Effects of the T-Type Ca\textsuperscript{2+} Channel Blocker Mibefradil on Repolarization of Guinea Pig, Rabbit, Dog, Monkey, and Human Cardiac Tissue

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Accepted for publication October 18, 1999 This paper is available online at http://www.jpet.org

ABSTRACT
At supratherapeutic doses (2- to 5-fold), the T-type Ca\textsuperscript{2+} antagonist mibefradil modifies the T/U wave of the human ECG. In this study, we show that this effect is observed in conscious monkeys and is duplicated by verapamil or diltiazem. We then evaluate the proarrhythmic risk of such alterations of cardiac repolarization by examining the actions of mibefradil on cardiac action potentials (APs). In isolated cardiomyocytes from guinea pigs or humans, mibefradil dose dependently shortens the plateau of the AP; this effect is similar to other Ca\textsuperscript{2+} antagonists and opposite to drugs having class III antiarrhythmic properties. The metabolites of mibefradil, singly or in combination, also shorten APs. In isolated rabbit hearts, noncardiodepressant concentrations of mibefradil have no effect on monophasic action potentials (MAPs), whereas cardiodepressant levels produce a slight nonsignificant lengthening. In hearts of open-chest bradycardic dogs, mibefradil has no effect on MAP dispersion or on QT interval and shortens MAPs slightly; although high doses produce atrioventricular block, likely through Ca\textsuperscript{2+} antagonism, arrhythmias are never observed. In contrast, d-sotalol lengthens QT interval and MAPs, increases dispersion, and produces arrhythmias. Together, these in vitro and in vivo results suggest that mibefradil carries no proarrhythmic risk despite changes in T/U wave morphology. Although these changes resemble those observed with class III compounds, they also are seen with nonproarrhythmic compounds such as verapamil and diltiazem. In conclusion, the classical models used in the present study could not link the changes in T/U wave morphology produced by mibefradil and verapamil to any experimental marker of proarrhythmic liability.

For the last 30 years, Ca\textsuperscript{2+} channel blockers (CCBs, also called Ca\textsuperscript{2+} antagonists) have been used in the treatment of hypertension, angina pectoris, and some cardiac arrhythmias (Epstein, 1997). Classical CCBs belong to three unrelated chemical classes: dihydropyridines (DHPs, such as amlodipine, felodipine, and nifedipine), phenylalkylamines (verapamil), and benzothiazepines (diltiazem). They all exert their antihypertensive action through the selective block of L-type Ca\textsuperscript{2+} channels. However, the use of classical CCBs is limited by several major side effects (negative inotropism, reflex tachycardia) as well as minor drawbacks (edema, headache, flushing, or constipation) (Epstein, 1997).

Mibefradil (Posicor or Ro 40-5967) is a novel CCB that belongs to a new chemical class (tetralol derivatives) and possesses the unique feature of blocking selectively T-type Ca\textsuperscript{2+} channels. Indeed, depending on the cell type, mibefradil blocks T-type Ca\textsuperscript{2+} channels 10 to 30 times more potently than L-type Ca\textsuperscript{2+} channels (Mishra and Hermsmeyer, 1994; Bénardeau and Ertel, 1998). Preclinical and clinical investigations have shown that mibefradil is a potent vasodilator with antianginal, antihypertensive, and anti-ischemic properties (Ertel and Clozel, 1997; Kobrin et al., 1997). In addition, mibefradil is better tolerated than classical CCBs because it has much lesser side effects: at therapeutic doses, mibefradil decreases heart rate slightly but it neither reduces cardiac contractility nor induces reflex tachycardia (for reviews of the properties of mibefradil, see Ertel and Clozel, 1997; Kobrin et al., 1997). However, pharmacokinetic interactions between mibefradil and drugs metabolized through the cytochromes P-450 3A4 and 2D6 have led to the withdrawal of mibefradil from the clinic.

The beneficial pharmacological and hemodynamic actions of mibefradil are likely a consequence of its pharmacological selectivity for T-type over L-type Ca\textsuperscript{2+} channels and its functional selectivity for the vasculature over the myocardium and for coronary over peripheral vessels (Ertel and Clozel, 1997).
Like the DHPs, the vascular selectivity of mibefradil likely derives from its preferential block of Ca\(^{2+}\) channels (both T-type and L-type) in depolarized tissues. However, the basis of its coronary selectivity remains obscure.

Among the side-effects reported for supratherapeutic doses of mibefradil in humans (200 and 250 mg/day compared with the recommended 50 or 100 mg/day), one was particularly intriguing. It was shown that mibefradil could induce changes in the morphology of the T/U wave complex of the ECG, notably a decrease in T wave amplitude, an increase in U wave amplitude, and a fusion of T/U wave complexes.

Overall, these changes were variably interpreted. Although some cardiologists saw them as clear prolongations of the QT interval, others viewed them as increases in U wave amplitude combined with no change or even a slight shortening of the QT interval. In many cases, the fusion of the T and U waves was such that automatic QT measurement algorithms (and even trained cardiologists) were unable to accurately define the end of the T wave (Giles, 1997; Kobrin et al., 1997).

The incidence and morphology of these changes were similar to those observed with verapamil or diltiazem but they were very different from those observed with class III antiarrhythmics and other QT-prolonging compounds (Giles, 1997; Kobrin et al., 1997). Nevertheless, such changes clearly indicate that mibefradil modifies ventricular repolarization.

To understand this alteration of cardiac repolarization, we examined the actions of mibefradil on the surface ECG of conscious animals and on the cardiac action potential (AP) at three levels of integration: 1) APs in isolated cardiac myocytes, 2) monophasic APs (MAPs) in isolated hearts, and 3) MAPs in hearts of open-chest anesthetized animals. When pertinent, mibefradil was compared with conventional CCBs and with QT-prolonging compounds and, in some preparations, metabolites of mibefradil were tested.

Materials and Methods

ECG Measurements in Conscious Squirrel Monkeys

Animals. The study was performed in normotensive squirrel monkeys (Saimiri sciureus) weighing 700 to 800 g. All monkeys were maintained under identical conditions, had free access to normal chow and water, and lived under a 12-h light/dark cycle. The animals were handled according to the “Position of the American Heart Association on Research Animal Use.”

