Laboratory Investigation

Specific $I_{K1}$ Blockade: A New Antiarrhythmic Mechanism?

Effect of RP58866 on Ventricular Arrhythmias in Rat, Rabbit, and Primate

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Background. The effectiveness of blockade of the inwardly rectifying $K^+$ current ($I_{K1}$) in prevention of arrhythmias is unknown. We have examined the antiarrhythmic potential of a new selective $I_{K1}$ blocker, RP58866, in rat, rabbit, and primate (marmoset) isolated hearts in the settings of acute ischemia and reperfusion.

Methods and Results. In concentration–response studies ($n$=12 per group), the drug reduced ischemia-induced ventricular fibrillation (VF) in rat from control incidence of 100 to 50%, 17% ($p<0.05$), and 0% ($p<0.05$) at 1, 3, and 10 $\mu$mol/L, respectively. RP58866 produced significant bradycardia at the 3- and 10-$\mu$mol/L concentrations and significant QT interval widening at all three concentrations ($p<0.05$). When rat hearts ($n$=12 per group) were paced (5 Hz) via the left atrium to prevent bradycardia, the antiarrhythmic effects of 10-$\mu$mol/L RP58866 were unmodified (ischemia-induced VF incidence was reduced by drug from 83% in control hearts to 8%; $p<0.05$). Similarly, pacing did not prevent the drug’s QT-widening activity at 90% repolarization ($QT_{90}$ was 64±3 msec in control hearts versus 128±17 msec in the presence of 10 $\mu$mol/L of drug; $p<0.05$). In separate groups of rat hearts ($n$=10 per group) subjected to 10 minutes of ischemia, reperfusion-induced VF incidence was reduced from 90% in control hearts to 10% ($p<0.05$), 0% ($p<0.05$), and 0% ($p<0.05$) by 1-, 3-, and 10-$\mu$mol/L RP58866. To examine whether drug actions were species-specific, we performed further studies in rabbit and primate using the middle concentration of RP58866 (3 $\mu$mol/L). Ischemia-induced VF incidence was too low in these species to assess the effects of the drug. However, RP58866 widened QT interval ($p<0.05$), slowed heart rate ($p<0.05$), and reduced the incidence of reperfusion-induced VF from 67% to 8% ($p<0.05$) in rabbit. Furthermore, in the more clinically relevant primate species (marmoset; $n$=9–12 per group), RP58866 (3 $\mu$mol/L) abolished ischemia-induced VT (36% incidence in control hearts; $p<0.05$) and significantly reduced the incidence of ischemia-induced ventricular premature beats from 91% to 33% ($p<0.05$). The drug was also effective against reperfusion VF in primates (incidence reduced from 64% in control hearts to 11%; $p<0.05$). As in rat and rabbit, RP58866 significantly widened QT interval in primate and caused bradycardia before and during ischemia. RP58866 had no significant influence on coronary flow in any species. Finally, in further studies on rat, QT widening by RP58866 was found to persist relatively unmodified in nonischemic hearts perfused with solution containing $K^+$ elevated to 8 $\text{mmol/L}$ to mimic the early ischemic milieu.

Conclusions. RP58866, a selective $I_{K1}$ blocker, is a potent and efficacious new antiarrhythmic drug in ischemia and reperfusion in rat, rabbit, and primate. When tested in rat, pharmacological activity was undiminished by cardiac pacing or elevation of extracellular $K^+$. (Circulation 1993;87:1979–1989)

KEY WORDS • ventricular fibrillation • myocardial ischemia • reperfusion • primates

Potassium ($K^+$) channel blockers and their use as antiarrhythmic agents have received much attention recently. Such drugs are generally designated as class III antiarrhythmics, because they prolong action potential duration (APD) and/or QT interval in the absence of significant effects on conduction velocity. However, until recently, all the available...
K⁺ channel blockers have been relatively nonselective, blocking more than one K⁺ channel, other channels, and receptors as well. Recently, new agents have been developed that not only affect APD selectively but affect particular cardiac repolarizing K⁺ currents selectively. These include tidesamil, which blocks the transient outward current (Iₒ); glibenclamide, which blocks the ATP-dependent K⁺ channel (I_KATP) and prolongs APD selectively during ischemia; UK66,914, which blocks the delayed rectifying K⁺ current (I_K)⁴,⁵; and RP58866, an inwardly rectifying K⁺ current (I_K') blocker.⁶–⁷ These agents make it possible to examine the value of selective blockade of an individual K⁺ current as a mechanism for suppression of arrhythmias.

Until recently, the majority of work on class III agents has focused on I_K blockers. Hitherto, it has proven difficult to unequivocally link blockade of a particular channel to arrhythmia suppression, since one cannot measure channel block and arrhythmia suppression continuously. We have shown that UK66,914, a new and highly selective I_K blocker, widens QT interval and reduces the incidence of reperfusion-induced arrhythmias by more than 50% in rabbit, a species possessing functional cardiac I_K,⁹ whereas in rat, a species deficient in I_K,¹⁰ the drug is devoid of activity.¹¹ This shows that I_K block is a specific mechanism for suppression of ventricular fibrillation (VF) during reperfusion. There is currently much interest in blockade of I_KATP and its effectiveness as an antiarrhythmic mechanism, but results are in conflict with some studies that show I_KATP blockers to be beneficial¹²–¹⁶ and others that find them ineffective.¹³,¹⁴ Block of I_K has been examined by Tsu­chihashi and Curtis using tidesamil.¹⁵ Although tidesamil had no effect on the incidence of ischemia- or reperfusion-induced VF in rat in vitro, it decreased the duration of VF once it had started. In rat in vivo, tidesamil reduces ischemia-induced VF incidence,¹⁶ although this occurs only at high doses beyond the range over which the drug is selective for I_K.²

