatrial node to isoproterenol. This blockade was entirely surmountable. Based on the rate and occupation theories of drug antagonism (23, 25, 26), our data suggests that the blockade is of a simple competitive type. The potency of MJ 1999 as a β-receptor blocking agent is expressed by its pA₂ value of 6.1, determined by the technique described by Schild (22). Our pA₂ value is remarkably similar to the pA₂ values obtained with MJ 1999 by Blinks (17) using kitten atrium, and by Patil (29) using an isolated tracheal-chain preparation of guinea pig. Comparison of the pA₂ values of MJ 1999 (6.1) with those obtained for propranolol (8.7) and pronethalol (7.4) (17), indicates that MJ 1999 is less potent as a β-receptor blocking agent than either propranolol or pronethalol. Furthermore, the similarity of the pA₂ values in different test systems, for any of these β-receptor blocking agents provides a basis for comparing the effects of equipotent blocking concentrations of MJ 1999, propranolol, and pronethalol.

**EFFECTS ON ELECTROPHYSIOLOGICAL PROPERTIES**

The effects of this drug on automaticity were assessed in several test systems, both in vivo and in vitro. The effects of MJ 1999 on automaticity of the His-Purkinje system in vivo were assessed in four dogs with complete atrioventricular block. The effects of various doses of MJ 1999 were evaluated on the
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The change in duration of action potential is plotted in milliseconds on the ordinate, and the change in effective refractory period is plotted in milliseconds on the abscissa. Values were obtained from five different experiments and expressed as the mean ± SE (bars that are not shown are enclosed within the symbols). The data show that in both fiber types the change in duration of action potential increased more than the change in effective refractory period, causing the points to shift to the left of the line of identity. However, the first significant shift of the points off the line of identity appeared at a lower concentration of MJ 1999 in Purkinje fiber (1 X 10^{-5} M) than in ventricular muscle fiber (1 X 10^{-4} M).

\( \Delta \text{APD} = \text{change in duration of action potential} \)
\( \Delta \text{ERP} = \text{change in effective refractory period} \)

Effect of various concentrations of MJ 1999 on the plot of the increase in duration of action potential (change in duration of action potential) as a function of the increase in the effective refractory period (change in effective refractory period). The change in duration of action potential is plotted in milliseconds on the ordinate, and the change in effective refractory period is plotted in milliseconds on the abscissa. Values were obtained from five different experiments and expressed as the mean ± SE (bars that are not shown are enclosed within the symbols). The data show that in both fiber types the change in duration of action potential increased more than the change in effective refractory period, causing the points to shift to the left of the line of identity. However, the first significant shift of the points off the line of identity appeared at a lower concentration of MJ 1999 in Purkinje fiber (1 X 10^{-5} M) than in ventricular muscle fiber (1 X 10^{-4} M).

\( \Delta \text{APD} = \text{change in duration of action potential} \)
\( \Delta \text{ERP} = \text{change in effective refractory period} \)

idioventricular rate and escape time. Only doses of 2.0 mg/kg caused slight slowing of the idioventricular rate and slight prolongation of the escape time.

MJ 1999, 1 X 10^{-4} M, did not consistently slow spontaneously beating isolated Purkinje fibers. Because of its variable and inconsistent effects on automaticity of the isolated Purkinje fibers, the sinoatrial node was selected for a more detailed examination of the effects of MJ 1999 on automaticity. Spontaneously beating right atrial preparations of rabbit were significantly slowed only by concentrations greater than 1 X 10^{-4} M and sinus arrest was not seen even at a concentration of 1 X 10^{-5} M. Concentrations of MJ 1999 that did not have much direct effect on rate of SA node were able to block the chronotropic effect of catecholamines. Further, our results in spontaneously beating isolated preparations of rabbit atrium and canine Purkinje fiber suggest that spontaneous liberation of catecholamines is minimal.

A comparison of the data obtained by A. Morales-Aguilera and E. M. Vaughan Williams for propranolol, using spontaneously beating right atrial preparations of rabbit (30), and our data for MJ 1999 would suggest that propranolol and MJ 1999, in equipotent \( \beta \)-receptor blocking concentrations, have similar depressant effects on the rate of SA node. For example, propranolol (2 X 10^{-4} M) caused a 22% decrease in the rate of SA node while MJ 1999 (7 X 10^{-5} M) caused a 28% decrease in the rate of SA node. Results obtained in studies on rats (7), isolated perfused cat and rabbit hearts (2, 4), cats (31), dogs (1, 7, 32) and man (7) show that effective \( \beta \)-receptor...
Effect of MJ 1999 (5 × 10⁻⁴M) on the membrane responsiveness of a canine Purkinje fiber. The abscissa represents transmembrane potential at the time of excitation in millivolts and the resultant _V_₇₀ of phase 0 in volts/second (ordinate). The S-shaped curve was calculated according to the formula of Weidmann with:

\[
\text{peak } V_{\text{m}} = 70 \text{ mV}
\]

blocking doses of MJ 1999 cause only small decreases in heart rate. These observations are in accord with our findings for the direct effects of MJ 1999 on automaticity.