Transmitter Implantation. The monkeys were anesthetized with an i.m. injection of 10 mg/kg alfaxalone/alfadolone acetate and 1.2 mg/kg climazolam. The surgery was performed under aseptic conditions. The combined ECG/blood pressure transmitter was implanted in the right subclavian vein and connected to a platform (RLA 3000) that was placed around a cage and connected to a multiplexer (RMX10); the digital signal was then transferred via a consolidation matrix (BCM100) to a dedicated personal computer (Compaq Prolinea 4/66). Blood pressure (BP) was calibrated with an ambient-pressure monitor (C11PR). BP (mean arterial pressure) and heart rate (HR) were recorded for 10 s every 10 min; the ECG was recorded for 10 s each hour with a sampling rate of 1000 Hz. The signal was analyzed with the Dataquest analysis program (Physiostat ECG Analysis; Data Sciences). BP was expressed in millimeters of mercury, HR in beats per minute, and the calculated intervals of the ECG signal in milliseconds. The QT interval was measured from the beginning of the QRS complex to the end of the T wave, including double T waves or U waves if they occurred. To calculate QTc, QT was corrected for HR with Fredericia’s formula: QTc = QT/RR\(^{1/2}\) (in seconds, where RR is the interval between two R waves).

Protocol. Six monkeys were included in this study. Each monkey was treated in succession with two to four drugs among the following: mibefradil (30 mg/kg/day p.o. in distilled water), verapamil (100 mg/kg/day p.o. in 5% gum arabic), diltiazem (30 mg/kg/day p.o. in 5% gum arabic), and placebo (5% gum arabic). The dose of mibefradil was selected as 3-fold the “therapeutic” dose in animals, i.e., 3-fold the dose used commonly to achieve 10 to 20% reduction in BP. The doses of verapamil and diltiazem were then chosen to obtain a decrease in BP similar to that seen with the high dose of mibefradil. For each drug, baseline ECG, BP, and HR were recorded for 24 h before drug application. Drug treatment lasted 2 days (compounds were given at ~8:00 AM) and was followed by at least 3 days of washout. On the second treatment day, the maximal changes in HR, QT interval, and BP for each dog were averaged within each treatment and these averages were used for further analysis. Monkey A received serially mibefradil, placebo, verapamil, and diltiazem; monkey B received mibefradil and diltiazem; monkey C received mibefradil, placebo, and verapamil; monkey D received placebo, mibefradil, verapamil, and diltiazem; monkey E received placebo, mibefradil, and verapamil; and monkey F received placebo, mibefradil, verapamil, and diltiazem.

The ECG at baseline and the ECG during treatment (indiscriminately day 1 or 2 because no obvious differences were observed) were coded and sent to a confirmed cardiologist for blind analysis of the repolarization. Any of the following changes were accepted as a morphological alteration of the T wave: amplitude increase, flattening, doubling (T-U wave), reversal, or widening.

Drugs. Alfaxalone/alfadolone acetate (Saffan) was from Glaxovet (Uxbridge, UK) and climazolam (Climasol) from Graub (Basel, Switzerland). Mibefradil was from Roche (Basel, Switzerland), verapamil from Calbiochem (La Jolla, CA), and diltiazem from Profaromaco (Milan, Italy).

Cellular Electrophysiology

Guinea Pig Ventricular Myocytes. Cell preparation. Single ventricular myocytes were enzymatically dissociated from the hearts of male Fullingsdorf Albino guinea pigs. A guinea pig (200–300 g) was sacrificed by cervical dislocation and the heart was rapidly excised, mounted on a Langenbein apparatus in a retrograde perfusion configuration, and perfused 5 min with a nominally Ca\(^{2+}\)-free Tyrode’s solution (see “Solutions and Drugs”). Then, 200 nM Ca\(^{2+}\), 0.6 mg/ml collagenase type I (~180 U/ml; Sigma Chemical Co., St. Louis, MO) and 0.09 mg/ml protease type XXIV (~1.6 U/ml; Sigma Chemical Co.) were added and perfused 30 min. Next, the enzymes were removed and perfusion continued for 15 min. Finally, the solution was changed to a K\(^{+}\)-rich solution, which was perfused 10 min. Parts of both ventricles were cut in small pieces and washed twice with fresh K\(^{+}\)-rich solution. The cell suspension was centrifuged at 35g, resuspended in M199 medium, centrifuged again, and resuspended in M199E medium supplemented with streptomycin (100 \(\mu\)g/ml), penicillin (100 I.U./ml), cAMP (100 \(\mu\)M), 3-isobutyl-1-methylyxanthine (100 \(\mu\)M), and 10% fetal calf serum (Life Technologies, Paisley, Scotland). Cells were plated in Petri dishes on CellTak coated coverslips (Becton Dickinson Labware, Mountain View, CA) and stored at 37°C in a CO2 incubator.

Action potentials. Experiments were performed at room tempera-
ture (RT = 22–25°C) or at 35°C. Membrane voltage was recorded with an EPC-9 amplifier (HEKA elektronik; Lambrecht/Pfalz, Germany) in current-patch-clamp mode, in the whole-cell configuration or with the nystatin-perforated-patch method. Because the spontaneous resting membrane potential of guinea pig ventricular myocytes is near ~80 mV, APs were elicited from this resting potential with short depolarizing current pulses (5–10 ms). Cells were selected if their AP duration at 95% repolarization (APD95) was stable and in the range 0.1 to 6 s at RT or 0.05 to 3 s at 35°C. To ensure that K+ and Ca2+ currents recovered completely from inactivation, the interval between consecutive APs was set to the greatest of 6 s and six times the APD95. Borosilicate glass capillaries (Dagan Corporation, Minneapolis, MN) were used to manufacture pipettes with a tip resistance of ~2 MΩ.

Human Atrial Myocytes. Cell preparation. Single atrial myocytes were enzymatically isolated from human right atrial appendages. In this study, the human cardiac tissue was obtained during surgeries performed at the Hôpital Marie-Lannelongue (Le Plessis Robinson, France, surgical team of J-Y Neveux) in accordance with the institutional guidelines for human subject research. We used tissue from three patients: a 67-year-old man with mitral insufficiency (no. 1), a 74-year-old woman with coronary insufficiency (no. 2), and an 8-year-old boy with aortic insufficiency (no. 3). Patient 3 had a dilated right atrium. Patients 1 and 2 received pharmacological medications (patient 1: spironolactone and furosemide; patient 2: candesartan (Candesartan-Cilexetil, Novartis), bepridil (Roche, Ro 23-6006) and Cd2+). The internal solution contained 107 mM K-glutamate, 20 mM MgCl2, 2 mM CaCl2, 1 mM EGTA, 5 mM Mg-ATP, and 0.1 mM Na2-GTP, pH 7.2 with KOH. The external solution contained 161 mM NaCl, 4 mM KCl, 5 mM CaCl2, 0.5 mM MgCl2, 10 mM HEPES, and 10 mM glucose, pH 7.4 with NaOH, and the appropriate test compounds.

