Effects of a Novel Cardioselective ATP-Sensitive Potassium Channel Antagonist, 1-[[5-[2-(5-Chloro-o-anisamido)ethyl]-β-methoxyethoxyphenyl]sulfonyl]-3-methylthiourea, Sodium Salt (HMR 1402), on Susceptibility to Ventricular Fibrillation Induced by Myocardial Ischemia: In Vitro and in Vivo Studies

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ABSTRACT

In the present study, a novel sulfonylthiourea, 1-[[5-[2-(5-chloro-o-anisamido)ethyl]-β-methoxyethoxyphenyl]sulfonyl]-3-methylthiourea, sodium salt (HMR 1402), was investigated using in vitro and in vivo systems. HMR 1402 inhibited rilmakalim-induced currents in rat and guinea pig myocytes (IC50 = 60 and 509 nM, respectively). Hypoxia-induced shortening of action potential duration at 90% repolarization was also significantly attenuated by HMR 1402 (68.1 ± 3.9% of control at 0.3 μM). In contrast, HMR 1402 had a smaller effect on pancreatic β-cells (rat insuloma cells, RINm5F) hyperpolarized with 100 μM diazoxide (IC50 = 3.9 μM, compared with glibenclamide IC50 = 9 nM). In a similar manner, hypoxia induced increases in coronary flow in isolated guinea pig hearts were only slightly reduced by HMR 1402. These data strongly suggest that HMR 1402 has pharmacological selectivity for cardiac myocytes and, therefore, may protect against ischemically induced ventricular fibrillation (VF) without the untoward effects of nonselective compounds. To test this hypothesis, VF was induced in 8 dogs with healed myocardial infarctions by a 2-min coronary occlusion during the last minute of exercise. On a subsequent day, the exercise plus ischemia test was repeated after HMR 1402 (3.0 mg/kg i.v., n = 4, infusion 4 μg/kg/min for 1 h before exercise, n = 4). This drug significantly reduced the incidence of VF protecting seven of eight animals (p = 0.0007) without altering plasma insulin, blood glucose, or the increases in mean coronary blood flow induced by either exercise or 15-s coronary occlusions. Thus, the ATP-sensitive potassium channel antagonist HMR 1402 can prevent ischemically induced VF without altering coronary blood flow or blood glucose.

Sudden cardiac death due to ventricular tachyarrhythmias remains the leading cause of death in most industrially developed countries, accounting for between 300,000 and 500,000 deaths each year in the United States alone (Abildstrom et al., 1999; Zheng et al., 2001). Although only a small number of these patients had a known history of heart disease before the collapse, up to 90% of these individuals were subsequently shown to have underlying coronary artery disease (Abildstrom et al., 1999). Therefore, myocardial ischemia almost certainly plays a crucial role in the induction of the lethal arrhythmias in these patients. It is well established that myocardial ischemia is accompanied by rapid increases in the extracellular potassium concentration (for reviews, see Billman, 1994; Coronel, 1994). The resulting depolarization of the surrounding tissue, reductions in action potential duration, and heterogeneity of repolarization facilitates reentrant conduction and the induction of ventricular fibrillation (Billman, 1994). An accumulating body of evidence demonstrates that the activation (opening) of ATP-sensitive potassium (KATP) channel is largely responsible for potassium efflux and the accompanying electrophysiological changes elicited by myocardial ischemia (for reviews, see Billman, 1994; Coronel, 1994). For example, the KATP antagonist glibenclamide attenuated extracellular potassium accumulation and prevented shortening of action potential duration provoked by hypoxia or myocardial ischemia (Benndorf et al., 1991; Nakaya et al., 1991; Dhein et al., 2000). In a

ABBREVIATIONS: KATP, ATP-sensitive potassium; SUR, sulfonylurea receptor; APD90, action potential duration at 90% repolarization; MES, 2-[N-morpholino(ethanesulfonic acid)monohydrate; LVDP, left ventricular diastolic pressure; LVP, left ventricular systolic pressure; HMR 1402,1-[5-[2-(5-chloro-o-anisamido)ethyl]-β-methoxyethoxyphenyl]sulfonyl]-3-methylthiourea; HMR 1883, 1-[5-[2-(5-chloro-o-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea.
similar manner, glibenclamide has also been shown to prevent ventricular fibrillation induced by myocardial ischemia in several animal models (Gwilt et al., 1992; Billman et al., 1993, 1998; Barrett and Walker, 1998; El-Reyani et al., 1999), as well as to reduce both the severity and number of arrhythmias in diabetic patients (Cacciapuoti et al., 1991; Davis et al., 1998; Aronson et al., 2003) and the incidence of ventricular fibrillation in noninsulin-dependent diabetic patients with acute myocardial infarction (Lomuscio et al., 1994). Because the $K_{ATP}$ channels are only activated when intracellular ATP levels fall (Deutsch et al., 1991; Edwards and Weston, 1993), as during ischemia, drugs that block this channel would have minimal effects on the nonischemic myocardium, and therefore should be free of the proarrhythmic effects noted for many antiarrhythmic drugs. However, $K_{ATP}$ channels are not located exclusively in the heart (Gribble et al., 1998; Gögelein et al., 1999; Gögelein, 2001). Indeed, glibenclamide blocks both pancreatic $K_{ATP}$ channels and coronary vascular smooth muscle $K_{ATP}$ channels, thereby promoting insulin release and hypoglycemia as well as reducing coronary perfusion (Gögelein et al., 1999; Gögelein, 2001), respectively. These noncardiac actions would limit the antiarrhythmic potential of glibenclamide in the clinic. Cardioselective compounds should have fewer side effects and would therefore provide a better therapeutic option than the nonselective ATP-sensitive potassium channel antagonist glibenclamide.

