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Title: The effects of a novel cardioselective ATP-sensitive potassium channel antagonist, HMR 1402, on susceptibility to ventricular fibrillation induced by myocardial ischemia: in vitro and in vivo studies.

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Running title: cardioselective K_{ATP} antagonists prevent ventricular fibrillation

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List of Abbreviations:

VF = ventricular fibrillation
K_{ATP} = ATP-sensitive potassium
APD_{90} = action potential duration at 90% repolarization
CF = coronary flow
HEPES = N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
PIPES = piperazine-N,N'-bis-[2-ethanesulfonic acid]
MES = (2-[N-morpholino(ethanesulfonic acid)monohydrate
HR = heart rate
SUR = sulfonylurea receptor
LVP = left ventricular pressure
LVDP = left ventricular diastolic pressure

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ABSTRACT

In the present study, a novel sulfonylthiourea HMR 1402 was investigated using in vitro and in vivo systems. HMR 1402 inhibited rilmakalim-induced currents in rat and guinea pig myocytes (IC_{50} = 60 nM and 509 nM, respectively). Hypoxia induced shortening of APD_{90} 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 (IC_{50} = 3.9 μM, compared to glibenclamide IC_{50} = 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 non-selective 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 hour before exercise, n=4). This drug significantly reduced the incidence of VF protecting 7 of 8 animals (P = 0.0007) without altering plasma insulin, blood glucose, or the increases in mean CBF induced by either exercise or 15s coronary occlusions. Thus, the ATP-sensitive potassium channel antagonist HMR 1402 can prevent ischemically-induced VF without altering coronary blood flow or blood glucose.

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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 prior to 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 re-entrant conduction and the induction of ventricular fibrillation (Billman 1994). An accumulating body of evidence demonstrates that the activation (opening) of ATP-sensitive potassium (K_{ATP}) channel is largely responsible for potassium efflux and the accompanying electrophysiological changes elicited by myocardial ischemia (for reviews see Billman1994; Coronel 1994). For example, the K_{ATP} antagonist, glibenclamide, attenuated extracellular potassium accumulation and prevented shortening of action potential duration provoked by hypoxia or myocardial ischemia (Nakaya et al., 1991; Bendorf et al., 1991;Dhein et al., 2000). In a 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; Billman et al., 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., 1996; Aronson et al., 2003) and the incidence of ventricular fibrillation in non-insulin

dependent diabetic patients with acute myocardial infarction (Lomuscio et al., 1994). As 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 non-cardiac actions would limit the anti-arrhythmic potential of glibenclamide in the clinic. Cardioselective compounds should have fewer side effects and would therefore provide a better therapeutic option than the non-selective 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 (Gögelein, 2001; Gögelein et al., 1999; Inagaki et al., 1995). The functional channel forms as a hetero-octomer composed of a tetramer of the pore and 4 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 and co-workers (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, while Kir 6.1/SUR2B is located on vascular smooth muscle. In a similar manner, Liu et al (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 non-selective ATP-sensitive channel antagonist glibenclamide. The novel sulfonylurea compound HMR 1402 1-[[5-[2-(5-chloro-o-anisamido)ethyl]-β-methoxyethoxyphenyl]sulfonyl]-3-methylthiourea, sodium salt (figure 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.

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 which contained the following bathing solution (in mM): 136 NaCl, 3.3 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, $MgSO_4$, % glucose, 10 HEPES, pH adjusted to 7.4 with NaOH, gassed with 100% O_2. The bath temperature was maintained at 37^o 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-Hugtetten, 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 APD_{90} was recorded 30 min. after the administration of rilmakalin. HMR 1402, 30 nM to 20 µM, was then added (in the presence of rilmakalim) and APD_{90} was recorded 30 min later. Finally, the effects of hypoxia on APD_{90} were evaluated. After a 30 min equilibration period, hypoxia was induced by gassing the bathing solution with 100% N and removing glucose from the bathing solution. 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 APD_{90} was recorded for 60 min.