Whole-cell currents. Calcium currents were recorded with an Axoclamp 200A amplifier (Axon Instruments, Foster City, CA). Currents were low-pass filtered at 5 kHz and digitized by a LabMaster A/D converter (Scientific Solutions, Solon, OH); series resistance was compensated but not capacitive or leakage current. Ca2+ currents were elicited every 7 s, from a holding potential of ~80 or ~60 mV. Cell capacitance is estimated from the capacitative current elicited by a 10-nA depolarizing pulse from a holding potential of ~80 mV.

Solutions and Drugs. Cell preparations. Ca2+-free Tyrode’s solution was prepared as follows: 135 mM NaCl, 5.4 mM KCl, 1 mM MgCl2, 0.33 mM NaH2PO4, and 10 mM HEPES, pH 7.5 with NaOH. K+-rich solution was prepared as follows: 140 mM K-glutamate, 1 mM EGTA, 5 mM MgCl2, 10 mM HEPES, and 1 mM glucose, pH 7.4 with KOH. Ca2+-free Krebs-Ringer solution was prepared as follows: 35 mM NaCl, 16 mM NaHCO3, 25 mM NaHCO3, 4.75 mM KCl, 1.2 mM KH2PO4, 10 mM HEPES, 10 mM glucose, 134 mM saccharose, pH 7.4 with NaOH. HEPES-buffer solution was prepared as follows: 130 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 25 mM HEPES, and 5 mM glucose, pH 7.4 with NaOH.

APs. The external and internal (pipette) solutions were designed to allow the activation of all currents known to be activated during a cardiac cycle and to avoid sustained changes in intracellular free Ca2+. The internal solution contained 107 mM K-glutamate, 20 mM MgCl2, 5 mM CaCl2, 11 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid-K (BAPTA-K), 0.9 mM CaCl2, 1 mM MgCl2, 20 mM HEPES, 5 mM K2-ATP, and 0.1 mM Na2-GTP, pH 7.2 with KOH. The external solution contained 161 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 0.5 mM MgCl2, 10 mM HEPES, and 10 mM glucose, pH 7.4 with NaOH, and the appropriate test compounds.

Rabbit Isolated Hearts

Surgical Preparation. Hearts were obtained from albino rabbits of either sex, weighing ~1 kg, and they were perfused with the Langendorff method with the following solution: 118 mM NaCl, 3 mM KCl, 22 mM NaHCO3, 1.1 mM MgCl2, 0.4 mM NaH2PO4, 1.8 mM CaCl2, 5 mM dextrose, 2 mM pyruvate, and 0.038 mM creatine. Under a dissecting microscope, the atria were removed and a pair of stimulating electrodes was sutured on the His bundle. A recording electrode was placed in the subendocardium of the left ventricle near the septum and a balloon was sutured into the left ventricle. The preparation was then transferred to the SCREENIT computer sys-
tem (Hondeghem, 1994), where a pair of epicardial stimulating electrodes, a recording electrode (measuring mostly ventricular myocytes), and a K⁺ reference electrode were positioned.

Protocol. Once the heart was mounted on the experimental station, the computer checked whether the following parameters were within normal range: electrode offset and noise, threshold stimulation current (acceptable, <300 μA; normal, ~100 μA), optimum preload and developed pressure, automaticity cycle length (acceptable, >1 s; normal, >15 s), coronary perfusion (acceptable, >10 ml/min; normal, 13–17 ml/min), and cardiac activation time (acceptable, <50 ms; normal, ~40 ms). If all parameters were not within normal range, the preparation was rejected.

The experiment was started ~1 h after surgery. After a control period of 60 min, 0.1 μM drug was applied for 60 min, then 1 μM for another 60 min, then 10 μM for the last 60 min. Thirty minutes into each condition, the preparation was thoroughly checked and various electrophysiological and hemodynamic parameters [monophasic action potential duration (MAPD), conduction time, refractory period, threshold stimulation, susceptibility to arrhythmia, left ventricular developed pressure, and coronary perfusion rate] were measured. In particular, threshold stimulation current was measured as the minimum current required to make the preparation follow five consecutive beats at a cycle length of 400 ms. The intraventricular balloon was inflated to preload and maximum developed pressure, conduction time, and AP duration at 50% repolarization (APD₅₀) were measured for cycle lengths of 2000, 1500, 1000, 750, 500, 300, and 250 ms.

Measurements. Data were captured by a 12-bit analog-to-digital converter; MAPs and left ventricular pressure were sampled at 1 kHz. Statistical analysis was done with MDCSTAT (Micro Data Collection Inc., Novato, CA) and Primer of Biostatistics (McGraw-Hill Book Company, New York). For comparison between means, an ANOVA was used and significance was evaluated with a Scheffe test (Primer of Biostatistics).

Dog Model of Arrhythmia

Surgical Preparation. Adult beagles (15–30 kg; 17 females, 5 males) were anesthetized with thiopental (25 mg/kg i.v.). After tracheal cannulation, anesthesia was maintained by halothane inhalation (0.9–1.2%) and inspiratory CO₂ (Beckman LB2) was adjusted to physiological values through mechanical ventilation (Harvard Pump). The 3-lead ECG (I, II, III) and femoral BP (Statham P23 XL) were recorded continuously.

The heart was exposed by a medial sternotomy and it was suspended in a pericardial cradle. Bradycardia (~40 beats/min) was obtained by simultaneous clamp of the sinus node area and β-adrenergic block (propranolol; 0.5 mg/kg i.v.). Two wire electrodes were sutured to the epicardial surface of the right atrium for atrial stimulation. A bipolar 6F stimulation electrode was pushed through the right jugular vein and located in the right ventricle to allow ventricular stimulation.

Four unipolar MAPs were measured simultaneously by differential amplification and low-pass filtering (<500 Hz) of the signals between a 4-pole active electrode (4P multipolar, GTSM, Castelnaudary, France) and a reference electrode (electrode catheter filled with 3 M KCl). The reference electrode was inserted 2 to 3 mm below the epicardial surface and away from any coronary vessel. The active electrode was pushed through the anterior wall of the left ventricle just after the main branch of the left anterior descending artery, close to the septum. The four MAPs were therefore recorded from regions located on the same axis (perpendicular to the heart surface) and spaced 1.5 mm from one another. Care was taken to place the most distal pole of the active electrode just inside the left ventricular cavity, in proximity to the endocardial surface. The opened chest was covered with a plastic sheet and intrathoracic heat was maintained with a heating blanket and an infrared heating lamp.

Measurements. QT interval duration was measured between the beginning of the QRS complex and the end of the T wave. For mapping of the MAPs, a bipolar 6F stimulation electrode was inserted through the right jugular vein and located in the right ventricle to allow ventricular stimulation. After the heart was mounted on the experimental station, the computer checked whether the following parameters were within normal range: electrode offset and noise, threshold stimulation current (acceptable, <300 μA; normal, ~100 μA), optimum preload and developed pressure, automaticity cycle length (acceptable, >1 s; normal, >15 s), coronary perfusion (acceptable, >10 ml/min; normal, 13–17 ml/min), and cardiac activation time (acceptable, <50 ms; normal, ~40 ms). If all parameters were not within normal range, the preparation was rejected.