Several different ATP-sensitive potassium channel subtypes have been identified. The ATP-sensitive potassium channel consists of a pore-forming subunit coupled to a sulfonylurea receptor (Inagaki et al., 1995; Gribble et al., 1998; Gögelein, 2001). The functional channel forms as a heteroctomer composed of a tetramer of the pore and four sulfonyl receptor subunits. At present, two different pore-forming subunits have been identified, both of which produce an inward rectifier potassium current (Kir 6.1 and Kir 6.2; Liu et al., 2001). Three different sulfonylurea receptor subtypes have been isolated: SUR1 (on pancreatic islet cells), SUR2A (on cardiac tissue), and SUR2B (on vascular smooth muscle) (Gribble et al., 1998; Gögelein et al., 1999; Gögelein, 2001). Thus, six different potassium channel pore and sulfonylurea receptor combinations are possible. Suzuki et al. (2001) recently demonstrated that Kir 6.2 and Kir 6.1 were required for cardiac and vascular smooth muscle ATP-sensitive potassium channel activity, respectively. They concluded that Kir 6.2/SUR2A most likely forms the cardiac cell membrane ATP-sensitive potassium channel, whereas Kir 6.1/SUR2B is located on vascular smooth muscle. In a similar manner, Liu et al. (2001) demonstrated that the mitochondrial $K_{ATP}$ channel most closely resembles Kir6.1/SUR1 subtype. It should therefore be possible to develop compounds that selectively inhibit (or activate) a particular ATP-sensitive potassium channel subtype. A drug that selectively blocks the Kir 6.2/SUR2A subtypes should prevent ischemically induced changes in cardiac electrical properties (e.g., reductions in action potential duration), and thereby protect against arrhythmias without the untoward side effects noted for the nonselective ATP-sensitive channel antagonist glibenclamide. The novel sulfonylurea compound 1-[5-[2-(5-chloro-0-anisamido)ethyl]-$\beta$-methoxyethoxyphenyl)sulfonyl]-3-methylthioure, sodium salt (HMR 1402) (Fig. 1) was developed to block myocardial $K_{ATP}$ channels selectively. It was, therefore, the purpose of this series of studies to first evaluate the effects HMR 1402 on $K_{ATP}$ channels in vitro, using cardiac and pancreatic preparations, and then to investigate the effects of HMR 1402 on the susceptibility to ventricular fibrillation using an unanesthetized canine model of sudden death.

Materials and Methods

The principles governing the care and use of animals as expressed by the Declaration of Helsinki, and as adopted by the American Physiological Society, were followed at all times during this study. In addition, The Ohio State University or the Aventis Pharma Institutional Animal Care and Use Committee approved all the procedures used in this study.

In Vitro Studies

Experiments with Papillary Muscles. Pirbright white guinea pigs of either sex (Charles River Wiga, Sulzfeld, Germany), weighing 300 to 500 g, were killed by cervical dislocation and exsanguination. The hearts were rapidly removed and the right papillary muscles were excised and mounted in an organ bath that contained the following bathing solution: 136 mM NaCl, 3.3 mM KCl, 2.45 mM CaCl$_2$, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 5 mM glucose, 10 mM HEPES, pH adjusted to 7.4 with NaOH, gassed with 100% O$_2$. The bath temperature was maintained at 37°C. The muscles were stimulated with rectangular pulses of 1 V and 1-ms duration at the rate of 1 Hz. The action potential was obtained using standard microelectrode techniques. Briefly, a glass microelectrodes containing 3 M KCl was inserted into the cell, and the signal was amplified (microelectrode amplifier type 309; Hugo Sachs, March-Hugetten, Germany) and recorded with a computer system. The following parameters were measured: the cell's resting potential, the action potential duration at 90% repolarization (APD$_{90}$), the upstroke velocity, and action potential amplitude. After a 30-min equilibration period, the $K_{ATP}$ channel opener rilmakalim was added (Terzic et al., 1994) (3...
μg/ml, dissolved in 1,2-propanediol, pH of the solution was adjusted to 6.0 with NaOH. MES 5 mM was used as the buffer instead of HEPES. The APD90 was recorded 30 min after the administration of rilmakalim. HMR 1402, 10 mM to 20 mM (after centrifugation in the presence of rilmakalim), and APD90 was recorded 30 min later. Finally, the effects of hypoxia on APD90 were evaluated. After a 30-min equilibration period, gassing the bathing solution with 100% N2 and removing glucose from the bathing solution induced hypoxia. In addition, the pH was adjusted to 6.5 (PIPES was used as the buffer instead of HEPES). After 60 min of hypoxia, HMR 1402 was added to the bath and APD90 was recorded for 60 min.

**Patch-Clamp Experiments.** Ventricular myocytes were isolated from both Sprague-Dawley rats (Sprague-Dawley, Moellegard, Denmark) and Pirkbright white guinea pigs (Charles River Wiga) as have been described previously (Hamill et al., 1981; Gogelein et al., 1998). Briefly, the animals were anesthetized with ether and sacrificed by cervical dislocation. The hearts were excised, retrogradely perfused via the aorta at 37°C, first with nominally Ca2+-free Tyrode's solution (143 mM NaCl, 5.4 mM KCl, 0.5 mM MgCl2, 0.25 mM NaH2PO4, 10 mM glucose, 5 mM HEPES, pH adjusted to 7.2 with NaOH); and then with Tyrode's solution containing 20 μM Ca2+ and 0.3 mg/ml collagenase type CLS II (Biochrom KG, Berlin, Germany). After 5 to 10 min of collagenase treatment, the ventricles were cut into small pieces in storage solution (50 mM L-glutamic acid monopotassium salt, 40 mM KCl, 20 mM taurine, 20 mM KH2PO4, 1 mM MgCl2, 10 mM glucose, 10 mM HEPES, 0.2 mM EGTA, pH adjusted to 7.2 with KOH). Then, myocytes were dispersed by gentle shaking and finally by filtration through a nylon mesh (365 μm). Thereafter, the cells were washed twice by centrifugation at 90g for 5 min and kept in storage solution at room temperature.

Whole-cell currents were recorded in the tight-seal whole-cell mode of the patch-clamp technique (Yazawa et al., 1990). In addition, cell potentials were recorded in the whole cell mode when the amplifier (EPC-9 patch-clamp amplifier; HEKA, Lambrecht, Germany) was switched to the current-clamp mode (clamp current 0 pA). Patch pipettes were pulled from borosilicate glass capillaries with 0.3-mm wall thickness and 1.5-mm outer diameter, and their tips were heat-polished. Pipettes had a series resistance of approximately 3 MΩ, which was compensated by 40% by means of the EPC's compensation circuit. The cell capacitance was calculated from the current response after applying a voltage pulse from the holding potential (~80 mV) to ~70 mV. The capacitance of the cell studies was approximately 170 pF. Current-voltage relations were measured by applying voltage pulses from ~140 to +80 mV. The voltage clamp potential was recorded at 0-mV clamp potential was evaluated. At this voltage, most of the time- and voltage-dependent currents are inactive. In all experiments, the calcium channel antagonist nifedipine (5 μM) was present to block the L-type calcium current. The inward-rectifying current Iki was measured at ~120-mV clamp potential, when voltage ramps from ~140 to +80 mV were applied within 500 ms. The fast and slow component of the delayed outward current (IKr and IKs, respectively) were recorded by applying voltage pulses of 3-s duration from a holding potential of ~80 to ~10 mV (IKr) for 200 ms (to inactivate the fast Na+ channels), and then a pulse to +60 mV was applied for 5 s. The pipette solution contained 140 mM KCl, 10 mM NaCl, 1.1 mM MgCl2, 1 mM EGTA, 1 mM Mg-ATP, 10 mM HEPES, pH 7.2 adjusted with KOH, and the bathing solution was 140 mM NaCl, 4.7 mM KCl, 1.0 mM MgCl2, 1.3 mM CaCl2, 10 mM glucose, 10 mM HEPES, pH 7.4 adjusted with NaOH. The experiments were performed at 37 °C. The outward movements of positive ions (from the cell to the extracellular side) are depicted as positive currents. The sign of the electrical potential refers to the cytosolic side with respect to the grounded extracellular side.