However, we did not find the narrow difference between the concentrations of MJ 1999 required to cause β-receptor blockade and significant slowing of rate in kitten atria studied at 32.5°C (17). In our experiments on rabbit SA node, significant slowing occurred only with concentrations greater than 1 × 10⁻⁴M. Thus, although the β-receptor blocking potency of MJ 1999 was similar in the two test systems, the onset of significant slowing in kitten atria occurred at a concentration one hundredfold less and the degree of slowing was greater than that seen in rabbit atria. To exclude temperature as the major factor accounting for the difference between the two studies, we performed four experiments on tissues at 32.5°C ± 0.5°C, but we were still unable to demonstrate significant slowing at concentrations lower than 1 × 10⁻⁴M.

For the present discussion we defined the effective therapeutic ratio as the difference in molar concentration between the pA₂ value and the lowest concentration of the β-receptor blocking agent causing significant slowing of spontaneous rate of SA node. It has been stated that propranolol and pronethalol have a higher effective therapeutic ratio than MJ 1999 (17). However, our studies and those of Stanton and Levy (2, 4) would suggest that...
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this is not so; and thus the differences in the effective therapeutic ratio described above would suggest that the therapeutic ratio may be species dependent. Therefore, studies of this ratio in various experimental models may have little predictive value for man.

MJ 1999 in concentrations as high as 5 x 10^-5 M had no effect on the resting potential, amplitude and overshoot of phase 0 of the action potentials recorded from ventricular muscle and Purkinje fibers. Similarly, in Purkinje fiber, both peak V_max of phase 0 depolarization and membrane responsiveness were unaffected by this high concentration of the drug. Thus, both the inward sodium current and the availability of the sodium carrier over a wide range of transmembrane voltage difference were unaffected by high concentrations of MJ 1999. These results contrast with those obtained with propranolol and pronethalol. Davis et al. (16) reported that propranolol at a concentration of 1 x 10^-5 M caused a decrease in the magnitude, overshoot and maximum upstroke velocity of phase 0 of depolarization recorded from both ventricular muscle and Purkinje fibers. Both propranolol (1 x 10^-5 M and 4 x 10^-5 M) and pronethalol (4 to 9 x 10^-6 M) (13, 14) depress membrane responsiveness.

MJ 1999, in concentrations from 1 x 10^-5 M to 1 x 10^-3 M, had a marked effect on the duration of action potentials recorded from both ventricular muscle and Purkinje fibers. This effect was: (a) more marked in Purkinje fibers than in ventricular muscle fibers; (b) concentration dependent; and (c) more apparent at slower rates of stimulation. Greater change of Purkinje fiber repolarization relative to repolarization in ventricular muscle is also seen after application of other agents such as diphenylhydantoin (20) and propranolol (16). The prolongation of the action potential by MJ 1999 occurred as a result of lengthening of phases 2 and 3, particularly the terminal part of phase 3. A delay in onset of g_{Ks} (33) or an increase in inward current flowing through a "slow channel," carried either by calcium or sodium ions (34), might account for the prolongation of phases 2 and 3. Wood et al. (35) have suggested that an increase in slow inward calcium current (I_{Ca,ss}) might be responsible for increased tension. Augmentation of a slow I_{Ca,ss} might account for the aftercontraction seen in papillary muscle of kitten when the action potential was prolonged by MJ 1999 (36). Further, this could explain the increased tension of the aftercontraction, caused by MJ 1999, when the external calcium concentration was increased (36).

In contrast to the effects seen with MJ 1999, Davis et al. (16) showed that propranolol accelerated repolarization of both canine Purkinje and ventricular muscle fibers. Hoffman et al. (15) showed that pronethalol shortened phase 2 but on the other hand it prolonged phase 3. Thus, these three agents which share the property of β-receptor blockade have markedly different effects on repolarization of canine ventricular muscle and Purkinje fibers.

The effects of high catecholamine concentrations (either epinephrine or isoproterenol) on Purkinje fibers previously exposed to 1 x 10^-5 M MJ 1999 were surprising. Under these conditions catecholamines further prolonged the action potential. This effect has several possible explanations. First, if one assumes the presence of several β-receptor sites, then this β-receptor blocking agent might not be able to achieve total blockade, leaving a portion of the receptor sites exposed permitting this effect on the action potential. On the other hand, catecholamines might have an effect on cardiac cellular membranes which is separate from their usual action on the β-receptor site(s). Recent work by Vassort et al. (37) has shown that epinephrine increases the amplitude and duration of the plateau of the frog's atrial potential, possibly by increasing a slow inward current carried either by calcium or sodium ions.