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complex recorded on the ECG (lead II). The dependence of the ventricular repolarization on cycle length was studied at steady state by changing the atrial cycle length at 3-min intervals (1500 → 1000 → 600 ms, or reverse). The same cycle lengths were used for ventricular stimulation to look for retrograde conduc- tion through the AV node. Between measurements, heart rate was kept as low as could maintain a diastolic BP >50 mm Hg; ECG and MAPs were continuously monitored and they were recorded in case of arrhythmias. Torsades de pointes (TdP) were defined as polymorphic ventricular tachycardias of eight complexes or more, with rotation of the QRS spikes, with or without degeneration into ventricular fibrillation. No resusci- tation was performed.

Protocol. Three groups of dogs were included in the study; group 1 (n = 5) received mibefradil in ethanol solution, group 2 (n = 5) received only the alcohol vehicle, and group 3 (n = 8) received d-sotalol in aqueous solution. Group 3 was not submitted to ventric- ular stimulation. Control ECG, MAPs, and BP were recorded start- ing 30 min after electrode implantation and dosage was in- creased every 60 min. Measurements in drug were performed 30 min infused 60 min after electrode implantation and dosage was in- creased every 60 min. Measurements in drug were performed 30 min after starting the infusion. Experimental doses were 0.9, 1.8, and 3.6 mg/kg/h for mibefradil and 1.15, 2.25, and 4.5 mg/kg/h for d-sotalol. The lowest dose of mibefradil is in the therapeutic range, whereas the two highest doses are supratherapeutic. Propranolol chlorhy- drate was obtained from Sigma Chemical Co., and d-sotalol chlorhy- drate was obtained from Bristol-Myers Squibb (Logne, France).

Data are summarized as means ± S.E. For each group, the effects of dose, cycle length, and depth of recording on MAPD and MAPX were analyzed with multifactorial ANOVA. Comparisons between drugs were performed with ANOVA on baseline values and varia- tions from baseline. Student’s t test adjusted for multiple compari- sons (Bonferroni method) was used when appropriate. The rate of occurrence of AV block was tested for each dose and cycle length with exact probabilities. Statistical significance was set at P < .05.

Table 1 also shows that both mibefradil and verapamil produce an increase in QT interval (60% larger for mibefradil than verapamil, on average) that is associated with a de- crease in HR only for mibefradil. In Fig. 1, for example, mibefradil slows HR substantially (RR from 265 to 357), diltiazem slows HR minimally (RR from 261 to 271) and verapamil accelerates HR (RR from 317 to 284). However, mibefradil increases QT from 119 to 151 (32 ms), diltiazem has no effect (from 114 to 114), and verapamil increases QT

### Results

#### Monkey ECG Telemetry

In humans, mibefradil, verapamil, and diltiazem induce similar changes in the morphology of the T/U wave complex of the ECG (Giles, 1997; Kobrin et al., 1997). We have ob- served comparable changes in animals, including dogs, rab- bits, rats, and monkeys. Figure 2 shows typical recordings from monkeys equipped with a telemetry ECG apparatus; these animals are thus conscious and freely moving. Figure 2A shows ECIs at baseline and after treatment with 30 mg/kg mibefradil for 2 days. At baseline, the T wave appears normal but, after treatment, one can observe what could be described either as a biphasic T wave or as the appearance of a U wave partly merged with the T wave. Regardless, ven- tricular repolarization is obviously affected. Figure 2B shows ECIs from the same animal at baseline and after treatment with 100 mg/kg verapamil for 2 days: clearly, verapamil induces ECG modifications that are similar to those observed with mibefradil. Finally, Fig. 2C shows ECIs from the same animal at baseline and after treatment with 30 mg/kg dilti- azem for 2 days: again, diltiazem induces large ECG modifi- cations. Among six monkeys treated in succession with two to four drugs (mibefradil, verapamil, diltiazem, and placebo) in varying sequences, the incidence of changes in repolarization (determined in blind fashion) was four of six for mibefradil (67%), three of five for verapamil (60%), three of four for diltiazem (75%), and one of five for placebo (20%) (Table 1). Thus, the three CCBs have a similar incidence of repolarization disturbances.

#### Table 1

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Fig. 2. Mibefradil, verapamil, and diltiazem induce similar changes in the repolarization pattern of the monkey ECG. A, 30 mg/kg/day mibe- fradil, baseline and day 2. B, 100 mg/kg/day verapamil, baseline and day 2. C, 30 mg/kg/day diltiazem, baseline and day 2. All ECGs are from the same monkey.
from 117 to 131 (14 ms). Due to the differential effects on HR and QT, the corrected QT interval (QTc, Fredericia’s formula; Table 1) is increased by both mibefradil and verapamil but not by diltiazem (45% more for mibefradil than verapamil, on average). In Fig. 1, mibefradil increases QTc from 185 to 213 (28 ms), diltiazem has no effect (from 178 to 176), and verapamil increases QTc from 172 to 199 (27 ms). Assuming that Fredericia’s correction can be applied to squirrel monkeys, this result demonstrates that the decrease in HR does not explain fully the lengthening of QT interval by mibefradil. Finally, as expected, all three compounds decrease BP at these high doses, with verapamil producing the largest effect.

Guinea Pig Ventricular Action Potentials

Mibefradil and Its Metabolites Shorten Guinea Pig Ventricular APs. Fig. 3 shows typical APs recorded in enzymatically isolated guinea pig ventricular myocytes at RT (Fig. 3A) or at 35°C (Fig. 3B). In both cases, APs are characterized by a resting potential near −80 mV (−82.4 ± 0.2 mV (n = 94) at RT and −83.5 ± 0.4 mV (n = 13) at 35°C). On triggering of the AP by a current pulse, a very rapid upstroke is followed by a high and sustained plateau between +40 and +10 mV. Among all the cells studied, there was never a notch or a significant fast repolarizing phase following the peak of the AP, consistent with the absence of transient outward K+ currents (IKt) in these cells (Varro et al., 1993). Eventually, the plateau is terminated by a rapid repolarization to the initial resting potential. At RT, APD40 and APD95 were 1080 ± 105 and 1198 ± 108 ms, respectively (n = 94); at 35°C, APD40 and APD95 were 792 ± 218 and 868 ± 224 ms, respectively (n = 13). Such variation in the APDs can be expected from cells isolated from all layers of both ventricular walls (Antzelevitch et al., 1991; Anyukhovsky et al., 1996; Noguchi et al., 1997).