**Studies with RINm5F Cells.** RINm5F cells were maintained in RPMI 1640 tissue culture media, containing 11 mM glucose, supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin. Cells were seeded out every 2 to 3 days onto petri dishes and kept in a humidified atmosphere of 95% O2 and 5% CO2 at a temperature of 37°C. For patch-clamp experiments, cells were isolated by incubation in a Ca2+-free medium containing 0.25% trypsin for about 3 min. Single cells and clusters of two to three cells were obtained after trypsinization. The isolated clusters were placed on the ice until use. The tight-seal whole-cell patch-clamp technique was applied to single cells. The same pipette and bathing solutions as described in the previous section were also used during these studies.

**Investigation of Coronary Flow.** As above-described guinea pigs of either sex were killed, the hearts were quickly removed, cannulated via the aorta, and a latex balloon was placed into the left ventricle. The hydrostatic pressure in the balloon was adjusted to 10 mm Hg. The hearts were perfused in the Langendorff mode with a perfusion pressure of 55 mm Hg. Coronary flow was recorded with a type E blood flow transducer (Hellige, Freiburg, Germany). Perfusion solution was 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 25 mM NaHCO3, 1.2 mM MgSO4, 1.2 mM NaH2PO4, 5 mM glucose, 2 mM pyruvate, gassed with 95% O2 and 5% CO2. The temperature of the perfusion medium and the temperature of the isolated heart were maintained at 37°C. After equilibration in control solution for about 60 min, HMR 1402 was added at its lowest concentration (1 μM). After 15 min, the recordings were taken and the next higher drug concentrations were applied. The experiments were then repeated under hypoxic conditions. Hypoxia was induced by gassing the perfusion solution with 20% O2, 75% N2, and 5% CO2. After 30 min of hypoxia, the lowest concentration of the drug (0.1 μM) was added. After 15 min, the recordings were taken, and the next higher drug concentrations were applied under continuous hypoxia.

**In Vivo Studies.** Surgical Preparation of the Canine Model of Sudden Death. A total of 24 heartworm-free mongrel dogs (Kaiser Lake Kennels, Kaiser Lake OH), weighing 15 to 20 kg (18.0 ± 4.0 kg), were used in this study. The animals were anesthetized and instrumented as described previously (Billman et al., 1998). Briefly, 24 h before surgery, a transdermal fentanyl patch that delivers 100 μg/h for 72 h (Duragesic; Janssen Pharmaceutica, Titusville, NJ) was placed on the left side of the animal’s neck and secured with tape. On the day of surgery, the dogs received 15 mg (1 ml i.m.) of morphine sulfate (Elkins-Sinn, Cherry Hill, NJ) and thiopental sodium (20 mg/ml i.v.; Baxter Healthcare, Glendale, CA) to induce anesthesia. Each dog was given between 17 and 20 g/kg thiopental sodium, depending upon the individual response. The dogs were intubated and surgical plane of anesthesia was maintained by the inhalation of isoflurane (1–1.5%; Baxter Healthcare). Using strict aseptic procedures, a left thoracotomy was made in the 4th intercostal space. The heart was exposed and supported by a pericardial cradle. The left circumflex coronary artery was dissected free of the surrounding tissue. Both a 20-MHz pulsed Doppler flow transducer and a hydraulic occluder were then placed around this vessel. A pair of silver-coated copper wires was also sutured on the epicardial surface of the heart and used to obtain a ventricular electrogram. One electrode was placed in the potentially ischemic area (lateral left ventricular wall, an area perfused by the left circumflex artery) and a nonischemic region (right ventricular outflow tract or anterior left ventricle proximal to the occluder). A two-stage occlusion of the left anterior descending artery was then performed approximately one-third the distance from its origin to produce an anterior wall myocardial infarction. This vessel was partially occluded for 60 min and then tied off. The leads to the cardiovascular instrumentation were tunneled under the skin to exit on the back of the animal’s neck.

In addition to the fentanyl patch described above, morphine sulfate (1.0 mg/kg s.c.) was given as needed to control any postoperative pain. The long-lasting local anesthetic 0.25% bupivacaine HCl (Abbott Diagnostics, North Chicago, IL) was also injected in each of three sites (0.5 ml) to block the intercostal nerves in the area of the incision to minimize discomfort to the animals. Each animal was placed on antibiotic therapy (500 mg of amoxicillin; Teva Pharmaceuticals, Sellersville, PA) twice daily for 7 days. The animals were
placed in a quiet recovery area and were returned to their home cage once they the effects of the anesthesia had dissipated. To minimize the incidence of arrhythmias, the dogs received 100 mg of lidocaine HCl (i.m.; Elkins-Sinn) before surgery, which was supplemented (60 mg i.v.) before each stage of the two-stage coronary occlusion. The dogs also received 500 mg of procatinamide HCl (i.m.; Abbott Diagnostics) before the surgery.

**Exercise Plus Ischemia Test: Classification of the Dogs.** The studies began 3 to 4 weeks after the production of the myocardial infarction (Fig. 2, flow chart). The animals were trained to run on a motor-driven treadmill. The susceptibility to ventricular fibrillation (VF) was tested as described previously (Billman et al., 1998). Briefly, the animals ran on a motor-driven treadmill while workload increased every 3 min for a total of 18 min or until a heart rate of 70% of maximum (approximately 210 beats/min) had been achieved. During the last minute of exercise, the left circumflex coronary artery was occluded, the treadmill stopped and the occlusion maintained for an additional minute (total occlusion time 2 min.). The exercise plus ischemia test reliably induced ventricular flutter that rapidly deteriorated into VF. Therefore, large metal plates (11 cm diameter) were placed across the animal’s chest so that electrical defibrillation (Zoll defibrillator; Zoll Medical, Burlington, MA) could be achieved with a minimal delay but only after the animal was unconscious (10–20 s after the onset of VF). Of the 24 animals that underwent surgery, four animals (17%) died acutely or within the first 72 h after infarction. An additional two animals could not be classified due to failure of the occluder. Thus, the exercise plus ischemia test was performed on 18 of the original 24 animals. The occlusion was immediately released if VF had occurred. Eleven dogs developed VF (susceptible), whereas the remaining seven did not (resistant). Three susceptible animals were not successfully defibrillated and as such were not available for additional studies.