Until recently, there has been very little information on the utility of I_K block as an antiarrhythmic mechanism. This is primarily a consequence of the lack of selective agents available to probe this channel. In the present study, our aim was to investigate the effect of I_K blockade on arrhythmias and QT interval using the novel I_K blocker RP58866 (Figure 1). This agent has been characterized pharmacologically. At concentrations up to 30 µmol/L, RP58866 is a highly selective blocker of I_K and has no effect on inward currents (I_Na and I_o) or other potassium currents [I_K and I_KATP]; it prolongs APD, QT interval, and atrial, nodal, and ventricular refractory periods with no effects on conduc­tion velocity or excitability, and it has no effect on resting membrane potential.⁵–⁸,¹⁷ Thus, at concentrations up to 30 µmol/L, RP58866 is a highly selective tool for probing the utility of I_K block as an antiarrhythmic mechanism.

We investigated the effects of RP58866 in three species: rat, rabbit, and marmoset. Rat was used as a first-line bioassay. Since rat ventricle possesses no functional I_K,¹⁰,¹⁸ and since neither I_K blockers¹⁹ nor selective I_K blockers²⁰ possess antifibrillatory activity in the perfused rat heart, the preparation is particularly suited to detecting potentially antiarrhythmic effects of I_K blockade because, although RP58866 (<30 µmol/L) does not block I_K or I_o,²⁰ use of rat provides additional reason for excluding I_K or I_o block as mechanisms potentially contributing to any antiarrhythmic effects of the drug. Rabbit was used to confirm actions on reperfusion arrhythmias in a second species. Marmoset was used to determine whether the drug at a concentration effective in rat could reduce ischemia- and reperfusion-induced arrhythmias in primate hearts.

Our preliminary findings have been presented in part to the American Heart Association,²¹,²² the British Pharmacological Society,²³ and the International Society for Heart Research.²⁴

Methods

The perfusion technique, methods for induction of ischemia and reperfusion, methods for verification and quantification of occluded-zone size, and the techniques for recording, quantifying, and analyzing data have all been described previously.²⁵–²⁷ All experiments were performed in accordance with the American Physiological Society guidelines and the United Kingdom Home Office “Guide on the Operation of the Animals (Scientific Procedures) Act 1986.”

Animals and General Experimental Methods

Rats (male Wistar; Bantin and Kingman, UK; 250–300 g) were anesthetized with pentobarbitone (60 mg·kg⁻¹ i.p.). Hearts were excised 30 seconds after an intravenous injection of 250 units sodium heparin and placed into ice-cold perfusion solution containing (in mmol/L): NaCl 118.5, NaHCO₃ 25.0, MgSO₄ 1.2, NaH₂PO₄ 1.2, CaCl₂ 1.4, KCl 3.0, and glucose 11.1. Hearts were perfused according to the Langendorff method,²⁶ with perfusion solution delivered at 37°C and pH 7.4. All solutions were filtered (5-µm pore size) before use. Perfusion pressure was constant and equivalent to 100 cm H₂O. A unipolar electrogram (ECG) was recorded by implanting a stainless steel wire electrode into the center of the region to become ischemic, with a second electrode connected to the aorta. ECG configuration, including QT interval at 100% and 90% repolarization, was evaluated as described previously.²⁷ A traction-type coronary occluder consisting of a silk suture (Mersilk, 4-0) threaded through a polyethylene guide was used for coronary occlusion. The suture was positioned loosely around the left main coronary artery beneath the left atrial appendage. Regional ischemia was induced by tightening the occluder and reperfusion by releasing it.

To exclude the possibility that any beneficial effects seen in rat studies are the result of the unusual cardiac electrophysiology of the rat,¹⁵,²⁹ we repeated essential parts of the studies in rabbit, which has markedly different cardiac action potential morphology, heart rate, and intracellular cation content.³⁰
Rabbits (male New Zealand White; Froxfield, UK; 0.9–1.2 kg; n=12 per group) were anesthetized by means of pentobarbitone (60 mg·kg⁻¹ i.v.) with sodium heparin (250 units i.v.). Perfusion was as described for the rat except that the Ca⁴⁺ concentration of the perfusion solution was raised to 2.8 mmol/L to raise susceptibility to arrhythmias, as described previously. The ECG was recorded in the same way as for rat studies.

To examine the actions of RP58866 in an animal species presumed to be more closely allied to human, studies were performed in primate. Marmosets (male Callithrix jacchus; 300–350 g; n=9–12 per group) were anesthetized with Saffan (a mix of 0.9% α-vaxilone plus 0.3% a-dolone, of which we gave 2 mL/kg i.m.). The heart was arrested and exsanguinated in vivo by insertion of a 23-gauge needle into the left ventricle for infusion of ice-cold Krebs' solution (constituents as described above for rat studies). This was done primarily because the animals were used jointly in separate studies requiring in vivo exsanguination of other organs (e.g., brain). Cardiac excision and Langendorff perfusion were performed as described above for rat and rabbit.