At RT (Fig. 3A) or at 35°C (Fig. 3B), 10 μM mibefradil shortens the AP by abbreviating the plateau without any effect on the rapid terminal repolarization: this action is consistent with the block of L-type Ca2+ channels. The reduction in APD95 induced by 10 μM mibefradil is 52 ± 6% (n = 14) at RT and 65 ± 8% (range 50–83%; n = 4) at 35°C. Mibefradil does not modify the upstroke velocity significantly between −80 and 0 mV but slows it slightly above 0 mV, with a concomitant decrease in the peak amplitude of the AP (Fig. 3); this could already result from block of L-type Ca2+ channels but it also could indicate a slight inhibition of Na+ channels. Mibefradil has no effect on the resting potential, indicating that it does not block markedly the inwardly rectifying K+ current, IK1.

The AP shortening induced by 10 μM mibefradil occurs rapidly after drug application and is partially or entirely reversible (Fig. 3, A and C). In general, prolonged applications tended to be less reversible than brief ones. To test the possibility that mibefradil may have a second, slower action on APs, we performed a few experiments with the nystatin-perforated patch-clamp technique to increase cell stability: mibefradil could then be applied for 7 up to 25 min (n = 3). In
all cases, the rapid initial AP shortening is never followed by another slow-onset action; in particular, the APD\textsubscript{95} remains stable (Fig. 3D).

On chronic treatment, the steady-state concentration of mibefradil in plasma is in the range of 1 to 10 \(\mu\text{M}\) but a large fraction (99–99.5\%) is bound to plasma proteins; thus, the free concentration is in the range of 5 to 100 nM. In addition, mibefradil accumulates \(<10\)-fold in tissues. Because some compounds (e.g., droperidol) induce a shortening of APs at high doses but a lengthening at lower concentrations (Adamantidis et al., 1993), we examined the action of mibefradil below 10 \(\mu\text{M}\) (0.1 nM–10 \(\mu\text{M}\)). We also tested 30 \(\mu\text{M}\) and 40 \(\mu\text{M}\) mibefradil in two cells; at these high concentrations, the cells were lost in a few seconds but we could still observe only a rapid reduction of the APD\textsubscript{95}, of the AP amplitude, and of the upstroke velocity. We find that mibefradil dose dependently shortens APs in this preparation and that the dose response is well fit with a Michaelis-Menten equation with one binding site and an IC\textsubscript{50} value of 90 nM (Fig. 4, ○). However, because very high concentrations were not usable, the maximal reduction is weakly defined in this dose response and the IC\textsubscript{50} value could actually be as high as 400 nM. Nevertheless, this range of values is consistent with the IC\textsubscript{50} reported for the inhibition of L-type Ca\textsuperscript{2+} channels in the same cells (230 nM) (Liang-Min and Osterrieder, 1991). Similar results were obtained in two experiments at 35°C with 0.1 and 1 \(\mu\text{M}\) (Fig. 4, □).

In humans, mibefradil is metabolized into several by-products, among which four primary and four secondary metabolites have been identified (Wiltshire et al., 1997). Because these metabolites (particularly the primary ones and especially metabolite G; Fig. 1) can reach significant levels in vivo, representing 50 to 80\% of the circulating drug-related material (Casas et al., 1997), we examined whether they could affect APs differently from mibefradil itself. At 10 \(\mu\text{M}\), the four primary metabolites of mibefradil (G, E1, H1, and K; Fig. 1) shorten the AP like mibefradil (Fig. 5). The reductions in APD\textsubscript{95} are G, 44 ± 22\% (n = 3); E1, 48 ± 5\% (n = 3); H1, 46 ± 12\% (n = 4); and K, 55 ± 6\% (n = 2). It is worth noting that these metabolites reduce AP amplitude and upstroke velocity more than mibefradil, suggesting that they block Na\textsuperscript{+} channels more potently than their parent compound. The four secondary metabolites (B3, C1, E2, and B1; Fig. 1) are present at much lower levels in plasma and they induce little or no change in the APD (<10\%) when tested at 10 \(\mu\text{M}\) (B1) or 1 \(\mu\text{M}\) (B3, C1, E2) (data not shown); thus they seem to have negligible activity on ion channels.

Because it was unreasonable to build dose responses for all metabolites, we decided to test a mixture of mibefradil and its eight metabolites at relative concentrations similar to those found in human plasma (see Materials and Methods). For mibefradil concentrations of 0.01, 0.1, or 1 \(\mu\text{M}\) in the final perfusate, we measured AP shortenings similar to those obtained with mibefradil alone (Fig. 4, △). Thus, the presence of its metabolites does not affect the action of mibefradil on APs.

Collectively, these results show that mibefradil dose dependently shortens guinea pig ventricular APs, consistent with a block of L-type Ca\textsuperscript{2+} channels. We never observed either a lengthening or a crossover (shortened APD\textsubscript{90} and lengthened APD\textsubscript{95}) of the APs.

**Classical CCBs Shorten Ventricular APs Similarly to Mibefradil.** We compared the action of mibefradil with those of classical CCBs, such as amlodipine, nisoldipine, verapamil, and Cd\textsuperscript{2+}. Figure 6 shows that all CCBs significantly shorten APs. Their actions are very similar to that of mibefradil, although Cd\textsuperscript{2+} and the DHPs may induce a slightly faster and larger reduction of the early plateau than mibefradil or verapamil. At RT, APD\textsubscript{95} is shortened 66 ± 6\% by 10 \(\mu\text{M}\) amlodipine (n = 3), 71 ± 4\% by 1 \(\mu\text{M}\) nisoldipine (n = 6), 45 ± 2\% by 10 \(\mu\text{M}\) verapamil (n = 7), and 67 ± 5\% by 100 \(\mu\text{M}\) Cd\textsuperscript{2+} (n = 7) compared with 52 ± 6\% by 10 \(\mu\text{M}\) mibefradil.

**QT-Lengthening Compounds Prolong Ventricular APs.** Most conventional CCBs (DHPs, verapamil, diltiazem, and bepridil) block not only L-type Ca\textsuperscript{2+} channels but also repolarizing K\textsuperscript{+} channels (Gotoh et al., 1991; Lefèvre et al., 1991; Wegener and Nawrath, 1996; Avdolon et al., 1997). In some cases (e.g., bepridil), block of K\textsuperscript{+} currents is potent enough that repolarization disturbances can be observed in vitro (prolonged APs and after-depolarizations in isolated cells or tissues) as well as on clinical treatment (lengthened QT interval and TdP in the surface ECG) (Somberg et al., 1985; Osaka et al., 1988). Other proarrhythmic compounds (e.g., quinidine, sotalol, and cisapride) also produce similar repolarization disturbances, due to block of repolarizing K\textsuperscript{+} channels. At micromolar concentrations, mibefradil also appears to block such currents, either native or recombinant (Randall, 1995; Chouabe et al., 1998). Yet, mibefradil does not modify the surface ECG like class III antiarrhythmics or the above-mentioned drugs but rather affects it like the CCBs verapamil and diltiazem (Giles, 1997; Kobrin et al., 1997).