**Fig. 2.** A flow chart illustrating the sequence of events for the in vivo studies. MI, myocardial infarction.

The remaining susceptible animals (n = 8) then received one or more of the following treatments on subsequent days: 1) The exercise plus ischemia test was repeated after pretreatment with the HMR 1402 (3.0 mg/kg i.v., n = 4). The drug was dissolved in 2 to 3 ml of 0.9% saline and injected in a cephalic vein as a bolus given approximately 3 min before exercise (i.e., 20 min before the occlusion). 2) Sudden death testing was also repeated after infusion of HMR 1402. A catheter was placed in a cephalic vein and the drug was infused for 3 h beginning 2.5 h before the onset of exercise and continuing throughout the duration of the exercise plus ischemia test. The drug was infused at 4 μg/kg/min (n = 4). The exercise plus ischemia test was also repeated in one animal at a lower dose, 2 μg/kg/min. 3) Finally, a second control the exercise plus ischemia test (saline, n = 6) was repeated 1 week after the last drug test. At least 5 days elapsed between each exercise plus ischemia test. Blood samples (8–10 ml, heparinized collection tubes) were obtained from a cephalic vein at the end of each study. Plasma samples were stored at –20°C for future analysis. The concentration of HMR 1402 was determined by high-performance liquid chromatography-UV.

**Reactive Hyperemia and Plasma Glucose/Insulin Measurements.** The K<sub>ATP</sub> channels are located not only on cardiac tissue but also on vascular smooth muscle and pancreatic islet cells (Gögelein et al., 1999). Therefore, the effects of HMR 1402 on plasma glucose/insulin levels and the regulation of coronary blood flow (response to a brief interruption in coronary blood flow) were also evaluated. The animals (n = 4) were placed into an insulin clamp at approximately 3 min before exercise (i.e., 20 min before the occlusion), and the left circumflex coronary artery was occluded three or four times for 15 s. At least 2 min (or until coronary flow had returned to preocclusion baseline) elapsed between occlusions. The occlusions were repeated 5 min after HMR 1402 (3.0 mg/kg i.v.). Finally, the effects HMR 1402 (3.0 mg/kg i.v., n = 4) and glibenclamide (1.0 mg/kg i.v., n = 4) on blood glucose and plasma insulin were evaluated. The animals were fasted at least 24 h before the administration of the drugs. Blood samples (1 ml) were drawn from a cephalic vein before and 10, 20, 30, 60, 120, and 240 min after the injection of K<sub>ATP</sub> antagonists. Time control samples (i.e., after the injection of saline) were obtained from the same animals the next day. Blood glucose levels were determined using a glucometer (Accu-Chek instant monitor; Roche Diagnostics, Indianapolis, IN). Plasma insulin was measured using a commercially available radioimmunoassay kit (Serano, Freiburg, Germany).

**Data Analysis.** All the in vivo data were recorded on a model 2800S eight-channel recorder (Gould Instrument Systems Inc., Cleveland, OH) and a model M-30 FM tape recorder (Teac, Tokyo, Japan). Coronary blood flow was measured using a flowmeter model 545 C-4 (Bioengineering, University of Iowa, Iowa City, IA). The electrogram data were averaged over 3 to 5 s before the onset of the occlusion and at the 60-s time point (or before VF onset) after the onset of the occlusion. To determine the reactive hyperemia response, the total area between the peak coronary blood flow and return to baseline was measured for each 15-s occlusion, and the percentage of repayment was calculated. The reactive hyperemia response to each occlusion was averaged to obtain one value for each animal. All the hemodynamic (or plasma glucose/insulin) data were then analyzed using a two-factor analysis of variance with repeated measures. When the F ratio was found to exceed a critical value (p < 0.05), Newman-Keuls multiple range test was used to compare the means. The effects of the drug interventions on ventricular fibrillation were determined using Fisher’s exact test.

In the in vitro data were compared using Student’s t-test either for paired or unpaired observations. Differences were considered significant at p < 0.05. The values for half-maximal inhibition (IC<sub>50</sub>) and the Hill coefficient were calculated by fitting the data points of the concentration/response curves to the logistic function:

\[ f(x) = \frac{(a - d)}{(1 + x/c)^s} + d \]
where $a$ represents the plateau value at low drug concentration and $d$ the plateau-value at high drug concentration; $c$ represents the IC$_{50}$ value and $n$ the Hill coefficient.

All data are reported as the mean ± S.E.M. Cardiac arrhythmias, PR interval, and QT interval were evaluated at a paper speed of 100 mm/s. The QT interval was corrected for heart rate (QTc) using Bazett’s method (QT/RR$^{1/2}$) and Fridericia’s method (QT/RR$^{1/3}$).

**Results**

**In Vitro Studies**

**Guinea Pig Papillary Muscle Studies.** The effects of HMR 1402 on the action potential parameters under control (i.e., normoxic) conditions are displayed in Table 1. The actions of the drugs were recorded 30 min after application. The data show that HMR 1402 (2 μM) had no significant effects on the APD$_{90}$, resting potential, amplitude of phase 1 of the action potential, and on the upstroke velocity. There was a slight increase in APD$_{90}$ that can be explained by a time-dependent effect that was independent of the presence of HMR 1402. In a separate set of experiments, we observed an increase of APD$_{90}$ from 188 ± 8 to 199 ± 7 ms ($n = 5$) after 30 min in the absence of drugs.

The effects on the rilmakalim-induced shortening of the action potential are shown in Fig. 3A. The application of rilmakalim (3 μg/ml, external pH 6.0) caused a pronounced shortening of the APD$_{90}$, which was accompanied by a slight decrease in the action potential amplitude. HMR 1402 was then added in the presence of rilmakalim. A typical example is shown in Fig. 3A; the addition of HMR 1402 (100 nM) caused significantly prolonged APD in the presence of rilmakalim. This drug elicited a pronounced dose-dependent prolongation of the APD (i.e., an inhibition of the rilmakalim effect). The concentration relation fitted by the logistic function is displayed in Fig. 3B. The concentration for the half-maximal inhibition (IC$_{50}$) for HMR 1402 was 98 nM with a Hill coefficient of 1.6. It should be noted that the addition 2 μM HMR 1402 in the presence of rilmakalim (Fig. 3B) elicited a longer action potential than was noted under control conditions. This effect can at least partially be explained by prolongation of the APD$_{90}$ caused by lowering the pH to 6.0.