Patch-clamp Experiments

Ventricular myocytes were isolated from both Spague Dawley rats (Moellegard, Denmark) and Pirbright white guinea pigs (Charles River Wiga, Sulzfeld, Germany) as have been previously described (Hamill et al., 1981; Gögelein et al., 1998). Briefly, the animals were anesthetized with ether and sacrificed by cervical dislocation. The hearts were dissected and retrogradely perfused via the aorta at 37°C: first with nominally Ca^{2+} free Tyrode solution (in mM): 143 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 0.25 NaH_2PO_4, 10 glucose, 5 HEPES, pH adjusted to 7.2 with NaOH; secondly with Tyrode solution containing 20 µM Ca^{2+} and 0.3 mg/ml collagenase type CLS II (Biochrom KG, Berlin, Germany). After 5 - 10 min of collagenase treatment, the ventricles were cut into small pieces in storage solution [(in mM): 50 L-glutamic acid monopotassium salt, 40 KCl, 20 taurine, 20 KH_2PO_4, 1 $MgCl_2$, 10 glucose, 10 HEPES, 0.2 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 90 g 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 cells studies was approximately 170 pF. Current-voltage (I/V) relations were measured by applying voltage ramps from -140 mV to + 80 mV. The current 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 I_{K1} was measured at –120 mV clamp potential, when voltage ramps from –140 mV to +80 mV were applied within 500 ms. The fast and slow component of the delayed outward current (I_{Ks} and I_{Kr}, respectively) were recorded by applying voltage pulses of 3 s duration from a holding potential of -80 mV to –10 mV (I_{Kr}) for 200 ms (to inactivate the fast Na^+ channels), then a pulse to +60 mV was applied for 3 sec. The pipette solution contained (in mM): 140 KCl, 10 NaCl, 1.1 $MgCl_2$, 1 EGTA, 1 Mg-ATP, 10 HEPES, pH=7.2, adjusted with KOH, and the bathing solution was (in mM): 140 NaCl, 4.7 KCl, 1.0 $MgCl_2$, 1.3 $CaCl_2$, 10 glucose, 10 HEPES, pH=7.4, adjusted with NaOH. The experiments were performed at 35 ± 1°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% (vol/vol) fetal calf serum, 2 mM glutamine and 50 µg/ml gentamycine. Cells were seeded out every two to three days onto petri dishes and kept in a humidified atmosphere of 95% O_2 and 5% CO_2 at a temperature of 37^o C. For patch-clamp experiments, cells were isolated by incubation in a Ca^{2+}-free medium containing 0.25% trypsin for about 3 min. Single cells and clusters of 2-3 cells were obtained after centrifugation with 800 rpm and were stored on 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 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 mmHg. The hearts were perfused in the Langendorff mode with a perfusion pressure of 55 mmHg. Coronary flow was recorded with a Hellige type E blood flow transducer (Freiburg, Germany). Perfusion solution (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 $MgS0_4$, 1.2 NaH_2PO_4, 5 glucose, 2 pyruvate, gassed with 95% O_2 and 5% CO_2. The temperature of the perfusion medium and the temperature of the isolated heart were maintained at 37^o 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% O_2, 75% N_2 and 5% CO_2. 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 - 20 kg (18.0 ± 0.4 kg) were used in this study. The animals were anesthetized and instrumented as previously described (Billman et al., 1998). Briefly, 24 hours before surgery, a transdermal fentanyl patch that delivers 100 μg/h for 72 hr (Duragesic, Jansen Pharmaceutica, Titusville, NJ) was place on the left side of the animal's neck and secure with tape. On the day of surgery, the dogs received 15 mg (1 ml, i.m.) morphine sulfate (Elkins-Sinn, Cherry Hill, NJ) and thiophental sodium (Baxter Healthcare, Glendale CA; 20 mg/ml, i.v.) to induce anesthesia. Each dog was given between 17 and 20 gm/kg of thiopental sodium depending upon the individual response. The dogs were intubated and surgical plane of anesthesia was maintained by the inhalation of isofurane (1 to 1.5%, Baxter Healthcare, Glendale CA). Using strict aseptic procedures, a left thoracotomy was made in the fourth 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 were 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 non-ischemic region (right ventricular out flow 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 in order to produce an anterior wall myocardial infarction. This vessel was partially occluded for 20 minutes 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 post-operative pain. The long-lasting local anesthetic 0.25% bupivacaine HCl (Abbott Laboratories, North Chicago, IL) was also injected in each of three sites (0.5 ml) to block the intercostals nerves in the area of the incision to minimize discomfort to the animals. Each animal was placed on antibiotic therapy (500 mg amoxocillin, 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 lidocaine HCl (i.m.; Elkins-Sinns, Cherry Hill, NJ) 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 procainamide HCl (i.m; Abbott Laboratories, North Chicago, IL) before the surgery.

Exercise-plus Ischemia Test: Classification of the Dogs

The studies began 3-4 weeks after the production of the myocardial infarction (see flow chart, figure 2). The animals were trained to run on a motor-driven treadmill. The susceptibility

to ventricular fibrillation (VF) was tested as previously described (Billman et al. 1998). Briefly, the animals ran on a motor driven treadmill while workload increased every 3 minutes for a total of 18 minutes 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 M series 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, 4 animals (17%) died acutely or within the first 72 hours after infarction. An additional 2 animals could not be classified due to failure of occluder. Thus, 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) while the remaining 7 did not (resistant). Three susceptible animals were not successfully defibrillated and as such were not available for additional studies.

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 2-3 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 hr beginning 2.5 hr 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 1 animal at a lower dose, 2 μg/kg/min. 3) Finally, a second control the exercise plus ischemia test (saline, n = 6) was repeated one week after the last drug test. At least 5 days elapsed between each exercise plus ischemia test. Blood samples (8-10 ml, heparinzed collection tubes) were obtained from a cephalic vein at the end of each study. Plasm samples were stored at -20°C for future analysis. The concentration of HMR 1402 was determined by HPLC/UV.

Reactive Hyperemia and Plasma Glucose/insulin Measurements

The K_{ATP} 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 unrestrained on a laboratory table, and the left circumflex coronary artery was occluded three or four tomes for 15 s. At least 2 min. (or until coronary flow had returned to pre-occlusion 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 hr 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_{ATP} 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, Boehringer Mannheim, 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 Gould Model 2800S eight-channel recorder (Cleveland OH) and a Teac Model M-30 FM tape recorder (Tokyo Japan). Coronary Blood Flow was measured using a University of Iowa Bioengineering flow meter model 545 C-4 (Iowa City IA). The electrogram data were averaged over 3-5 s before the onset of the occlusion and at the 60 s time point (or before VF onset) after the onset of the occlusion. In order 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 percent 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-Keul's 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.