To verify that APs in isolated guinea pig ventricular myocytes can be used to recognize drugs able to prolong repolarization in vivo, we compared mibefradil with some such drugs (Fig. 7). Reproducibly, 20 \(\mu\text{M}\) quinidine or 50 nM terfenadine more than double APD\textsubscript{95} (Fig. 7, A and B), even in cells where
mibefradil shortens APD\textsubscript{95} (Fig. 7A): this can be expected from drugs that block K\textsubscript{1} channels more potently than Ca\textsuperscript{2+} channels. Similarly, bepridil can increase APD\textsubscript{95} (Fig. 7C). Yet, its effect is generally less substantial and it is not observed in every cell. In fact, in some cells, bepridil actually reduces APD\textsubscript{95}, consistent with its dual action on ionic currents. However, we could not find a means to predict, from control parameters, how a given cell would respond to bepridil. Thus, guinea pig ventricular APs are indeed sensitive to QT-prolonging compounds, even in the case of multiple actions. Yet, as mentioned, mibefradil never prolonged APs in 56 cells tested.

**Human Atrial APs**

Human and guinea pig cardiac APs display clear differences. In particular, their kinetics is distinct, reflecting different balances of inward and outward currents (Coraboeuf and Nargeot, 1993). Consequently, we wanted to confirm in a human preparation the results obtained in guinea pigs. Because human ventricular myocytes are in very limited supply, we used myocytes isolated from right atrial appendages obtained during cardiovascular surgery.

Human atrial APs at RT are characterized by, in succession, a rapid upstroke (Na\textsuperscript{+} current, INa), a rapid partial repolarization (K\textsuperscript{+} current, IK\textsubscript{to}), a plateau (Ca\textsuperscript{2+} current, IC\textsubscript{aL}), and a late slow repolarization [K\textsuperscript{+} current, IK\textsubscript{co}, and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange current, I(Na\textsuperscript{+}/Ca\textsuperscript{2+})] (Fig. 8). The amplitudes and durations of the plateau and of the late repolarization are somewhat variable among cells. Mibefradil [1 \mu M (n = 3) and 10 \mu M (n = 4)] reduces the APD during the plateau (APD\textsubscript{40}, from \textminus15 to \textminus85\%; n = 7) but induces minor changes during the late repolarization (APD\textsubscript{95} <\textpm 5\%; n = 7). Thus, complete repolarization is neither lengthened nor shortened.

In isolated human atrial myocytes, the amplitude of the Ca\textsuperscript{2+} current, IC\textsubscript{aL}, is often small because of the dephosphorylation of L-type Ca\textsuperscript{2+} channels. We also tested mibefradil on APs under conditions where we used the \beta-adrenergic agonist isoproterenol (1 \mu M), to rephosphorylate L-type Ca\textsuperscript{2+} channels and increase IC\textsubscript{aL}. Isoproterenol increased the AP plateau duration in every cell (12 of 12) and decreased its amplitude in many cells (8 of 12); isoproterenol also increased APD\textsubscript{95} slightly in most cells (9 of 12) (Fig. 9A). These actions are consistent with increases in the amplitudes of both IC\textsubscript{aL} and IK\textsubscript{co}, which partially counteract each other. Under these conditions, 10 \mu M mibefradil always reduces both APD\textsubscript{40} and APD\textsubscript{95} (51.5 ± 8.3 and 4.1 ± 1.2\%, respectively; n = 9; Fig. 9A).

Because the action of mibefradil on human cardiac Ca\textsuperscript{2+} channels had not been reported previously, we also used voltage-clamp to measure directly the block of IC\textsubscript{aL}. Figure 9B shows that 1 \mu M mibefradil added on top of 1 \mu M isoproterenol reduces the peak of IC\textsubscript{aL} by 43.6 ± 7.4\% (n = 4).

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**Fig. 5.** The four direct metabolites of mibefradil shorten ventricular APs like their parent drug. Pulse interval: 10 (A), 18 (B), 12 (C), and 6 s (D).
Rabbit Isolated Hearts

The T/U wave of the ECG reflects the repolarization of the entire left ventricle. This tissue is an integrated system made of different cellular types with distinct electrophysiological properties (Antzelevitch et al., 1991); thus, as an ensemble, the ventricle may respond to a drug in a very different way than isolated cells. To examine the action of mibefradil in an intact heart, we used the Langendorff-perfused rabbit heart, where we have previously tested hundreds of compounds (Hondeghem, 1994). In this preparation, we measure simultaneously MAP parameters in two locations (left ventricular septum and epicardium), conduction time, refractory period, threshold stimulation, susceptibility to arrhythmia, left ventricular developed pressure, and coronary perfusion rate.

At concentrations of 0.1 and 1 μM, mibefradil has no effect on MAPDs recorded near the left ventricular septum (Fig. 10A, □, ▲) or on the epicardium (data not shown). In addition, the drug does not affect ventricular developed pressure significantly (Fig. 10B, □, ▲). At 10 μM, mibefradil dramatically decreases developed pressure (Fig. 10B, ◊) and increases conduction time (60 ± 12% at 300-ms cycle length), indicating that this concentration is well above the therapeutic range. There also may be a slight increase in MAPD at very slow cycle lengths (Fig. 10A, ◊) but this effect is not statistically significant and very small compared with that produced by “torsadogenic” agents such as quinidine (Hondeghem, 1994).

Dog Model of Arrhythmia

At the final level of integration, we examined in a dog bradycardia model of arrhythmia the actions of mibefradil and of the class III antiarrhythmic d-sotalol, which delays cardiac repolarization and induces TdP. We recorded the duration of MAPs in multiple cardiac layers (epicardium to endocardium), their transmural dispersion (MAPX), the duration of the QT interval (QTD), and the incidence of arrhythmia.