**TABLE 1**

Effects of HMR 1402 and action potential parameters under normoxic conditions

<table>
<thead>
<tr>
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<th>Control (n = 5)</th>
<th>HMR 1402 (2 μM) (n = 5)</th>
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<tbody>
<tr>
<td>APD$_{90}$ (ms)</td>
<td>177.6 ± 6.8</td>
<td>185.2 ± 7.2</td>
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<tr>
<td>Resting potential (mV)</td>
<td>-78.8 ± 1.0</td>
<td>-80.4 ± 1.8</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>114.0 ± 4.5</td>
<td>113.4 ± 4.2</td>
</tr>
<tr>
<td>Upstroke velocity (V/s)</td>
<td>118.4 ± 11.8</td>
<td>114.4 ± 12.6</td>
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Fig. 3. A, effect of HMR 1402 on rilmakalim-induced shortening of the action potential in guinea pig right papillary muscle. The APD shortened markedly in the presence of 3 μg/ml rilmakalim. Subsequent addition of 0.1 μM HMR 1402 attenuated the rilmakalim-induced reductions in APD. The external pH was 7.4 under control conditions, but was adjusted to 6.0 in the presence of rilmakalim and HMR 1402. B, concentration-response effect of HMR 1402 on the rilmakalim-induced shortening of the APD$_{90}$ at external pH 6.0. The rilmakalim-induced shortening of the APD$_{90}$ is considered as 100%. The numbers in brackets indicate the numbers of experiments.
In three experiments performed in the absence of rilmakalim, the ADP₉₀ prolonged from 180 ± 6 to 205 ± 5 ms when the pH was changed from 7.4 to 6.0 for 90 min.

To obtain a more physiological shortening of the action potential duration, guinea pig right papillary muscles were exposed to a hypoxic solution (free of glucose and oxygen, pH adjusted to 6.5 in the hypoxic solution). Under hypoxia, the ADP₉₀ shortened from 176 ± 7 to 48 ± 5 ms (n = 12) within 30 min. This time course of hypoxia-induced ADP₉₀ shortening is consistent with previously published observation (Benndorf et al., 1991; Nakaya et al., 1991; Gögelein et al., 1998). The subsequent addition (i.e., after 60 min of hypoxia) of HMR 1402 caused a clear prolongation of the ADP₉₀, as is shown for HMR 1402 in Fig. 4. The concentration for half-maximal inhibition of the hypoxia-induced shortening of APD fell between 1 and 10 μM (Fig. 4). It has been previously demonstrated that the efficacy of sulfonylurea drugs to block the KATP channel may be impaired by long duration hypoxia (Venkatesh et al., 1991). In contrast, HMR 1402 significantly attenuated the hypoxia-induced ADP shortening even after 60 min of hypoxia in the present study.

**Patch-Clamp Studies.** Typical whole-cell current recordings from a guinea pig cardiomyocyte are shown in Fig. 5. This figure demonstrates a pronounced increase in the current after application of 10 μM rilmakalim at pH 7.4, whereas the reversal potential did not change significantly. As shown previously with guinea pig and rat cardiomyocytes (Gögelein et al., 1998), the current increased in both the outward and inward direction. HMR 1402 was applied in increasing concentrations when the rilmakalim-induced current had reached steady state. This drug produced a dose-dependent inhibition of the current. The whole-cell currents recorded at 0-mV clamp potential both from guinea pig and rat are shown in Fig. 6. Half-maximal inhibition of the rilmakalim-activated K_ATP current by HMR 1402 was 509 and 60 nM, respectively. A higher concentration of HMR 1402 was required to inhibit the rilmakalim induced current in ventricular myocytes compared with the concentration to attenuate rilmakalim induced current in guinea pig papillary muscle (see above). This discrepancy is likely due to the different extracellular pH values used in the studies. In agreement with these findings, the structurally closely related compound HMR 1883 was more potent at the low pH value of 6.0 that at a physiological pH 7.4. This difference was assumed to be due to the fact that sulfonylurea drugs become more ionized at an acidic pH (Gögelein et al., 1998).

A representative example of the effects of HMR 1402 on the current-voltage curve for a single guinea pig cardiomyocyte is shown in Fig. 7. It is clear that HMR 1402 (10 μM) did not alter this voltage-current curve, suggesting that this drug has little or any effect on potassium currents. Indeed, in separate patch-clamp experiments 100 μM HMR 1402 decreased the transient outward current I_{to} 6 ± 2% (n = 5); the inward rectifying current I_K, 6 ± 9% (n = 5); and the slow component of the delayed outward current I_{Ks} by 17 ± 7% (n = 4). It should be noted that there was a time-dependent run-down of the I_{Ks} current by 14 ± 7% (n = 4) in the absence of the drug, indicating that HMR 1402 did not block this current significantly. These data support the results obtained in guinea pig papillary muscle (Table 1), where HMR 1402 had no effect on the action potential duration, resting potential, action potential amplitude, and upstroke velocity. Thus, it can be concluded that HMR 1402 has no significant effects on other K⁺ channels or the fast Na⁺ channel at concentrations that blocked the K_ATP channel.

**Experiments with Pancreatic β-Cells (RINm5F).** The cell potential of RINm5F cells were also investigated with
Effects of HMR 1402 in Langendorff-perfused guinea pig hearts under normoxic and hypoxic conditions

**TABLE 2**

The numbers in brackets indicate the number of experiments.

Whole-cell mode patch-clamp techniques. Under resting conditions the potential was varied between −20 and −60 mV (−45 ± 2 mV, n = 54) and the depolarizing spikes were often observed. The addition of 100 μM diazoxide to the bathing solution resulted in a stable hyperpolarization of the cell membrane (−79 ± 1 mV, n = 54). Therefore, all experiments were performed in the presence of diazoxide. As displayed in Fig. 8, the addition of HMR 1402 failed to alter membrane potential at concentrations below 1 μM. The HMR 1402 concentration for half-maximal depolarization of these pancreatic cells was 3.9 μM with a Hill coefficient of 1.7. In contrast, we previously demonstrated that the addition of glibenclamide to the cells that had been incubated in a diazoxide-containing solution elicited a concentration-dependent depolarization of the cell membrane potential (IC_{50} = 9 nM) (Gögelein et al., 1998).