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_{50}) and the Hill coefficient were calculated by fitting the data points of the concentration/response curves to the logistic function:

$$f(x) = (a-d) / (1+x/c)^n + d$$

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were *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 ± SEM. 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 ms 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 figure 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 figure 2a, 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 figure 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. In three experiments performed in

the absence of rilmakalim the APD_{90} prolonged from 180 ± 6 ms to 206 ± 5 ms when the pH was changed from 7.4 to 6.0 for 90 min.

In order 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 APD_{90} shortened from 176 ± 7 to 48 ± 5 ms (n = 12) within 30 min. This time-course of hypoxia-induced APD_{90} shortening is consistent with previously published observation (Nakaya et al., 1991; Bendorf 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 APD_{90}, as is shown for HMR 1402 in figure 4. The concentration for half-maximal inhibition of the hypoxia-induced shortening of APD fell between 1 μ and 10 μM (figure 4). It has been previously demonstrated that the efficacy of sulfonylurea drugs to block the K_{ATP} channel may be impaired by long duration hypoxia (Venkatesh et al., 1991). In contrast, HMR 1402 significantly attenuated the hypoxia-induced APD 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 figure 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 zero

mV clamp potential both from guinea pig and rat are shown in figure 6. Half-maximal inhibition of the rilmakalim-activated K_{ATP} current by HMR 1402 was 509 nM and 60 nM, respectively. A higher concentration of HMR 1402 was required to inhibit the rilmakalim induced current in ventricular myocytes as compared the concentration to attenuate rilmaklim induced reduction in the action potentil duration in guinea pig papillary muscle (see above). This discrepancy is likely due to the different extracelluar pH values used in the studies. In agreement with these findsings, 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 figure 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 100μM HMR 1402 decreased the transient outward current I_{to} 6 ± 2% (n = 5), the inward rectifying current I_{K1} 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.

<u>Experiments with pancreatic β-cells (RINm5F)</u>

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The cell potential of RINm5F cells were also investigated with whole-cell mode patch clamp techniques. Under resting conditions the potential was varied between –20 mV 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 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 figure 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 (CF) 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 (HR), and left ventricular developed pressure (dp/dt_{max}). The coronary flow decreased further at 10 µM HMR 1402, whereas at higher concentrations (30 µM 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_{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 figure 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 figure 9b and 9c, 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

The Effects of HMR 1402 on the Susceptibility to Ventricular Fibrillation

In agreement with previous studies (Billman et al, 1993; Billman et al., 1998), ventricular flutter that rapidly deteriorated into ventricular fibrillation was reproducibly induced in the susceptible animals with each presentation of both control exercise plus ischemia tests. The control exercise plus ischemia test provoked similar heart rate changes (1^{st} occlusion control 205.3 ± 9.8,occlusion 225.3 ± 17.7 beats/min; 2^{nd} occlusion control 199.7 ± 13.1, occlusion 248.3 ± 20.2 beats/min) with a similar time to VF onset (1^{st} occlusion 51.8 ± 7.5 s, 2^{nd} 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 pre-treatment 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 the exercise plus ischemia test obtained from the same animal before and after pretreatment with HMR 1402 are displayed in figure 10. HMR 1402 when

given as a bolus (n = 4, 3.0 mg/kg, i.v.) protected 3 of 4 susceptible animals while the infusion of HMR 1402 (4 μg/kg/min.) protected all four animals treated. It should be noted that the lower infusion rate, 2 μg/kg/min, did not prevent VF in an animal that was protected at 4 μg/kg/min. Thus, a total of 7 of 8 animals (p = 0.0007, a 87.5% reduction) were protected from ventricular arrhythmias. As 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 4. Ventricular flutter/fibrillation was prevented in all trials in which the plasma concentration exceeded 1.0 μg/ml. In contrast, VF was recorded in all trial in which a lower plasma concentration had been achieved (0.39 and 0.51 μg/ml).

The heart rate and coronary blood flow responses to exercise before and after HMR 1402 are displayed in figure 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 altered any of the resting cardiovascular variables investigated in this study and are displayed in table 3. The effects of glibenclamide and HMR 1402 on plasma glucose and insulin levels are shown in figure 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).

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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 action potential duration (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, Billman et al., 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

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on APD_{90}, 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 K_{ATP} channel antagonist HMR 1883 or the non-selective K_{ATP} 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 K_{ATP} 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 (IC_{50} for HMR 1402: 98 nM, IC_{50} for HMR 1883: 0.6 µM) (Gögelein et al., 1998). HMR 1402 also inhibited the rilmakalim-induced K_{ATP} 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 (IC_{50} = 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 (IC_{50} = 8 nM) than in guinea pig (IC_{50} = 20 nM) (Gögelein et al., 1998). As in the papillary muscle preparation, HMR 1402 is more potent in blocking rilmakalim-activated K_{ATP} current than HMR 1883 (Gögelein et al., 1998), especially in rat ventricular myocytes. Similarly, hypoxia consistently elicited a marked reduction in APD_{90} 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 of HMR 1883 had no significant effect (Gögelein et al., 1998), while 0.3 μM of HMR 1402 produced a significant inhibition of reductions in APD_{90} induced by hypoxia. The effect of HMR 1402 at a concentration of 10 μM was also more pronounced than that observed with 20 μM of HMR 1883 (Gögelein et al., 1998). Thus, one may conclude that HMR 1402 is more potent in blocking rilmakalim-activated and hypoxia-activated K_{ATP} 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_{ATP} channels promote 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 μM 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 μM 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 (figure 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) while 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_{ATP} 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 ß-cells (RINm5F cells) in which the K_{ATP} channels were activated by diazoxide. HMR 1402 only partially inhibited the effect of diazoxide on the cell membrane potential (IC_{50}=3.9 µM). This inhibition was somewhat more potent than that of HMR 1883 (IC_{50} approximately 20 µM) (Gögelein et al., 1998, Manning-Fox et al., 2002) but considerably less than that of glibenclamide (IC_{50}=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 (figure 12). Thus, the in vitro and in vivo data strongly suggest that HMR 1402 acts preferentially on cardiac K_{ATP} 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 7 of 8 animals tested. In agreement with these findings, both the non-selective K_{ATP} antagonist glibenclamide and the cardioselective K_{ATP} HMR 1883 protected