In our preparation, d-sotalol dose dependently lengthens QT (P < .001) and increases MAPD (P < .001) and MAPX (P < .001) at medium or long cycle lengths (1.0 and 1.5 s). This is illustrated in Fig. 11 for MAPX (A and B), endocardial MAPD (C and D), and QT (E and F). At a short cycle length (0.6 s), d-sotalol dose dependently lengthens QT (P < .001) and increases MAPD (P < .001) but not MAPX. Finally, d-sotalol has no significant effect on AV conduction at any cycle length; for example, at a 1-s cycle length, AV conduction time is 129 ± 5 ms at baseline and 128 ± 4 ms at the highest dose. The increased MAPX at longer cycle lengths is due to a more important lengthening of MAPs from the deep subendocardial layers. Finally, d-sotalol is clearly associated with

![Fig. 6. Classical CCBs shorten guinea pig ventricular APs like mibefradil. The DHP amlodipine (A, 10 μM), the phenylalkylamine verapamil (B, 10 μM), and the inorganic ion Cd²⁺ (C, 100 μM) produce very similar effects, although the action of amlodipine and Cd²⁺ may be more pronounced. Pulse intervals of 6 s.](#)
ventricular arrhythmias (Fig. 11G). At the intermediate dose, the effect is still moderate and we only observed three triplets in one animal. However, at the highest dose and the longest cycle length, the increases in endocardial MAPD \((145 \pm 27\) ms) and in MAPX \((68 \pm 18\) ms) were associated with ventricular tachycardia in three dogs, including several runs of typical TdP in two animals. In a fourth animal, the arrhythmias were limited to numerous premature beats and two couplets. Thus, our model clearly demonstrates the proarrhythmic liability of \(d\)-sotalol.

In contrast to \(d\)-sotalol, mibefradil has essentially no effect on the various repolarization parameters, at any cycle length, apart from a small \((25\) ms) but significant \((P < .001)\) decrease in MAPD at all recording depths (Fig. 11). This decrease is probably not drug related because a similar one is observed in the control group. In both groups, there were no arrhythmias. The most noticeable action of mibefradil is to impair AV conduction; in particular, mibefradil increases AV conduction time at all doses \((P < .001)\). At a 0.6-s cycle length, we observed second-degree AV block in two dogs at the intermediate dose and in all dogs at the highest dose. Retrograde conduction is even more sensitive to mibefradil: during ventricular stimulation at a 0.6-s cycle length, we observed second-degree retrograde conduction block in half the dogs at the lowest dose and in all dogs at the intermediate dose. Retrograde block also was observed in one control animal but this was the only conduction disturbance in the control group.

In addition, the highest dose of mibefradil induced a major BP drop in three dogs; this effect was associated with complete AV block and no significant effect was observed in the remaining animals. In contrast, BP remained stable in the control group, whereas the highest dose of \(d\)-sotalol induced a slight increase in systolic BP (from 105 \(\pm 5\) to 120 \(\pm 7\) mm Hg at a 1.5-s cycle length; \(P < .01\)).

**Discussion**

Mibefradil, at supratherapeutic doses, changes the morphology of the T/U wave of the ECG in humans (Giles, 1997; Kobrin et al., 1997) and animals (Fig. 2). Furthermore, at concentrations that could be reached in tissues at supratherapeutic doses, mibefradil blocks delayed-rectifier K⁺ channels in vitro (Chouabe et al., 1998). In this work, we examine whether these observations indicate a proarrhythmic risk. This question arises because many proarrhythmic compounds induce ECG changes (e.g., lengthening of QT interval and broadening and augmentation of T and/or U waves) that bear similarities with the effects of mibefradil (Weissenburger et al., 1991; Vos et al., 1995). Yet, drugs without proarrhythmic liability, such as verapamil and diltiazem, also modify the T/U wave in humans (Giles, 1997; Kobrin et
al., 1997) and animals (Fig. 2), indicating that such changes are not a definite indication of proarrhythmic risk.

It is widely accepted that the T wave reflects the repolarization of the cardiac left ventricle but there are diverse interpretations for the U wave, ranging from a late repolarization of specific cardiac regions to a mechanical artifact. However, most agree that T/U wave morphological changes indicate modifications of ventricular repolarization. Unfortu-
isolated rabbit hearts. Because this last methodology can be from atria because ventricular cells were unavailable, and hearts. Therefore, we also examine human cardiomyocytes, isolated, they may respond differently from cells in intact hearts. MAPD increases slightly.

Fig. 10. Effects of mibefradil on MAPD and on the peak left ventricular developed pressure in isolated rabbit hearts. Mibefradil has little effect on MAPD (A), except for the highest concentration tested where a slight, nonsignificant increase may be seen for very slow cycle lengths. However, at that concentration, contractility is drastically reduced (B), indicating that this concentration is well above therapeutic levels and suggesting that the observed increase may be irrelevant. The number of hearts per data point is shown in parenthesis for each drug concentration; at 10 μM, 50% of the hearts have completely failed and the remaining are near failing.

nately, we do not know how to evaluate the risk of a T/U wave alteration with or without increase in QT interval. Computer simulations can calculate the shape of an ECG produced by a finite series of APs (Winslow et al., 1993). However, from an alteration of the ECG, they cannot deduce the underlying modifications of APs. Nevertheless, a proarrhythmic liability probably results from a lengthening of APs that is heterogeneous in space or in time (Berger et al., 1997). Such lengthening favors early after-depolarizations that, in presence of heterogeneous refractoriness, may trigger local reentrant tachycardia. For these reasons, we investigate whether mibefradil modifies APs.

We use four preparations in this study. First, we examine guinea pig ventricular myocytes, which permit an accurate measurement of APs in individual cells. However, this preparation has two drawbacks: because these cells are not human, drugs may have different effects, and because they are isolated, they may respond differently from cells in intact hearts. Therefore, we also examine human cardiomyocytes, from atria because ventricular cells were unavailable, and isolated rabbit hearts. Because this last methodology can be automated, it allows a high throughput of drugs and much comparative data exists. Here, the concerns are that the hearts are isolated, perfused with artificial solutions, and not human. Finally, we investigate APs in open-chest dogs. This semi-intact but time-consuming preparation provides direct information on arrhythmias.

In guinea pig ventricular myocytes, 1 nM to 10 μM mibefradil shorten APs (Figs. 3 and 4). The EC50 is 90 to 400 nM, similar to the 230 nM reported for the IC50 on L-type Ca2+ channels in these cells (Liang-Min and Osterrieder, 1991). In addition, mibefradil and classical CCBs shorten APs in similar ways (Fig. 6). Because ICaL sustains the AP plateau, these results suggest that mibefradil shortens APs by blocking L-type Ca2+ channels. Mibefradil also may block Na channels weakly because high concentrations reduce the amplitude and velocity of the AP upstroke (Fig. 3). This agrees with a report that mibefradil blocks INa ~300-fold more weakly than ICaL (Liang-Min and Osterrieder, 1991). Finally, the metabolites of mibefradil, alone or in combination, either act like mibefradil or have no effect (Fig. 5).