**Experiments with Langendorff-Perfused Hearts.** The effects of HMR 1402 on coronary flow were investigated using Langendorff-perfused guinea pig hearts. As shown in Table 2, under normoxic conditions, HMR 1402 (1 μM) produced a slight but statistically significant decrease in the coronary flow but did not alter any other parameter, including left ventricular systolic pressure (LVP), left ventricular diastolic pressure (LVDP), heart rate, and left ventricular developed pressure (dp/dt\text{max}). The coronary flow decreased further at 10 μM HMR 1402, whereas at higher concentrations (30 and 100 μM) frequently produced transient increases in coronary flow. In a similar manner, HMR 1402 concentrations of 10 μM and above elicited decreases in LVP and dp/dt\text{max} but did not alter either LVDP or heart rate.

Similar responses were noted during hypoxia. Typical recordings of the coronary flow response to hypoxia are shown in Fig. 9A. This figure shows that hypoxia induced a pronounced increase in coronary flow, which was dose dependently antagonized by HMR 1402. The data are summarized in Fig. 9, B and C, showing the mean values of the coronary flow and percent inhibition by HMR 1402, respectively. The concentration of half-maximal inhibition for HMR 1402 was below 10 μM, and complete inhibition was achieved at 30 μM. In contrast, we previously reported that 1 μM glibenclamide completely inhibited hypoxia-induced increases in coronary flow (Gögelein et al., 1998).

**In Vivo Studies**

**Effects of HMR 1402 on the Susceptibility to Ventricular Fibrillation.** In agreement with previous studies (Billman et al., 1993, 1998), ventricular flutter that rapidly deteriorated into ventricular fibrillation was reproducibly induced in the susceptible animals with each presentation of control exercise plus ischemia tests. The control exercise plus ischemia test provoked similar heart rate changes (first occlusion, control 205.3 ± 9.8; occlusion, 225.3 ± 17.7 beats/min; and second occlusion, control 199.7 ± 13.1; occlusion, 248.3 ± 20.2 beats/min) with a similar time to VF onset (first occlusion 51.8 ± 7.5 s, second occlusion 44.6 ± 6.3 s). The exercise plus ischemia test was repeated after the following treatment; HMR 1402 (3.0 mg/kg i.v., n = 4), HMR 1402 (i.v. infusion at 4.0 μg/kg/min for 180 min, n = 4). The coronary artery occlusion elicited a significant increase in heart rate, a response that was not altered by either pretreatment with HMR 1402 or glibenclamide (no drug, 212.3 ± 6.9; occlusion, 234 ± 9.0 beats/min; HMR 1402, 202.2 ± 13.6; occlusion, 247.8 ± 11.2). Representative recordings of the response to

**Table 2**

Effects of HMR 1402 in Langendorff-perfused guinea pig hearts under normoxic and hypoxic conditions

<table>
<thead>
<tr>
<th>Drug Dose</th>
<th>0</th>
<th>1 μM</th>
<th>10 μM</th>
<th>30 μM</th>
<th>100 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic conditions</td>
<td>(n = 7)</td>
<td>(n = 4)</td>
<td>(n = 5)</td>
<td>(n = 4)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td>89.0 ± 6.4</td>
<td>94.8 ± 5.3</td>
<td>73.2 ± 6.6*</td>
<td>72.8 ± 8.0*</td>
<td>74.7 ± 4.1*</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td>−0.3 ± 0.5</td>
<td>−1.0 ± 0.4</td>
<td>−0.5 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>4.0 ± 1.6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>218.3 ± 4.3</td>
<td>206.3 ± 7.3</td>
<td>205.6 ± 6.9</td>
<td>204.8 ± 11.1</td>
<td>213 ± 1.4</td>
</tr>
<tr>
<td>LV dP/dt (mm Hg/s)</td>
<td>1307.3 ± 92.1</td>
<td>1273.8 ± 106.6</td>
<td>1033.6 ± 55.1*</td>
<td>1028.6 ± 72.8*</td>
<td>1028.0 ± 31.9*</td>
</tr>
<tr>
<td>CF (ml/min)</td>
<td>7.6 ± 0.5</td>
<td>7.0 ± 1.0*</td>
<td>6.7 ± 0.8*</td>
<td>8.4 ± 0.8</td>
<td>8.4 ± 0.4*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug Dose</th>
<th>0</th>
<th>0.1 μM</th>
<th>1 μM</th>
<th>10 μM</th>
<th>30 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypos conditions</td>
<td>(n = 6)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 5)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td>41.0 ± 1.0</td>
<td>41.0 ± 1.0</td>
<td>40.3 ± 1.7</td>
<td>34.8 ± 1.2*</td>
<td>29.7 ± 3.3*</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td>0.5 ± 2.7</td>
<td>1.0 ± 5.0</td>
<td>2.7 ± 3.2</td>
<td>−0.6 ± 1.9</td>
<td>0.3 ± 1.8</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>212.7 ± 10.3</td>
<td>214.0 ± 11.9</td>
<td>206.3 ± 7.5</td>
<td>199.2 ± 14.1</td>
<td>185.7 ± 22.6</td>
</tr>
<tr>
<td>LV dP/dt (mm Hg/s)</td>
<td>708.0 ± 65.4</td>
<td>698.3 ± 132.4</td>
<td>647.7 ± 110.3</td>
<td>592.2 ± 43.9*</td>
<td>481.7 ± 38.4*</td>
</tr>
<tr>
<td>CF (ml/min)</td>
<td>18.0 ± 0.7</td>
<td>16.4 ± 0.7</td>
<td>15.9 ± 0.8*</td>
<td>14.0 ± 1.0*</td>
<td>11.1 ± 1.1*</td>
</tr>
</tbody>
</table>

CF, coronary flow; HR, heart rate; LV, left ventricular.