against ischemically induced malignant arrhythmias (see 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 maximum) in animals (Billman et al., 1993; Billman et al., 1998). Therefore, non-selective 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 re-entry (Billman 1994). Drugs that block this channel should be particularly effective anti-arrhythmic 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 rilamkalim or by hypoxia in vitro without major actions on either coronary flow or rat pancreatic β-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. Since 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.

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Footnotes

[2] = Aventis Pharma, Frankfurt, Germany

Legends for Figures

Figure 1. The chemical structures for HMR 1402 and glibenclamide.

Figure 2. A flow chart illustrating the sequence of events for the in vivo studies. MI = myocardial infarction, VF = ventricular fibrillation

Figure 3a. 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 of HMR 1402 attenuated the rilmakalimn-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. Figure 3b. Concentration- response effect of HMR 1402 on the rilmakalim-induced shortening of the APD_{90} at external pH of 6.0. The rilmakalim-induced shortening of the APD_{90} is considered as 100%. The numbers in brackets indicate the numbers of experiments.

Figure 4a. Effect of HMR 1402 on hypoxia-induced shortening of the action potential in guinea pig right papillary muscle. The APD shortened markedly in the presence of hypoxia. Note that HMR 1402 significantly attenuated this hypoxia-induced shortening of the action potential (data presented after 60 min of hypoxia pH = 7.4 for control and 6.5 otherwise). Figure 4b. Concentration-response effect of HMR 1402 on the hypoxia-induced shortening of the APD_{90} at external pH of 6.5. The hypoxia-induced shortening of the APD_{90} is considered as 100%. The number of experiments was 4 for each data point..

Figure 5. Whole-cell currents recorded in a guinea pig ventricular myocyte, demonstrating the activation of K_{ATP} current by rilmakalim (10 μM) and its inhibition by HMR 1402.

Figure 6. Concentration-response curves demonstrating the inhibition of rilmakalim (10 μM) - induced whole-cell current in isolated rat and guinea pig ventricular myocytes. On the ordinate, the inhibition of the current in percent of the rilmakalim-induced current is plotted. The IC_{50} values are indicated in the figure. The numbers in brackets represent the numbers of experiments. The bathing solution pH was adjusted to 7.4.

Figure 7. HMR 1402 (10 μM) has no significant effects on the current-voltage curve in a guinea pig ventricular myocyte. The current-voltage curve was recorded with NaCl-solution in the bath. A voltage ramp from –140 mV to +80 mV was applied.

Figure 8. Concentration-response curves, summarizing the depolarizing effects of HMR 1402 and glibenclamide in pancreatic β-cells (RINm5F) in the presence of 100 μM diazoxide. Glibenclamide data from our previous study (Gogelein et al., 1998) have been added to facilitate comparison. The numbers in brackets indicate the number of experiments.

Figure 9a. Representative recordings of coronary flow from a guinea pig heart during hypoxia. Note the large increase in coronary flow and the partial inhibition of this flow increase by HMR 1402. Figure 9b. The mean values of the coronary flow under normoxia (control), hypoxia and subsequent addition of HMR 1402. Figure 9c. Concentration-response (percent inhibition) effects of HMR 1402 on the hypoxia-induced increase in coronary flow.

Figure 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 flutterdespite similar changes in heart rate that were induced by the ischemia.

Figure 11. The effects of HMR 1402 on the heart rate and coronary blood flow response to exercise. Closed circles represent control (i.e., no drug) while 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.

Figure 12. The effect of HMR 1402 (3.0 mg/kg i.v.) and glibenclamide (1.0 mg/kg, i.v.,) on plasma insulin and blood glucose. Note that HMR 1402 (open circle, dashed line) failed to alter either of these variables. The response was not different from than provoked by saline injection (gray squares). In contrast, glibenclamide (1.0 mg/kg, i.v.) provoked significant (* $p < 0.01$) increases in plasma insulin with corresponding significant (* $p < 0.01$) reductions in blood glucose.

Table 1

The Effects of HMR 1402 and Action Potential Parameters Under Normoxic Conditions

	Control (n = 5)	HMR 1402 (2μM) (n = 5)
APD_{90} (ms)	177.6 ± 6.8	185.2 ± 7.2
Resting potential (mV)	-78.8 ± 1.0	-80.4 ± 1.8
Amplitude (mV)	114.0 ± 4.5	113.4 ± 4.2
Upstoke velocity (V/s)	118.4 ± 11.8	114.4 ± 12.6

The recording time was 30 min after HMR 1402 was applied.