Studies of rabbit atrial fibers showed that the resting potential, the amplitude, overshoot, and peak V_max of phase 0 of depolarization and the duration of the action potential were unaffected by concentrations of MJ 1999 between 1 x 10^-5 M and 5 x 10^-4 M.
On the other hand propranolol, $3 \times 10^{-6}$M, causes a decrease in amplitude, overshoot, and peak $V_{\text{max}}$ of phase 0 of action potentials recorded from rabbit atrial cells, without significantly affecting the resting potential. In addition, repolarization was actually accelerated (30). These effects of propranolol on atrium are similar to its effects on canine ventricular muscle and Purkinje fibers (16), but very different from those of MJ 1999.

MJ 1999 prolonged the effective refractory period of ventricular muscle and Purkinje fibers. Again, this effect was concentration dependent and was more marked in Purkinje fiber than in ventricular muscle fiber. Because similar degrees of prolongation of the effective refractory period and duration of action potential occurred, the increase in the duration of action potential was plotted as a function of the increase in the effective refractory period caused by MJ 1999. When the change in duration of action potential was compared to the change in effective refractory period, the points were significantly shifted to the left of the line of identity in both ventricular muscle and Purkinje fibers, at a concentration of $1 \times 10^{-4}$M in ventricular muscle fibers and $5 \times 10^{-4}$M in Purkinje fibers (see Fig. 10). An examination of the effects of certain cardioactive agents such as diphenylhydantoin (20), lidocaine (18, 19), propranolol (16, 18), and quinidine (38) on the relation between duration of action potential and effective refractory period have shown that the effective refractory period is lengthened in relation to the duration of action potential. The opposite change, that is the shortening of the effective refractory period relative to the duration of action potential, caused by MJ 1999 would help to explain observations made when premature stimuli were applied to the preparations of canine ventricular muscle and Purkinje fiber. In some experiments, during exposure to MJ 1999, membrane activation (during repolarization) was seen at lower transmembrane voltages than under control conditions. Thus, after exposure to MJ 1999, points were obtained at lower transmembrane voltages on the S-shaped membrane responsiveness curve (see Fig. 11). Propranolol and pronethalol have the opposite effect (15, 16). The fact that responses were obtained at low transmembrane voltage during MJ 1999 exposure where none were obtained under control conditions is difficult to interpret. These differences occurred only in the transmembrane voltage range between 55 to 70 mV. The time constant of reaching a steady-state value of $h (V_{\text{max}}/\text{peak } V_{\text{max}})$ is so rapid relative to the slope of repolarization that it can usually be neglected (28). However, the time taken to achieve a steady state of $h$ is longest at low transmembrane voltages, e.g., 55 to 65 mV. Therefore marked changes in the slope of repolarization in this voltage range could influence the $V_{\text{max}}$ measured at a given transmembrane voltage. MJ 1999, $5 \times 10^{-4}$M, did decrease the slope repolarization over this voltage range (55 to 70 mV) to about half the control value. This change may be sufficient to explain the responses obtained in MJ 1999 at low transmembrane voltages.

Toxic effects of MJ 1999 were seen at a concentration of $1 \times 10^{-3}$M in Purkinje fibers previously stretched to enhance automaticity. Under these circumstances phase 4 depolarization was depressed by MJ 1999 (see Fig. 12). Findings so markedly different from results obtained with normal isolated Purkinje fibers may be explained on the basis of alterations produced by stretch. These alterations might be responsible for the different effects on automaticity seen with MJ 1999. Toxic concentrations of MJ 1999 initially caused marked prolongation of phases 2 and 3 and then repetitive depolarizations originated from the plateau of an afterpotential, at a time when automaticity was depressed. These effects on repolarization are similar to those seen after application of aconitine to Purkinje fibers. In attempting to explain the effects of aconitine, Peper and Trautwein have postulated that aconitine induces a steady-state sodium current and also affects the dynamics of the rapid inward sodium current (39). Veratridine and $\text{Ba}^{2+}$ have similar effects on cultured heart cells (40). It was suggested.
that these effects result from an increase in resting sodium permeability, possibly by a sustained opening of activated sodium channels.

Toxic effects of MJ 1999 appeared at lower concentrations in fibers previously exposed to quinidine and then allowed to recover. Although this in-vitro experimental model may not be comparable to the clinical situation, it seems reasonable to be particularly cautious, when MJ 1999 and quinidine are simultaneously administered to patients.