Like most other CCBs, micromolar mibefradil blocks repolarizing K’ channels (Gotoh et al., 1991; Lefevre et al., 1991; Randall, 1995; Wegener and Navrath, 1996; Avdonin et al., 1997; Chouabe et al., 1998) but it does not block the inward-rectifier current IK1 (Nilius et al., 1997). Because we only observe shortening of APs, ICaL block appears to dominate IK block in cardiomyocytes.

In human atrial myocytes, the balance between the currents generating APs is different from that of guinea pig ventricular cells. In addition to IKur (Wang et al., 1993a), human atrial cells express two outward currents, IKdr (Wang et al., 1993a) and IKur, that strongly contribute to repolarization (Shibata et al., 1989; Coraboeuf and Nargeot, 1993; Wang et al., 1993b; Ferek and Giles, 1995). In these cells, mibefradil does not change APD50 but shortens the AP plateau (Fig. 8), consistent with a predominant block of ICaL, which governs plateau height and duration (Shibata et al., 1989; Coraboeuf and Nargeot, 1993; Li and Nattel, 1997). Indeed, any ICaL decrease, due to pathological conditions (Le Grand et al., 1994) or to chronic treatment with CCBs (Le Grand et al., 1991), shortens the plateau. As expected, mibefradil blocks ICaL in human myocytes at concentrations similar to other species (Fig. 9).

We cannot entirely exclude that block of other channels contributes to the action of mibefradil. Block of T-type Ca2+ channels could reduce the inward current early during the AP but this channel has never been observed in human cardiomyocytes. Block of IK1, if present, might partially counteract block of ICaL but remains dominated by it because APs do not lengthen at any time or dose. Finally, the absence of change in APD95 suggests that mibefradil does not affect the Na+/Ca2+ exchanger, which participates in late repolarization (Bénaréau et al., 1996).

In summary, in isolated cells, mibefradil and metabolites do not induce an AP lengthening but a dose-dependent and reversible shortening. We now want to confirm this result in more intact preparations, where anatomical heterogeneities and interactions are taken into account.

In isolated rabbit hearts, mibefradil (≤1 μM) has no effect on MAPs recorded in two locations (left ventricular septum and epicardium). At 10 μM, mibefradil slows conduction and decreases developed pressure (Fig. 10), indicating that this is above therapeutic range. At 10 μM, MAPD increases slightly.
at slow rates but the effect is not statistically significant. This is in sharp contrast with quinidine and almokalant, which produce marked increases in MAPD and actual arrhythmias in our model (Hondeghem 1994; unpublished observations). That mibefradil does not produce the deleterious effects of quinidine suggests that ICa,L block overcomes IK block for mibefradil only.

In open-chest dogs, therapeutic and supratherapeutic doses of mibefradil do not induce arrhythmias, unlike sotalol (Weissenburger et al., 1991; Vos et al., 1995), d-sotalol (this study), and astemizole (Weissenburger et al., 1999). Unlike mibefradil, these compounds increase QT (Fig. 11 and Vos et al., 1995) by blocking repolarizing K+ currents (for review, see Matyus et al., 1997). Similarly, class III antiarrhythmics increase the duration and dispersion of MAPs; again, this is not seen with mibefradil. Thus, our model demonstrates the proarrhythmic liability of d-sotalol and astemizole, whereas mibefradil is like placebo apart from producing AV block through Ca2+ antagonism. In fact, Ca2+ antagonism may oppose potential “class III” effects. For example, in sheep Purkinje fibers, verapamil suppresses the APD-prolonging and EAD-producing actions of d-sotalol (Hiromasa et al., 1988). One might argue that the same effect is observed in humans, contributing to the safety of verapamil and mibefradil; however, proarrhythmic compounds can induce TdP in humans even if mibefradil or verapamil are coadministered. Therefore, Ca2+ antagonism is not sufficient to completely prevent such arrhythmias.

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**Fig. 11.** Effects of d-sotalol and mibefradil on the transmural dispersion of MAPs (MAPX), the endocardial MAPD, the QT-interval, and the incidence of ventricular arrhythmia (number of dogs exhibiting couplets, triplets, ventricular tachycardia, or TdP) in anesthetized dogs. d-Sotalol increases markedly MAPX (A, B), MAPD (C, D), and QT-interval (E, F) at both short (600 ms) and long (1500 ms) cycle lengths and the incidence of arrhythmia (G). In contrast, mibefradil has little effect on any of these parameters, aside from a very slight reduction in MAPD and QT interval.
One of the premises of cardiac electrophysiology is that the QT interval reflects, in a more or less complex manner, the duration of ventricular APs. How can drugs such as mibe-
fradil and verapamil increase QT without prolonging APs? Or, phrased differently, what is the cause of T/U wave alteration if not AP prolongation? Although we have no definite answer, we can propose a hypothesis. First, it is clear that the ECG represents a gradient of current flow in the myocardium, not an absolute current. If, anytime during ventricular repolarization, all repolarizing cells are evenly distrib-
uted, there is no ECG deflection. Then, there is no reason to assume that repolarization is finished at the end of the T wave; indeed, the U wave may indicate further repolarization and its variability may be related to variations in cardiac geometry. In this case, changes in T/U morphology indicate changes in repolarization geometry and can result as well from lengthening or shortening of APs. This was demonstrated in a computer model of cardiac electrophysiology (Denis Noble, personal communication).

Finally, it is difficult to surmise the electrophysiological effects of comedicated mibebradil with cardiac-acting drugs such as digoxin or amiodarone. In the MACH-1 trial, mortality was higher with mibebradil (27.0%) compared with placebo (24.6%) and most additional deaths could be attributed to sudden deaths (22 of 31) (Robert Pordy, personal commu-
nication). However, mibebradil increased the mortality of patients cotreated with digoxin or amiodarone but decreased that of other patients, suggesting that comedication has det-
ritional electrophysiological effects. How much was due to increased plasma levels of digoxin/amiodarone or to “electro-
physiological interactions” remains unknown.

In conclusion, mibebradil (like verapamil) manifests activities in vivo (changes in T/U wave) and in vitro (block of K+ channels) that are often linked to a proarrhythmic risk. Nev-
evertheless, this potentiality is not confirmed in the clinic (no higher risk of TD and mibebradil monotherapy) or in our models (no lengthening or dispersion of APs, no arrhythmias in animals). Thus, mibebradil can be grouped with verapamil and amiodarone, two other compounds that affect repolariza-
tion and block K+ channels but have little or no proarrhythmic risk.

Acknowledgments

We are grateful to Stéphane Hatem and Jean-Jacques Mercadier for hosting us in their laboratories during our human tissue exper-
iments and to the surgical team of J-Y Neveux for supplying the tissue. We also thank Michael Weber, Patrick Hess, Evelyne Dornbierer, Bruno Hespel, and Eliane Fliegeli for excellent technical assistance.

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Mol Pharmacol 51:721–726.


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