Table 2

The Effects of HMR 1402 in Langendorff-perfused Guinea Pig hearts under Normoxic and Hypoxic Conditions

A. Normoxic Conditions

	Drug Dose (μM)				
	0 (n = 7)	1μM (n = 4)	10μM (n = 5)	30μM (n = 4)	100μM (n = 6)
LVP (mmHg)	89.0 ± 6.4	94.8 ± 5.3	73.2 ± 6.6*	72.8 ± 8.0*	74.7 ± 4.1*
LVDP (mmHg)	-0.3 ± 0.5	-1.0 ± 0.4	-0.5 ± 0.3	1.3 ± 0.3	4.0 ± 1.6
HR (beats/min)	218.3 ± 4.3	206.3 ± 7.3	205.6 ± 6.9	204.8 ± 11.1	213 ± 1.4
LV dP/dt (mmHg/s)	1307.3 ± 92.1	1273.8 ± 106.6	1033.6 ± 55.1*	1026.8 ± 72.8*	1028.0 ± 31.9*
CF (ml/min)	7.6 ± 0.5	7.0 ± 1.0*	6.7 ± 0.8*	8.4 ± 0.8	8.4 ± 0.4*

Table 2 (continued)

B. Hypoxic Conditions

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

LVP = left ventricular pressure, LVDP = left ventricular diastolic pressure, HR = heart rate, LV = left ventricular, and CF = coronary flow, * $P < 0.05$ from no drug condition (i.e., 0 μM)

Table 3

Effect of HMR 1402 on Resting Cardiovascular Parameters

<u>Heart Rate (beats/min)</u>	
No Drug	124.1 ± 10.5
HMR 1402	112.5 ± 12.1
<u>PR Interval (ms)</u>	
No Drug	88.1 ± 4.6
HMR 1402	88.8 ± 5.8
<u>QRS Duration (ms)</u>	
No Drug	33.8 ± 2.6
HMR 1402	38.8 ± 2.3
<u>QT Interval (ms)</u>	
No Drug	206.2 ± 13.1
HMR 1402	228.1 ± 8.8
<u>QTc Interval, Bazett's Method (ms)</u>	
No Drug	293 ± 17.6
HMR 1402	308 ± 16.8
<u>QTc Interval, Fridericia's Method (ms)</u>	
No Drug	260.1 ± 15.0
HMR 1402	278.1 ± 12.0

Table 4

Plasma Concentration of HMR 1402

Animal #	Dose (i.v.)	Plasma Level (μg /ml)	Result
1a	2 μg/kg/min	0.504	VF
1b	4 μg/kg/min	1.246	No Arrhythmias
2	4 μg/kg/min	1.190	No Arrhythmias
3	4 μg/kg/min	1.275	No Arrhythmias
4	4 μg/kg/min	No sample	No Arrhythmias
5	3.0 mg/kg	2.283	No Arrhythmias
6	3.0 mg/kg	1.502	No Arrhythmias
7	3.0 mg/kg	5.336	No Arrhythmias
8	3.0 mg/kg	0.394	VF

VF = ventricular flutter that deteriorated into ventricular fibrillation.

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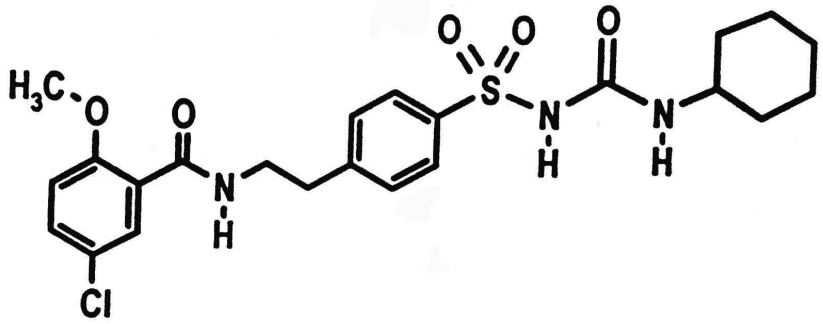
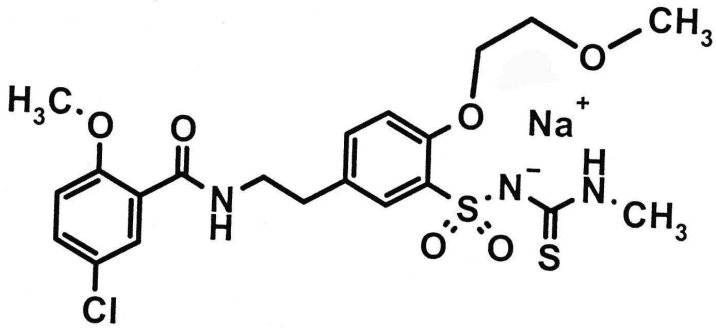
Figure 1