Experimental Protocols

Ischemia-induced arrhythmias in unpaced rat hearts. The first protocol was designed to allow identification of antiarrhythmic activity of RP58866 and used the isolated rat heart model. Hearts (n=12 per group) were perfused for an initial 5 minutes with salt solution (constituents described above), then solution was switched in a randomized, blinded fashion to one of four solutions modified by addition of stock solutions to contain 0-, 1-, 3-, or 10-μmol/L RP58866. The 0-μmol/L stock solution was vehicle (deionized water). After a further 5 minutes of perfusion, the left coronary artery was occluded. After 30 minutes of ischemia, the occluder was released to achieve reperfusion.

Reperfusion-induced arrhythmias in unpaced rat hearts. Owing to the expected high incidence of sustained VF in the control group during 30 minutes of ischemia, a separate study was performed to investigate the effect of the drug on reperfusion-induced arrhythmias. Hearts (n=10 per group) were perfused, randomized to one of four solutions (0-, 1-, 3-, or 10-μmol/L RP58866), and subjected to regional ischemia as above. In these hearts however, the occluder was released to achieve reperfusion after a shorter duration of ischemia (10 minutes). This duration of ischemia was used because, although it is associated with a high susceptibility to reperfusion-induced VF, it is sufficiently brief to preclude a high incidence of sustained ischemia-induced VF, which would otherwise interfere with assessment of reperfusion-induced arrhythmias in this model.

Arrhythmias in paced rat hearts. In previous studies we found that tacrine, a nonselective I₅₃ blocker, produces sinus bradycardia, so we anticipated the possibility of bradycardia with RP58866. To examine the role of any bradycardia in modulating the antiarrhythmic actions of RP58866, we performed additional studies in paced rat hearts (n=12 per group). Methods were as for unpaced hearts (above) except that RP58866 was investigated at the highest concentration only (10 μmol/L). Hearts were paced with a Harvard Student Stimulator using stainless steel electrodes impaled in the left atrium (twice threshold voltage; pulse width, 0.05 second). The stimulation frequency chosen (5 Hz) produces a heart rate slightly above that expected in unpaced control hearts after 10 minutes of regional ischemia. QT was measured at 90% repolarization (QT₉₀) in these experiments, since the end of repolarization was obscured, in some hearts, by the stimulus artifact, precluding accurate assessment of QT at 100% repolarization.

Potassium dependence of RP58866's effects on QT and RR in rat. Many class III agents lose their pharmacological activity in depolarized tissue and high-K⁺ environments. In previous studies, using the present rat model, we found that effects of certain potassium channel blocker drugs on QT interval are attenuated by extracellular K⁺ elevation. Since ischemia elevates extracellular K⁺ concentration, exaggeration or diminution of QT widening by perfusion with raised-K⁺ solution can give an indication of whether this important component of the ischemic milieu is likely to promote or antagonize effects of the drug. Thus, rat hearts (n=10 per group) were perfused with each of four modified solutions (8-mmol/L K⁺, 3-mmol/L K⁺, 3-mmol/L K⁺ plus 3-μmol/L RP58866, and 8-mmol/L K⁺ plus 3-μmol/L RP58866) for sequential 10-minute periods. During each period, three recordings were taken (after 1, 5, and 9 minutes of perfusion) of QT, RR, and PR intervals. PR was corrected for changes in RR (PRc) because (unlike QT) it is rate-dependent in rat.

Ischemia- and reperfusion-induced arrhythmias in rabbit hearts. Hearts (n=12 per group) were subjected to 30 minutes of ischemia as for rat studies. We were able to use the same rabbits for study of ischemia- and reperfusion-induced arrhythmias, because isolated hearts from this species have a much lower susceptibility to ischemia-induced sustained VF than rat. To limit animal use, we examined only two groups, control hearts and 3-μmol/L RP58866.

Ischemia- and reperfusion-induced arrhythmias in marmoset hearts. There are no published data on ischemia or reperfusion arrhythmias in marmoset. In the marmoset-studies, we followed a protocol similar to that for rat and used similar perfusion solution. Hearts were subjected to 30 minutes of ischemia by ligation of the left anterior descending coronary artery close to its origin at a position approximately equivalent to that for rat and rabbit studies. Two groups were studied, control hearts (n=12) and 3-μmol/L RP58866 (n=9).

Measurement of Occluded-Zone Size

Two independent methods were used to verify occlusion and to delineate the occluded zone from uninvolved tissue. First, coronary flow was measured by timed collection of coronary effluent in a graduated cylinder; occlusion was verified by comparing flow at 1 minute before occlusion with flow at 1 minute after occlusion and was quantified in terms of the percentage reduction in flow. Second, at the end of 5 minutes of reperfusion, readmission of flow was verified and the size of the formerly occluded zone was quantified by the disulfine blue dye exclusion method. Occluded-zone size was quantified as a percentage of total ventricular weight. Values of coronary flow in the uninvolved tissue and the reperfused zone were calculated from the total.
coronary flow and the weights of the occluded zone and the uninvolved zone, as described previously.26 We expected occluded-zone sizes of approximately 40% total ventricular weight in rat and rabbit.11 The deficiency of coronary collaterals in rat and rabbit40 means that occluded zone determined by dye is equivalent to zone by flow reduction.26 In marmoset (for which no data exist), we aimed for an occluded-zone size equivalent to that in the other species by placing the coronary occluder in an equivalent location. Collateral flow in marmoset was determined by comparing estimates of occluded-zone size measured by flow reduction and dye methods, since dye exclusion delineates the zone of underperfusion, whereas flow reduction defines the extent of global reduction. Collateral flow was calculated as milliliters per minute per gram of underperfused myocardium and was expressed as percent of flow in the uninvolved zone (delineated by dye).