Recent evaluation of the antiarrhythmic activity of β-receptor blocking agents have attempted to separate the antiarrhythmic effects caused by β-receptor blockade from those due to the nonspecific antiarrhythmic action of these drugs (9, 10). A. Morales Aguilera and E. M. Vaughan-Williams (30) have suggested that pronethalol and propranolol act as antiarrhythmic drugs through their ability to reduce the amplitude and rate of rise of the action-potential upstroke. These drugs can act as local anesthetics (9, 10, 30). Some β-receptor blocking agents may reduce the amplitude and V_max of phase 0 of the cardiac action potential and depress membrane responsiveness through mechanisms independent of β-receptor blockade (10). We have used the term “local anesthetic-membrane effects” to describe these effects on the action potential. However, we feel that the demonstration that an agent has “local anesthetic-membrane effects” need not mean that this drug will act as an antiarrhythmic agent. Effects of the drug on automaticity, refractoriness, and excitability may also contribute to its potential antiarrhythmic action.

Recent studies of the effectiveness of MJ 1999 in the treatment of experimental arrhythmias have provided conflicting results. Experiments carried out to determine the effectiveness of MJ 1999 against ventricular arrhythmias induced by hydrocarbon and epinephrine in the dog (5, 6, 8, 9) showed it uniformly successful in protecting the test animals. Similarly, MJ 1999 has been shown to be effective in abolishing aconitine-induced atrial arrhythmias (2, 8). On the other hand, conflicting reports on the efficacy of MJ 1999 in treating ventricular arrhythmias induced by ouabain infusion or coronary artery ligation have appeared (2, 6, 8, 9, 36, 41-43). In the case of ouabain-induced ventricular arrhythmias, an explanation for these differences may be that different animal species were used for the evaluation of MJ 1999's effects.

A further explanation of these conflicting results might be obtained by a consideration of the electrophysiological effects of MJ 1999. Most ventricular arrhythmias are assumed to occur as a result of altered automaticity or reentry or both (44, 45). Automaticity, apart from that enhanced by catecholamines, has been shown not to be greatly affected by MJ 1999. Arrhythmias occurring as a result of enhanced automaticity may not be abolished or suppressed by this drug, unless catecholamines are a significant factor in their genesis. On the other hand, reentrant arrhythmias may be initiated and or perpetuated by depressed conduction (46, 47). Under such circumstances, agents which prolong refractoriness, without altering conduction, may suppress the occurrence of such arrhythmias. Thus, an agent which prolongs refractoriness might terminate a reentrant arrhythmia by providing refractory tissue to the advancing wave of depolarization (45). On the other hand, an agent like MJ 1999 that does not alter conduction but prolongs the duration of action potential to a greater extent than the effective refractory period could have the opposite effect. Thus, the advancing wave of depolarization in a reentrant path could activate the membrane at lower transmembrane voltages, by virtue of the prolongation of the action potential. The decrease in the transmembrane voltage at the time of activation would result in a decrease in the maximum upstroke velocity of the action potential and slower propagation of the wave front, which might then sustain the reentrant circuit.

Propranolol, an agent which has been used in the treatment of angina pectoris (48) and
cardiac arrhythmias (49), has also been used in the treatment of myocardial infarction (48). Studies on the use of propranolol in acute myocardial infarction have shown more hypotension in those patients to whom propranolol was administered (48). Elliott and Stone (48) stated that "when myocardial infarction is associated with hypotension due to low cardiac output, heart block or marked bradycardia, the patient on anginal suppressing doses of propranolol cannot call on his circulatory reserve mechanisms."

Davis and Temte (18) suggest that the ability of propranolol to prevent local responses and decremental conduction may be a significant factor in its antiarrhythmic action. If this suggestion is correct, then our observations on the failure of MJ 1999 to prevent the propagation of premature graded responses might make this agent less effective as an antiarrhythmic agent. On the other hand, this agent has been shown to have little effect on resting potential, phase 0 of the action potential or membrane responsiveness. Thus, as membrane responsiveness is a principal determinant of cardiac conduction at slower transmembrane voltages, then MJ 1999 should not depress cardiac conduction, even when transmembrane voltage has been decreased by myocardial damage. In addition, this agent has been shown to have less negative inotropic effect (31, 32) than propranolol.

Recent reports suggest that this agent is effective in the treatment of angina pectoris (50, 51). In acute myocardial infarction the risk of developing significant congestive cardiac failure and conduction disturbances is not inconsiderable. An agent like MJ 1999 with less negative inotropic effects and less depressive effects on cardiac conduction would have fewer detrimental effects to the patient with myocardial infarction. Thus, despite the lack of certain antiarrhythmic effects, MJ 1999 might be preferred in the treatment of patients with angina pectoris in whom the risk of myocardial infarction while on therapy is great.

References
14. PARMLEY, W. W., AND BRAUNWALD, E.: Comparative myocardial depressant and antiarrhythmic properties of d-propranolol, dl-propranolol and
EFFECTS OF MJ 1999 ON CARDIAC TISSUE

43. KAUMANN, A. J., AND ARAMENDIA, P.: Prevention of ventricular fibrillation induced by coronary


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