CHEMICAL STRUCTURES OF HMR 1402 AND GLIBENCLAMIDE



GLIBENCLAMIDE



HMR 1402

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Figure 2

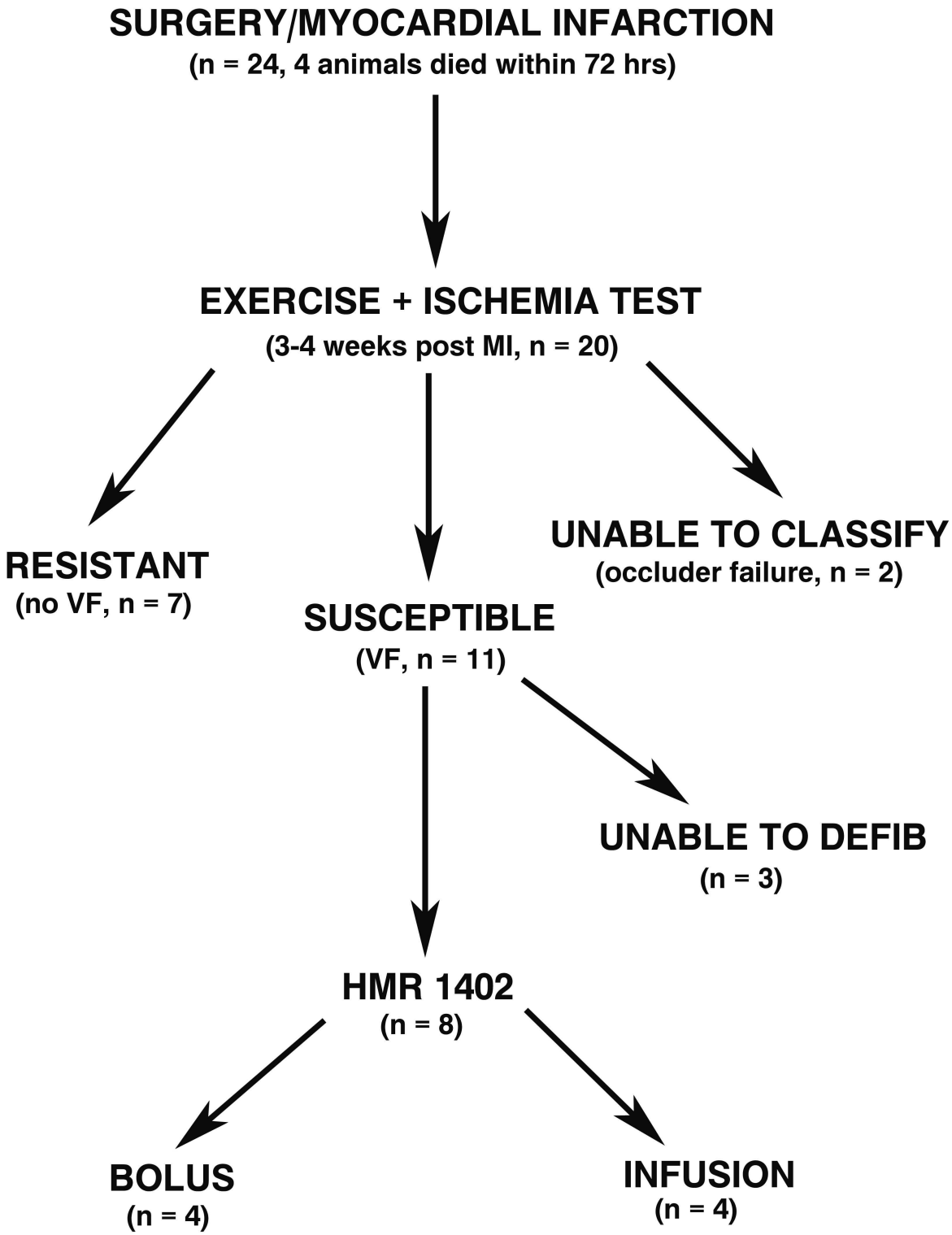

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Figure 3a