**Exclusion Criteria**

Any rat heart with a sinus rate of <250 beats per minute or a coronary flow of >18 mL/min or <8 mL/min at 5 minutes before the onset of ischemia (and before randomization) was excluded. Likewise, any rabbit with a sinus rate of <200 beats per minute or a coronary flow of >80 mL/min or <35 mL/min was excluded.31 Exclusion criteria for marmoset could not be defined before the study because no data base exists. Therefore, we applied le Chatlier’s principle and excluded any heart for which heart rate or coronary flow values lay more than 2 SD from the group mean. For all three species, any heart not in sinus rhythm during the 2 seconds before reperfusion was excluded from the reperfusion sample.

**Arrhythmia Diagnosis and ECG Analysis**

A digital storage-type oscilloscope (model DSO400, Gould) and a Gould chart recorder (model RS3200) were used in the identification and analysis of waveforms and diagnosis of arrhythmias. Arrhythmias were defined according to the Lambeth Conventions41 with slight modification.15 Ventricular premature beats (VPB) were defined as discrete and identifiable premature QRS complexes; a run of four or more VPBs was defined as ventricular tachycardia (VT). VF was defined as a signal from which individual QRS deflections vary in amplitude and coupling interval on a cycle-to-cycle basis. Very often, one type of arrhythmia may convert into another type without an intervening period of sinus rhythm; this has been amply illustrated (for one of the models used in the present study) by anecdote in published papers.26 When this conversion occurs, each type of arrhythmia is recorded as having been present during the period of assessment. During ischemia, VF generally initiates directly from sinus rhythm, whereas during reperfusion, VF generally begins after a run of VT of increasing complexity and irregularity.26 Use of fast chart speed recording or fast sweep speed oscilloscope monitoring in conjunction with strict adherence to the Lambeth Conventions41 with appropriate modifications15 is necessary to permit objective assessment of the classification, time of onset, and duration of ventricular tachyarrhythmias. Furthermore, blinded evaluation of all variables is mandatory.

From the ECG, the incidence and the time to onset of arrhythmias, the RR interval, and the QT interval measured at the point of 100% repolarization (or at 90% repolarization in paced heart studies) with on-screen cursors were obtained (see Figure 2). In isolated rat heart, QT is rate independent, but in rabbit, QT appears to vary with heart rate. For this reason, we have presented rat QT data uncorrected (QT), but have corrected rabbit data using Zbinden et al’s formula (QTZ = QT/RR). Appropriate QT correction for marmoset is unknown. For this reason, we have expressed QT uncorrected (QT), corrected by Zbinden’s formula (QTZ), and corrected by Bazett’s formula (QT = QT/√RR).

**Drugs and Materials**

RP58866 (batch number PHI 2050) was a gift from Rhone-Poulenc Rorer (France). It was stored as a 10⁻² mol/L stock solution in deionized water. All salts were reagent grade chemicals from Sigma Chemical Co. Water for preparing perfusion solution was supplied with a reverse osmosis system (Milli-RO 10 and Milli-Q 50, Millipore Ltd).

**Statistics**

Measurement of all variables was performed in a blinded manner, permitting use of sampling-based statistics. Gaussian-distributed variables were expressed as mean±SEM and were subjected to ANOVA. In rat, if treatment constituted a significant source of variance, each group was compared with the control by Dunnett’s test. In rabbit and marmoset studies in which only one drug concentration was used, Gaussian-distributed data were evaluated by unpaired t test. In high-K⁺ perfusion studies, ECG intervals were evaluated by paired analysis. The time to onset of first arrhythmia was log₁₀ transformed to generate a Gaussian-distributed variable as described previously.25 Binomially distributed variables were compared by χ² test with Yates’s correction where appropriate.44 A value of p<0.05 was taken as indicative of a statistically significant difference between values.

**Results**

**Ischemia and Reperfusion Studies in Rat**

RP58866 significantly reduced the incidence of ischemia-induced arrhythmias in a concentration-dependent manner. At the highest drug concentration, VT and VF were completely abolished (p<0.05), and VPB...
incidence was significantly reduced (Figure 3). In the 10-μmol/L group, four of 12 hearts were entirely free of arrhythmias during 30 minutes of ischemia. The onset of ischemia-induced VF was slightly delayed from 3.1±0.03 log₁₀ seconds in control hearts to 3.2±0.02 log₁₀ seconds in 1-μmol/L RP58866–treated hearts (p<0.05) (only two hearts of the 24 treated with the two higher concentrations of drug developed VF, so onset times could not be assessed in these groups). When ischemia-induced arrhythmias were analyzed in relation to their time course of occurrence, it was found that RP58866 reduced the occurrence of VPB, VT, and VF to a similar degree in each successive 5-minute interval, although the effects were statistically significant only

**FIGURE 3.** Bar graphs showing effect of 0-, 1-, 3-, and 10-μmol/L RP58866 on group incidences (%) of ischemia-induced ventricular fibrillation (VF) (solid bars), ventricular tachycardia (VT) (hatched bars), and ventricular premature beats (VPB) (open bars) in rat (n=12 hearts per group). *p<0.05 vs. control hearts.

**FIGURE 4.** Bar graphs showing effect of 0-μmol/L (panel A), 1-μmol/L (panel B), 3-μmol/L (panel C), and 10-μmol/L (panel D) RP58866 on the time course of occurrence of ischemia-induced ventricular fibrillation (solid bars), ventricular tachycardia (hatched bars), and ventricular premature beats (open bars) in rat (n=12 hearts per group).