*p < 0.05 from no drug condition (i.e., 0 μM).
the exercise plus ischemia test obtained from the same animal before and after pretreatment with HMR 1402 are displayed in Fig. 10. HMR 1402 when given as a bolus (\(n = 4\), 3.0 mg/kg i.v.) protected three of four susceptible animals, whereas the infusion of HMR 1402 (4 \(\mu\)g/kg/min) protected all four animals treated. It should be noted that the lower infusion rate, 2 \(\mu\)g/kg/min, did not prevent VF in an animal that was protected at 4 \(\mu\)g/kg/min. Thus, a total of seven of eight animals (\(p = 0.007\), a 87.5% reduction) were protected from ventricular arrhythmias. Because the response was similar with each route of administration (i.e., bolus versus

slow infusion), the data were combined in the subsequent analysis (see below). The plasma concentrations for HMR 1402 are displayed in Table 3. Ventricular flutter/fibrillation was prevented in all trials in which the plasma concentration exceeded 1.0 \(\mu\)g/ml. In contrast, VF was recorded in all trial in which a lower plasma concentration had been achieved (0.39 and 0.51 \(\mu\)g/ml).

The heart rate and coronary blood flow responses to exercise before and after HMR 1402 are displayed in Fig. 11. Neither the bolus injection nor the infusion of the HMR 1402 significantly altered the heart rate or mean coronary blood flow response to exercise.

**Effects of HMR 1402 on Resting Parameters.** HMR 1402 did not significantly alter any of the resting cardiovascular variables investigated in this study and are displayed in Table 4. The effects of glibenclamide and HMR 1402 on plasma glucose and insulin levels are shown in Fig. 12. In contrast to HMR 1402, glibenclamide elicited significant increases in plasma insulin that was accompanied by significant reductions in plasma glucose.

**Effects on Reactive Hyperemia.** The effects of HMR 1402 (\(n = 4\)) on the coronary blood flow response to brief (15 s) coronary artery occlusion were also examined. A large coronary blood flow was elicited by the release of a 15-s coronary occlusion (389.6 ± 84.7%) that was not significantly altered by HMR 1402 pretreatment (362.8 ± 62.4%; a 5.8 ± 5.4% reduction in the hyperemic response). In contrast, glibenclamide has been previously reported to reduce the hyperemic response to by approximately 30% (Billman et al., 1998).

**Discussion**

In the present study, the novel \(K_{ATP}\) channel antagonist HMR 1402 did not alter the cardiac action potential under control conditions but significantly attenuated the shortening of the APD\(_{90}\) induced either by the \(K_{ATP}\) channel agonist rilmakalim or by hypoxia. In a similar manner, the same concentration of this drug that attenuated these reductions in APD\(_{90}\) did not inhibit the activation of pancreatic or coronary vascular \(K_{ATP}\) channels in vitro. In conscious dogs, HMR 1402 prevented ischemically induced ventricular fibrillation without altering the increases in mean coronary blood flow induced either by submaximal exercise or by the reactive hyperemic response to brief (15-s) coronary artery occlusions. These findings were in marked contrast to glibenclamide that provoked large reductions in coronary blood flow (Billman et al., 1993, 1998). Finally, HMR 1402, in contrast to glibenclamide (Billman et al., 1998), did not alter plasma insulin concentrations. When considered together, these data suggest that the activation of cardiac \(K_{ATP}\) channels during myocardial ischemia plays an important role in both ischemically induced reductions in APD\(_{90}\) and in the genesis of ventricular fibrillation. The data further demonstrate that HMR 1402 preferentially blocks cardiac \(K_{ATP}\) channels without adversely affecting either coronary vascular or pancreatic \(K_{ATP}\) channels.

**HMR 1402 and Selectivity for Cardiac ATP-Sensitive Potassium Channels.** As noted above, at least six different \(K_{ATP}\) channels are possible. Recent evidence suggests that the SUR2A/Kir 6.2 combination is restricted to cardiac muscle (Suzuki et al., 2001; Manning-Fox et al., 2002). Thus, substances that preferentially inhibit this channel should
display selectivity for cardiac tissue. The present study demonstrates that 2 μM HMR 1402 had no significant effects on APD₉₀, the resting potential, the amplitude of the phase 1 of the action potential, or on the upstroke velocity. These data suggest that HMR 1402 has no significant effects on K⁺ channels (i.e., I_{K1}, I_{Kr}, and I_{Ks}) or Na⁺ channels in guinea pig papillary muscles. Indeed, patch-clamp experiments performed on either guinea pig or rat ventricular myocytes directly demonstrated that HMR 1402 (concentrations up to 100 μM) did not significantly affect cardiac K⁺ channel currents. Thus, as previously observed with either the cardioselective Kₐtp channel antagonist HMR 1883 or the nonselective Kₐtp antagonist glibenclamide (Gögelein et al., 1998; Manning-Fox et al., 2002), HMR 1402 does not affect other K⁺ channels in cardiac tissues under control conditions, and therefore no changes in the action potential duration and the QT-time of the ECG would be expected. Indeed, HMR 1402 did not alter QTc in conscious dogs in the present study.

In contrast, HMR 1402 potently blocked the rilmakalim-activated Kₐtp channels in guinea pig papillary muscle. At an external pH of 6.0, this inhibition was approximately 6.1 times more potent than that reported for HMR 1883 (IC₅₀ for HMR 1402, 98 nM; IC₅₀ for HMR 1883, 0.6 μM) (Gögelein et al., 1998). HMR 1402 also inhibited the rilmakalim-induced Kₐtp current with different potencies in rat and guinea pig myocytes. This finding contrasts with previous results with HMR 1883, which blocked the whole-cell current with comparable potency in both species (IC₅₀ = 800 nM in guinea pig and 700 nM in rat at pH 7.4) (Gögelein et al., 1998). Glibenclamide was also slightly more potent in rat (IC₅₀ = 8 nM) than in guinea pig (IC₅₀ = 20 nM) (Gögelein et al., 1998). As in the papillary muscle preparation, HMR 1402 is more potent in blocking rilmakalim-activated Kₐtp current than HMR 1883 (Gögelein et al., 1998), especially in rat ventricular myocytes. Similarly, hypoxia consistently elicited a marked reduction in APD₉₀ that was potently antagonized by HMR 1402. This inhibition of the hypoxia-induced shortening of the action potential duration was more potent for HMR 1402 than has been previously reported for HMR 1883 (Gögelein et al., 1998). For example, 0.5 μM HMR 1883 had no significant effect (Gögelein et al., 1998), whereas 0.3 μM HMR 1402 produced a significant inhibition of reductions in APD₉₀ induced by hypoxia. The effect of HMR

TABLE 3
Plasma concentration of HMR 1402

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose (i.v.)</th>
<th>Plasma Level</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2 μg/kg/min</td>
<td>0.504</td>
<td>VF</td>
</tr>
<tr>
<td>1b</td>
<td>4 μg/kg/min</td>
<td>1.246</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>2</td>
<td>4 μg/kg/min</td>
<td>1.190</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>3</td>
<td>4 μg/kg/min</td>
<td>1.275</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>4</td>
<td>4 μg/kg/min</td>
<td>No sample</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>5</td>
<td>3.0 mg/kg</td>
<td>2.283</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>6</td>
<td>3.0 mg/kg</td>
<td>1.052</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>7</td>
<td>3.0 mg/kg</td>
<td>5.336</td>
<td>No arrhythmias</td>
</tr>
<tr>
<td>8</td>
<td>3.0 mg/kg</td>
<td>0.394</td>
<td>VF</td>
</tr>
</tbody>
</table>

Fig. 10. Representative recordings from the same animal before and after pretreatment with HMR 1402 (3 mg/kg i.v.). Note that this drug prevented ventricular flutter despite similar changes in heart rate that were induced by the ischemia.