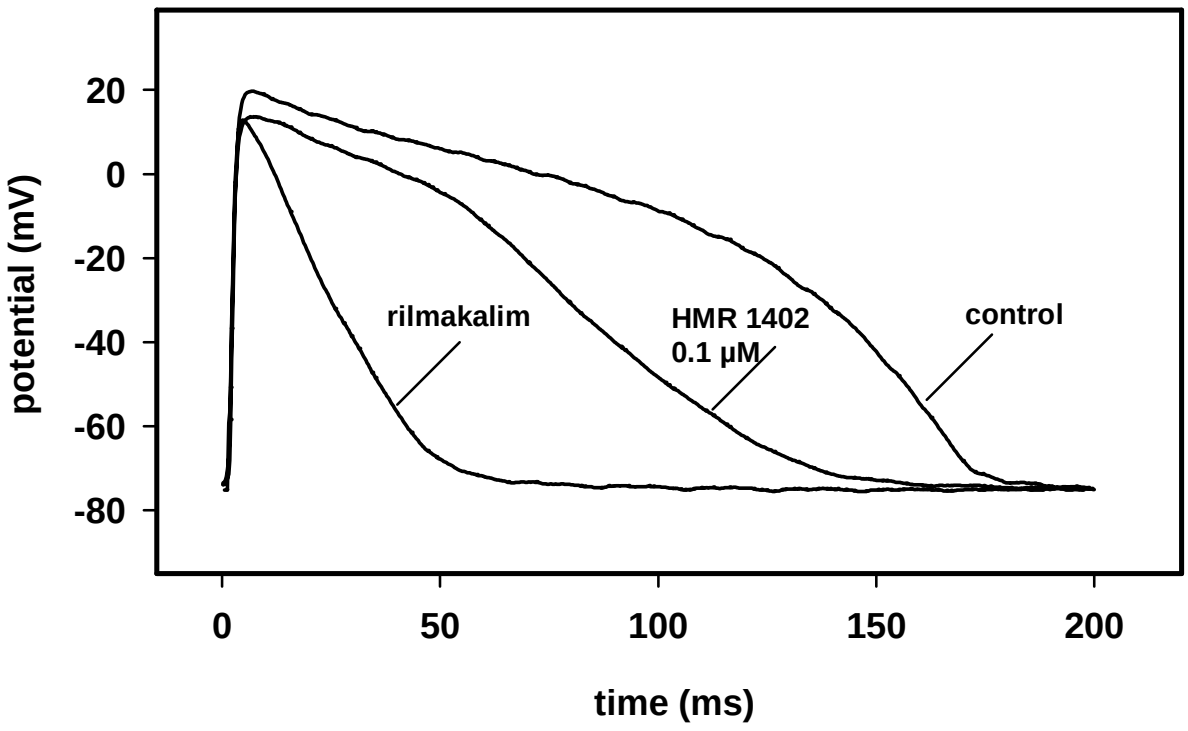

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Figure 3b

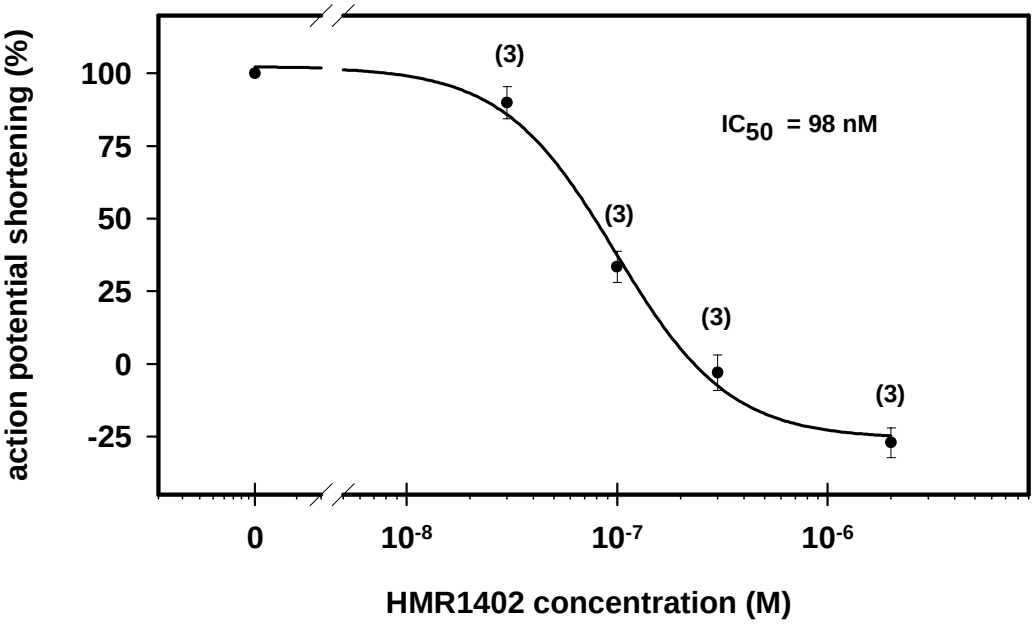

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Figure 4a

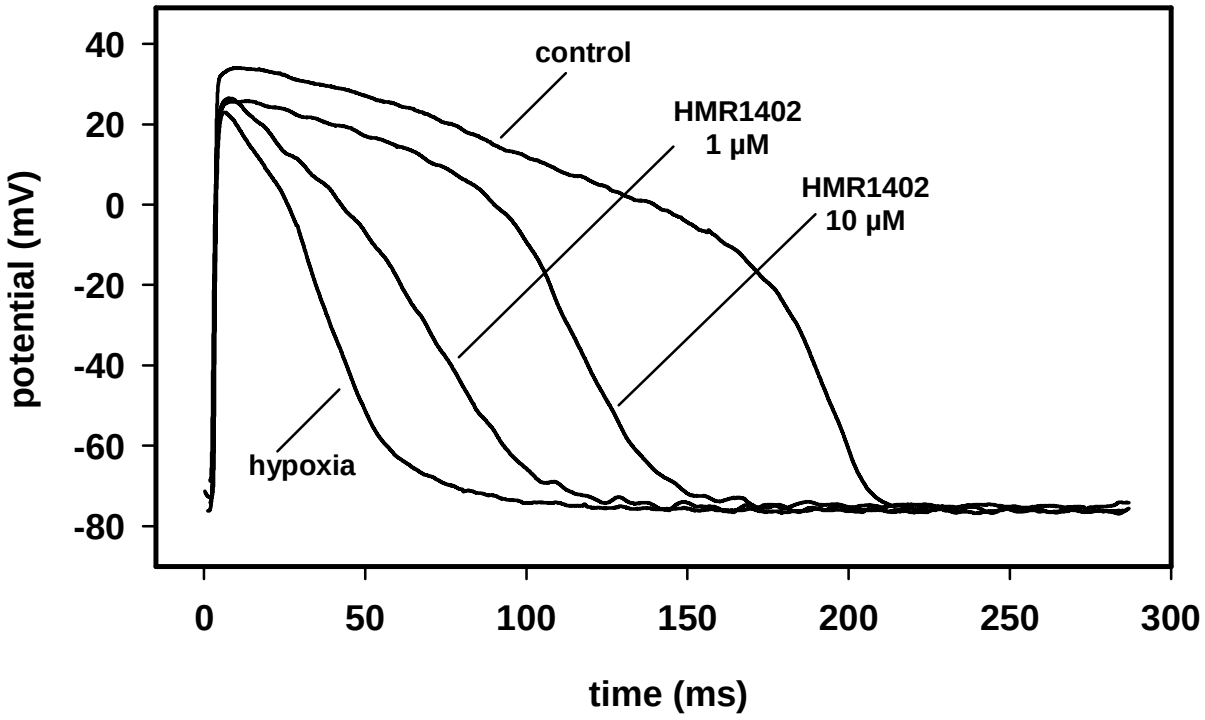