**FIGURE 5.** Bar graphs showing effect of 0-, 1-, 3-, and 10-μmol/L RP58866 on group incidences (%) of reperfusion-induced ventricular fibrillation (VF) (solid bars), ventricular tachycardia (VT) (hatched bars), and ventricular premature beats (VPB) (open bars) in rat (n=10 hearts per group). *p<0.05 vs. controls.
when control incidences were sufficiently high to permit “detection” of the effects (Figure 4).

RP58866 was also concentration-dependently effective against reperfusion-induced arrhythmias in rat (Figure 5). The 3-μmol/L concentration abolished VF, and the 10-μmol/L concentration abolished VF and VT and reduced VPB incidence (p<0.05). RP58866 significantly widened QT and RR intervals (Table 1) before and during ischemia.

RP58866 (examined at the highest concentration only) retained its antiarrhythmic actions on ischemia- and reperfusion-induced arrhythmias when hearts were paced at a frequency of 5 Hz (Figure 6). In paced hearts, the onset of the first ischemia-induced arrhythmia was significantly delayed by the drug from 2.70±0.02 to 3.0±0.04 log_{10} seconds. The drug also retained its ability to widen QT interval when hearts were paced (Table 2).

RP58866 had no effect on coronary flow or occluded-zone size. Coronary flow 1 minute before the onset of ischemia was 12±0.7, 10±0.8, 12±0.6, and 12±1.1 mL·min^{-1}·g^{-1} in control, 1-μmol/L, 3-μmol/L, and 10-μmol/L groups, respectively (p=NS), and recovery of coronary flow on reperfusion was 9±1.5, 12±2.0, 11±1.1, and 9±1.2 mL·min^{-1}·g^{-1} of reperfused tissue, respectively (p=NS). Occluded-zone sizes were similar in each group, values being 41±3%, 40±3%, 40±2%, and 42±2% of total ventricular weight with increasing concentrations of drug (p=NS). Thus, antiarrhythmic effects were not secondary to changes in flow or changes in occluded-zone size.

**Potassium Dependence of RP58866’s Effects on QT, RR, and PR Intervals in Rat**

The effects of elevation of extracellular K+ on the ability of RP58866 to widen QT interval were examined in the absence of ischemia in separate groups of rat hearts (Figure 7). Switching from 8-mmol/L K+ to 3-mmol/L K+ perfusion solution had no significant effect on QT (93±5 versus 99±8 msec). In 3-mmol/L K+ solution, 3-μmol/L RP58866 widened QT within 1 minute to 131±12 msec (p<0.05) and continued to widen it to 156±11 msec (p<0.05) after 9 minutes. When K+ was increased to 8 mmol/L in the presence of drug, there was a significant QT shortening (p<0.05) to 133±12 msec, although by 9 minutes after the change, QT had lengthened to 141±10 msec.

Switching from high to low K+ in the absence of drug widened RR from 206±9 to 221±7 msec (p<0.05). On introduction of RP58866, RR was further widened to 264±11 msec (p<0.05). When K+ was increased to 8 mmol/L in the presence of drug, no additional bradycardia was seen.

RP58866 had no effect on PR interval corrected for heart rate (PRc). PRc was significantly shortened, from 47±1 to 40±2 msec, after switching from 8 mmol/L to 3

### Table 1. Effect of RP58866 on QT Interval and RR Interval in Rat

<table>
<thead>
<tr>
<th>Time</th>
<th>Control hearts</th>
<th>1.0 μmol/L</th>
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<td>154±17*</td>
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<td>185±16</td>
<td>221±15*</td>
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**RR (msec)**

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<th>10.0 μmol/L</th>
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<td>+10</td>
<td>284±13</td>
<td>310±10</td>
<td>364±18*</td>
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Time refers to minutes before (−) and after (+) the onset of ischemia. *p<0.05 vs. time-matched control hearts.

### Table 2. Effect of RP58866 on QT Interval Measured at 90% Repolarization in Paced and Unpaced Rat Hearts

<table>
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<tr>
<td>+10</td>
<td>64±3</td>
<td>128±17*</td>
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</table>

**QT_{90} (msec) in paced hearts**

**QT_{90} (msec) in unpaced hearts**

<table>
<thead>
<tr>
<th>Time</th>
<th>Control hearts</th>
<th>RP58866</th>
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<tbody>
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<td>+1</td>
<td>53±3</td>
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<tr>
<td>+10</td>
<td>65±3</td>
<td>159±15*</td>
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*QT_{90}, QT interval measured at 90% repolarization. Time refers to minutes before (−) and after (+) the onset of ischemia. *p<0.05 vs. time-matched control hearts.
mmol/L of K⁺ in the absence of drug. Introduction of RP58866 had no effect on PR (39±3 msec), and raising K⁺ in the presence of drug caused PR to lengthen back to 50±4 msec (p<0.05).

**Effects of RP58866 in Rabbit**

Confirmatory studies were undertaken in rabbit, using a concentration of RP58866 (3 μmol/L) found to be effective in rat. Effects in rabbit were essentially similar to effects in rat. Control incidence of arrhythmias is very low in rabbit isolated heart during ischemia (Figure 8A), which precludes assessment of any antiarrhythmic activity a drug might possess in this setting, as demonstrated previously. This does, however, make the rabbit an ideal species in which to detect any drug-induced proarrhythmic events. Figure 6B shows that RP58866 had no proarrhythmic activity.