Fig. 11. Effects of HMR 1402 on the heart rate and coronary blood flow response to exercise. Closed circles represent control (i.e., no drug), whereas open circles represent the post HMR 1402 studies. Note that HMR 1402 did not alter either the heart rate or coronary blood flow response to exercise.
1402 at a concentration of 10 μM was also more pronounced than that observed with 20 μM HMR 1883 (Gögelein et al., 1998). Thus, one may conclude that HMR 1402 is more potent in blocking rilmakalim-activated and hypoxia-activated K<sub>ATP</sub> channels than HMR 1883 in either papillary muscle or in isolated myocytes.

ATP-sensitive potassium channels also play a significant role in the regulation of vascular muscle tone. The activation of K<sub>ATP</sub> channels promotes smooth muscle relaxation, thereby reducing arterial pressure and increasing coronary blood flow (Gögelein et al., 1999). Conversely, glibenclamide has been reported to inhibit hypoxia-induced increases in coronary flow (Daut et al., 1990; Gögelein et al., 1998), to reduce the hyperemic response to coronary occlusion (Billman et al., 1998), and to attenuate the active hyperemia elicited by exercise (Duncker et al., 1993; Billman et al., 1998). In the present study, 1 and 10 μM HMR 1402 slightly decreased the coronary flow in Langendorff-perfused guinea pig hearts under normoxic conditions but caused a paradoxical increase in coronary flow at 30 and 100 μM. This increase is likely due to additional, yet unexplained, effects of HMR 1402 on the coronary vascular system. On the other hand, HMR 1402 reduced hypoxia-induced increases in coronary flow at low concentrations (Fig. 8). However, glibenclamide (10 μM) provoked much larger reductions in coronary flow under normoxic conditions as well as hypoxic conditions (Gögelein et al., 1998; unpublished observations), whereas HMR 1883 had no effect under these conditions (Billman et al., 1998). Thus, in isolated guinea pig hearts, HMR 1402 was more potent in inhibiting hypoxia-induced vasodilation than HMR 1883 but was still much less potent than glibenclamide. Furthermore, in vivo, HMR 1402 did not alter either the reactive hyperemia induced by 15-s coronary artery occlusions or the increase in coronary artery blood flow elicited by exercise: a response that was nearly identical to HMR 1883 but considerably less than glibenclamide (Billman et al., 1998).

Finally, K<sub>ATP</sub> channels also regulate insulin secretion from pancreatic islet cells (Ashcroft et al., 1984); blocking these channels results in the secretion of insulin and a corresponding reduction in blood glucose. In the present study, the inhibitory effects of HMR 1402 were investigated in rat pancreatic β-cells (RINm5F cells) in which the K<sub>ATP</sub> channels were activated by diazoxide. HMR 1402 only partially inhibited the effect of diazoxide on the cell membrane potential (IC<sub>50</sub> of 3.9 μM). This inhibition was somewhat more potent than that of HMR 1883 (IC<sub>50</sub> of approximately 20 μM (Gögelein et al., 1998; Manning-Fox et al., 2002) but considerably less than that of glibenclamide (IC<sub>50</sub> of 9.3 nM) (Gögelein et al., 1998). In addition, HMR 1402 did not alter either plasma insulin or blood glucose levels in conscious dogs. This was in marked contrast to the pronounced hypoglycemia and the increase in plasma insulin provoked by glibenclamide (Fig. 12). Thus, the in vitro and in vivo data strongly suggest that HMR 1402 acts preferentially on cardiac K<sub>ATP</sub> channels and, unlike glibenclamide, has little or no effect on either coronary vascular or pancreatic tissue.

**HMR 1402 and Susceptibility to Ventricular Fibrillation.** In the present study, HMR 1402 significantly reduced the incidence of ventricular fibrillation, protecting seven of eight animals tested. In agreement with these findings, both the nonselective K<sub>ATP</sub> antagonist glibenclamide and the cardioselective K<sub>ATP</sub> HMR 1883 protected against ischemically induced malignant arrhythmias (Billman, 2002). However, it is important to emphasize that, in contrast to the actions of either HMR 1402 or HMR 1883, glibenclamide significantly reduced both exercise and reactive hyperemia-induced increases in coronary blood flow, as well
as depressed ventricular function (large reductions in left ventricular dP/dt max) in animals (Billman et al., 1993, 1998). Therefore, nonselective K_{ATP} channel antagonist may protect against ischemic arrhythmias but not without potentially significant adverse side effects.

In summary, the activation of cardiac K_{ATP} channels during myocardial ischemia promotes potassium efflux, reduction in action potential duration, and inhomogeneities in repolarization creating a substrate for reentry (Billman, 1994). Drugs that block this channel should be particularly effective antiarrhythmic agents. In the present study, the K_{ATP} channel antagonist HMR 1402 attenuated reductions in APD_{90} induced either by the K_{ATP} channel agonist ril-makalim or by hypoxia in vitro without major actions on either coronary flow or rat pancreatic \( \beta \)-cells. Similarly, HMR 1402 prevented ventricular fibrillation in animals known to be susceptible to malignant arrhythmias but did not increase plasma insulin, reduce blood glucose, or alter coronary blood flow regulation. These data strongly suggest that HMR 1402 acts preferentially on cardiac K_{ATP} channels. Because the K_{ATP} channel only becomes active as ATP levels fall (Deutsch et al., 1991; Edwards and Weston, 1993), HMR 1402 has the added advantage that it would only inhibit the activated channels located in ischemic tissue with minimal effects on normal tissue. Thus, selective antagonists of the cardiac K_{ATP} channel may represent a new class of ischemia-selective antiarrhythmic medications and, as such, should be free of the proarrhythmic effects that have plagued many currently available antiarrhythmic drugs.

**References**


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