Reperfusion-induced VF occurred with a high incidence (eight of 12) in control rabbit hearts (as expected), permitting analysis of the antiarrhythmic effects of RP58866. RP58866 significantly reduced the incidence of the most severe reperfusion-induced arrhythmias (VF and VT) but had no effect on VPB incidence (Figure 8B).

As in rat, RP58866 elicited significant bradycardia and caused significant widening of QT interval (corrected for alterations in rate; Table 3) but was devoid of effects on coronary flow (Table 3) in rabbit. Occluded-zone sizes were slightly higher than values in rat at 48±1% and 46±3% of ventricular weight in control and drug-treated hearts, respectively (p=NS).

**Effects of RP58866 in Primate**

RP58866 was examined in marmoset at the same concentration (3 μmol/L) as studied in rabbit. The actions were essentially similar in rat and rabbit. The slightly higher control incidence of VT and VPB during ischemia gave scope for assessment of antiarrhythmic activity in this setting. Both VT and VPB incidences were significantly reduced by drug (from 36% to 0% and from 91% to 33%, respectively; p<0.05). VF incidence was very low in marmoset during ischemia, but there was no proarrhythmia (Figure 7A). During reperfusion, RP58866 significantly reduced VF incidence, from 64% to 11%, but had no significant effects on either VT or VPB incidence (Figure 9B).

RP58866 elicited bradycardia and QT widening in marmoset (Tables 4 and 5). Changes were similar to those seen in rat and rabbit. QT was expressed in three different ways, since appropriate heart rate correction is not known for marmoset. QT uncorrected for heart rate was widened in marmoset to an extent similar to that in rat. For example, values 10 minutes after the onset of ischemia were increased by 70% in marmoset and 82% in rat by 3 μmol/L of drug (compare with time-matched control values). Likewise, QTc was widened to a similar extent in marmoset and rabbit 10 minutes after ischemia onset (by 18% and 16%, respectively). This indicates that when appropriate (species-dependent) correction for heart rate is applied, RP58866 affects QT similarly in different species.
RP58866 had no effect on flow before or during ischemia (Table 5) or on recovery of flow on reperfusion (13±1 mL · min⁻¹ · g⁻¹ of reperfused tissue versus 15±1 mL · min⁻¹ · g⁻¹ in control and drug-treated hearts, respectively; p=NS). Occluded-zone sizes were similar in control and drug groups whether measured by flow-reduction or dye-exclusion methods (in control group, 45±1% by dye method and 45±3% by flow reduction versus in drug-treated hearts, 47±3% by dye method and 46±3% by flow-reduction measurements; p=NS). The consistency between flow and dye measures of occluded-zone size, reminiscent of equivalent data for rat,26 indicates an absence of functional coronary collateral vessels in this species, as is the case for rat and rabbit.40 The similarity between control and drug groups for each of these estimates indicates an inability of RP58866 to increase collateral flow in this species. Findings were similar for rat and rabbit.

**Discussion**

Class III antiarrhythmic drugs represent a potential alternative to class I drugs in ischemic heart disease, given the problems associated with the latter45 and in view of the antiarrhythmic efficacy of agents such as d-sotalol.46-48 However, class III agents may have an inherent disadvantage in that their actions are commonly diminished with the short coupling intervals typical of ventricular tachyarrhythmias.49 This loss of activity at shorter cycle lengths is least prominent with amiodarone but appears to be a common feature of most class III agents,10,50,51 and it has been argued that therapeutic efficacy may be jeopardized as a result of it.52 The reason for this is not clear. However, it may not be a result of reverse use-dependence, as purported by Hondeghem and Snyders,52 since others have reported conventional use-dependence with class III agents.49,53

Class III agents are not a homogeneous class, however. Subclassification is possible in terms of the particular K⁺ channel blocked by the agent, according to the suggestion of the Sicilian gambit54 as discussed in more detail recently by Rosen et al55 and Colatsky.56 Most class III agents block Iₖ.49 However, some investigational agents, such as tacrine, also block Iₖ,32 The latter may represent a viable alternative to Iₖ blockers. However, their effectiveness as antiarrhythmics is not established.

The present study is the first to examine the potential of a new and highly selective Iₖ, blocker in prophylaxis of ischemia- and reperfusion-induced arrhythmias. We observed consistent antiarrhythmic effects in all three species studied (rat, rabbit, and marmoset), with effects being concentration-dependent where studied (in rat). Effects were accompanied by equivalent QT widening and bradycardia in each species.

The first question to consider is whether these effects were direct or secondary to “anti-ischemic” actions. An indirect antiarrhythmogenic action (e.g., via some anti-ischemic property) can be ruled out. The reasons for this are as follows. First, anti-ischemic interventions delay the onset of ischemia-induced and reperfusion-induced VF susceptibility (shown explicitly in the rat model57); they do not suppress arrhythmias throughout the time course of ischemia, in contrast to the findings with RP58866 in

### TABLE 3. Effect of RP58866 on QT₂ Interval, RR Interval, and Coronary Flow in Rabbit

<table>
<thead>
<tr>
<th>Time</th>
<th>Control hearts 3 μmol/L</th>
<th>Control hearts 3 μmol/L</th>
<th>Control hearts 3 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>0.62±0.02</td>
<td>0.61±0.01</td>
<td>260±8</td>
</tr>
<tr>
<td>-1</td>
<td>0.62±0.02</td>
<td>0.73±0.02*</td>
<td>294±5</td>
</tr>
<tr>
<td>+10</td>
<td>0.61±0.02</td>
<td>0.71±0.02*</td>
<td>336±17</td>
</tr>
</tbody>
</table>

QT₂, QT interval corrected by Zblinder's formula.⁶² Time refers to minutes before (−) and after (+) the onset of ischemia. *p<0.05 vs. time-matched control hearts.

#### Figure 9. Bar graphs. Panel A shows the effect of RP58866 on incidences (%) of ischemia-induced ventricular fibrillation (VF), ventricular tachycardia (VT), and ventricular premature beats (VPB) in perfused marmoset hearts. Control hearts (n=12) are represented by solid bars and 3 μmol/L. RP58866-treated hearts (n=9) by open bars. The drug significantly reduced the incidence of ischemia-induced VT and VPB. The incidence of VF was too low to permit assessment of antiarrhythmic activity. Panel B shows the effect of RP58866 on incidences (%) of reperfusion-induced VF, VT, and VPB in perfused marmoset hearts. Control hearts (n=12) are represented by solid bars and 3 μmol/L. RP58866-treated hearts (n=9) by open bars. *p<0.05 vs. control hearts (0 μmol/L). The drug significantly reduced the incidence of reperfusion-induced VF, but had no significant effect on VT or VPB.

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TABLE 4. Effect of RP58866 on QT Interval Corrected and Uncorrected in Primate

<table>
<thead>
<tr>
<th>Time</th>
<th>Control hearts</th>
<th>QT</th>
<th>Control hearts</th>
<th>QT_z</th>
<th>Control hearts</th>
<th>QT_z</th>
<th>Control hearts</th>
<th>QT_z</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>137±3</td>
<td>148±6</td>
<td>0.51±0.02</td>
<td>0.53±0.03</td>
<td>8±0.2</td>
<td>8±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td>151±6</td>
<td>231±14*</td>
<td>0.58±0.03</td>
<td>0.64±0.05</td>
<td>9±0.4</td>
<td>12±0.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+10</td>
<td>159±10</td>
<td>270±18*</td>
<td>0.56±0.02</td>
<td>0.66±0.04*</td>
<td>9±0.5</td>
<td>13±0.6*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

QT represents uncorrected QT interval; QT_z represents QT corrected for changes in heart rate by Zbinden's formula (see text); QT_z represents QT corrected by Bazett's formula (see text). Time refers to minutes before (−) and after (+) the onset of ischemia.

*p<0.05 vs. time-matched control hearts.

the rat (Figure 4). Second, pacing rat hearts failed to reverse the protective effects of the drug, as would be expected if anti-ischemic actions contributed to the antiarrhythmic effects. Therefore, the drug can be presumed to have affected arrhythmias as a consequence of a direct effect on cardiac electrophysiology.

We used the rat to examine the role of ventricular I_{K1} block versus other cellular mechanisms in mediating the antiarrhythmic effects. Since previous work has shown that UK66,914, a selective I_K blocker, has no effect on heart rate, QT interval, or arrhythmias in rat; since adult rat ventricle is deficient in functional I_{K1}; and since RP58866 does not block I_K over the concentration range used in the present study, then I_{K1} block can be excluded as a mechanism contributing to the drug's antiarrhythmic effects. Also, since previous work has shown that I_{to} block with tedisamil is ineffective in preventing VF in the isolated rat heart model; and since RP58866 does not alter the action potential shape in the "notch" region where I_{to} is active; then I_{to} block would not appear to be sufficient to explain any antiarrhythmic effect of the drug in rat. In addition, RP58866 has no effect on I_{es}, I_{KATP}, or T or L calcium currents, so block of these currents can be excluded as mechanisms contributing to the drug's effects on arrhythmias. However, RP58866 does have established effects on I_{K1}. These effects lead to APD widening at voltages negative to 1 mV. Furthermore, in contrast to UK66,914, RP58866 widens QT and prevents VF in rabbit and in rat (UK66,914 is effective in rabbit but has no pharmacological activity in rat). On the basis of these data, I_{K1} blockade appears to be a mechanism sufficient to account for the antiarrhythmic effects observed in rat in the present study.

An indication that the drug may be a specific I_{K1} blocker but not necessarily a completely selective blocker is that it caused pronounced bradycardia in all three species tested. This is puzzling, because although the periphery of the sinoatrial node has been shown to possess I_{K1}, the center of the node, which is responsible for determining heart rate, is believed to be devoid of this current. For this reason, effects on currents yet untested, such as the hyperpolarization-activated inward current (I_h), which appears to be important in determining pacemaker rate, and subtypes of I_{to} (I_{to1} and I_{to2}) cannot be ruled out completely. However, the important issue in relation to the present study is whether any such effects contributed to the suppression of arrhythmias produced by the drug via the resultant reduction in heart rate. Additional studies indicated that drug-induced bradycardia was not responsible for the antiarrhythmic effects seen, since cardiac pacing via the left atrium failed to diminish the drug's ability to reduce and abolish ischemia- and reperfusion-induced VF. Concomitant with this, QT interval remained widened by the drug in paced hearts. These findings suggest that the drug is protective primarily via its direct actions in the ventricles (cellular mechanism discussed above) independent of any additional benefit associated with the sinus bradycardia it elicits.

Ischemia elevates extracellular K^+ concentration, and the associated diastolic depolarization may diminish the pharmacological activity of K^+ channel blocking drugs. In the present study, we observed only slight attenuation of the QT-widening effects of RP58866 in hearts perfused with 8- versus 3-mmol/L K^+. This indicates that RP58866 loses little of its pharmacological activity in a high-K^+ environment equivalent to that of acute ischemia. This contrasts markedly with other class III agents, such as d-sotalol. If the antiarrhythmic activity of class III agents is mediated primarily via actions in the involved zone rather than in the adjacent uninvolved zone, then this action of RP58866 would appear to lend a potential advantage over other class III drugs.

In view of the caution expressed over data derived from rat studies, we sought to establish that RP58866 was effective in additional species. Studies in rabbit revealed good protection against reperfusion-induced arrhythmias accompanied by QT widening and bradycardia, findings equivalent to those in rat.

We could not examine antiarrhythmic effects against ischemia-induced arrhythmias in rabbit owing to the low incidence of such arrhythmias in control hearts, as we have found in previous studies in this species. However, this allowed us to examine possible proarrhythmic effects of the drug. RP58866 was devoid of such activity during ischemia and reperfusion. Carlsson et al. have shown that class III agents can induce torsade de points in rabbit when administered in conjunction with an alpha-agonist but in the absence of ischemia and reperfusion. It would appear from the present and previous studies that potassium channel blocking drugs such as

| Table 5. Effect of RP58866 on RR Interval and Coronary Flow in Marmoset |
|----------------|------------------------------|-----------------------------|-----------------------------|
| RR (msec) | Control hearts | RP58866 | Control hearts | RP58866 |
| 10 | 271±9 | 286±14 | 12±0.6 | 13±0.7 |
| 1 | 265±11 | 373±28* | 11±0.6 | 13±0.9 |
| 10 | 270±18 | 418±35* | 6±0.4 | 6±0.4 |

Time refers to minutes before (−) and after (+) the onset of ischemia.

*p<0.05 vs. time-matched control hearts.
RP58866 do not provoke torsade de pointes in rabbit hearts in the absence of "priming" with an α1-agonist.

The present study demonstrated, for the first time, pharmacological prevention of both ischemia- and reperfusion-induced arrhythmias in isolated perfused marmoset hearts. This model has not previously been used for such studies because of high costs. We were able to perform the present studies because a limited number of hearts were made available to us by colleagues in our department. The lack of data base with this model is a disadvantage. However, the model was used under the assumption that because it is primate, it is likely to be more "relevant" to humans. The lack of data base, however, does mean that we cannot say whether RP58866 is more or less effective than other drugs in the model. The important point from the primate studies is that the drug was effective in the model, reducing arrhythmias and widening QT interval in a manner equivalent to that seen in rat and rabbit. The model itself appears to be more similar to the rabbit than the rat model in terms of heart rate, QT interval, heart size, and susceptibility to ischemia- and reperfusion-induced arrhythmias. Occluded-zone sizes were similar in control and drug groups, whether measured by dye or flow reduction (as was the case for the other two species used). The latter indicates that the marmoset heart, like that of the rat and rabbit, is deficient in functional coronary collaterals.

Finally, although we have established that \( I_{K1} \) blockade is a mechanism sufficient to account for the effects of RP58866 on VF during ischemia and reperfusion by \( I_{K1} \) blockade, we have not addressed the mechanism by which \( I_{K1} \) blockade leads to VF suppression at the syncytial level (e.g., reentry, abnormal automaticity, flow of injury current). This is a difficult question to address. During a 30-minute period of ischemia in the isolated rat heart (the model used most intensively in the present studies), initiation of VF appears to occur via flow of injury current between ischemic and nonischemic tissue.\(^6\) \( I_{K1} \) blockade by RP58866 may be capable of preventing this from occurring either by reducing diastolic injury potential (by causing partial depolarization of nonischemic tissue) or by reducing systolic injury potential (by altering the shape of the terminal phase of the action potential). Ischemia-induced VFB and VT and reperfusion-induced VF appear to be initiated by mechanisms other than injury current flow,\(^6\) but as yet there is no proof of which of the possible mechanisms is most important.\(^5\) However, ischemia-induced VF may be initiated by a mechanism different from that for VT and VFPs.\(^6\) Moreover, modulation of VPBs preferentially at later (>20 minutes) versus earlier stages of ischemia may indicate that arrhythmogenic mechanisms may vary during a 30-minute period of ischemia as well as between different arrhythmia subtypes.\(^6\) Thus, the present observation that RP58866 appears to suppress ischemia-induced VF, VT, and VPBs and to do so equally well at different stages of a 30-minute period of ischemia indicates that it may be inappropriate to view the drug as having a selective action on any specific arrhythmogenic mechanism (e.g., flow of injury current versus reentry). Instead, the drug, via \( I_{K1} \) blockade, may have a broader spectrum of action on so-called syncytial arrhythmogenic mechanisms.

In conclusion, we have demonstrated the first observation of antiarrhythmic effects of a selective \( I_{K1} \) blocking drug in the setting of acute ischemia and reperfusion in three species, including a primate. \( I_{K1} \) blockade by RP58866, producing a widening of QT interval (which, unusually for a K\(^+\) channel blocker, appears to persist with heart rate maintained by pacing and in tissue exposed to elevated K\(^+\) concentration), may represent a useful new antiarrhythmic mechanism. Other as yet undiscovered pharmacological actions may contribute to the antiarrhythmic effects and the QT widening observed, although their invocation is not necessary.

Acknowledgments

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