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Figure 4b

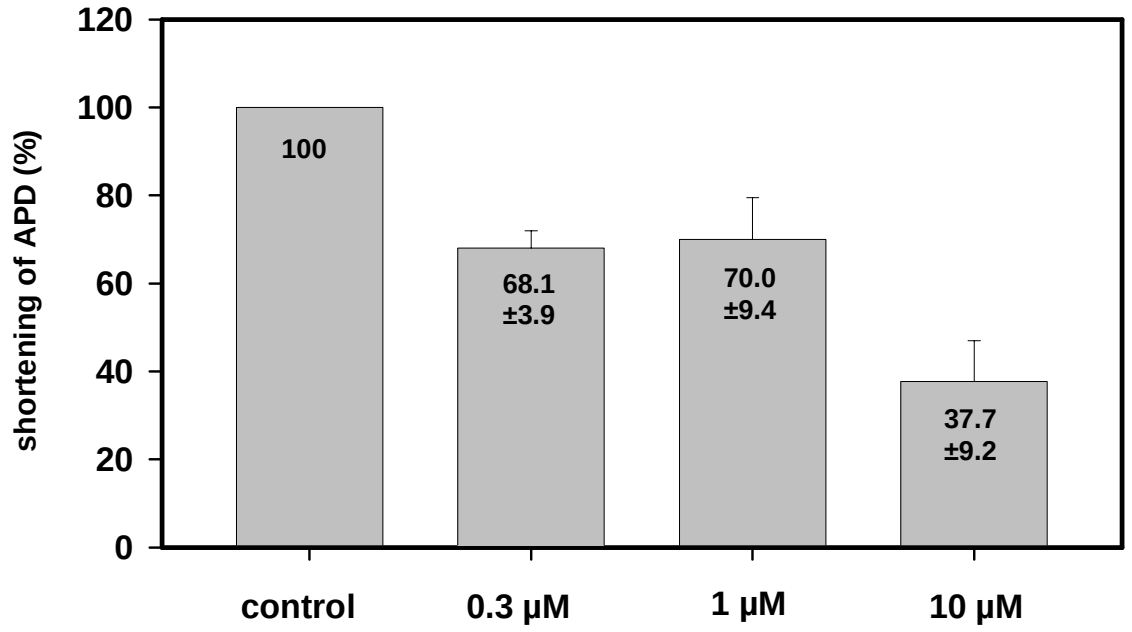

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Figure 5

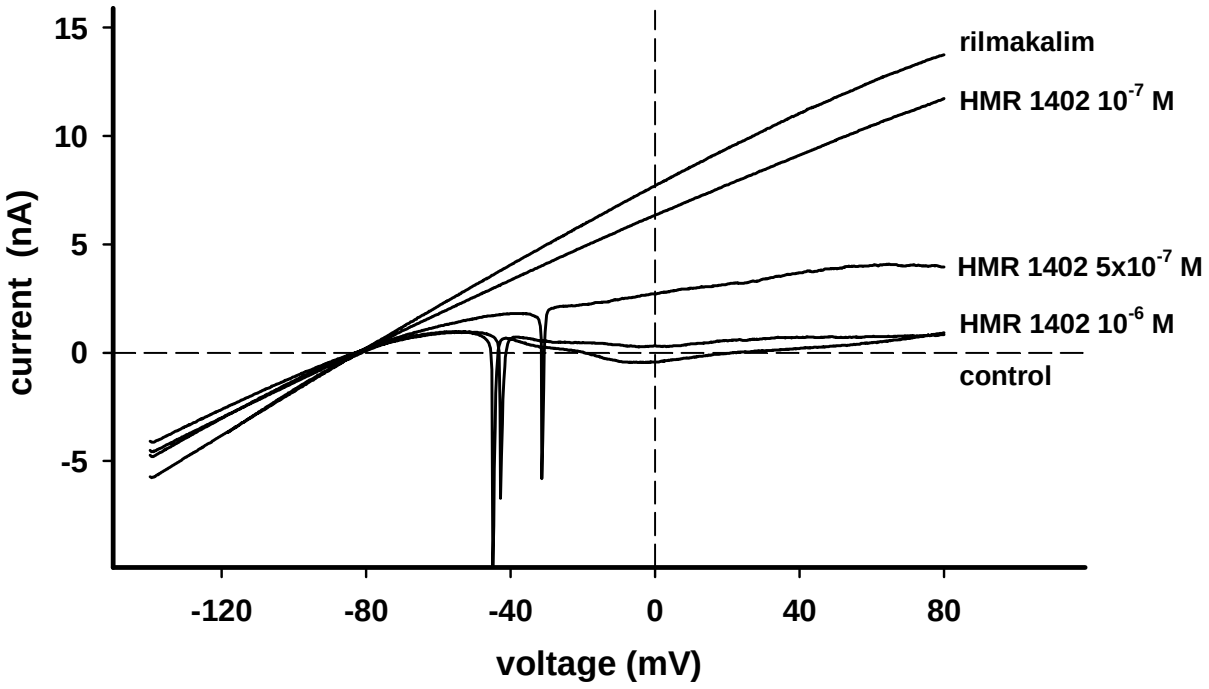

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Figure 6

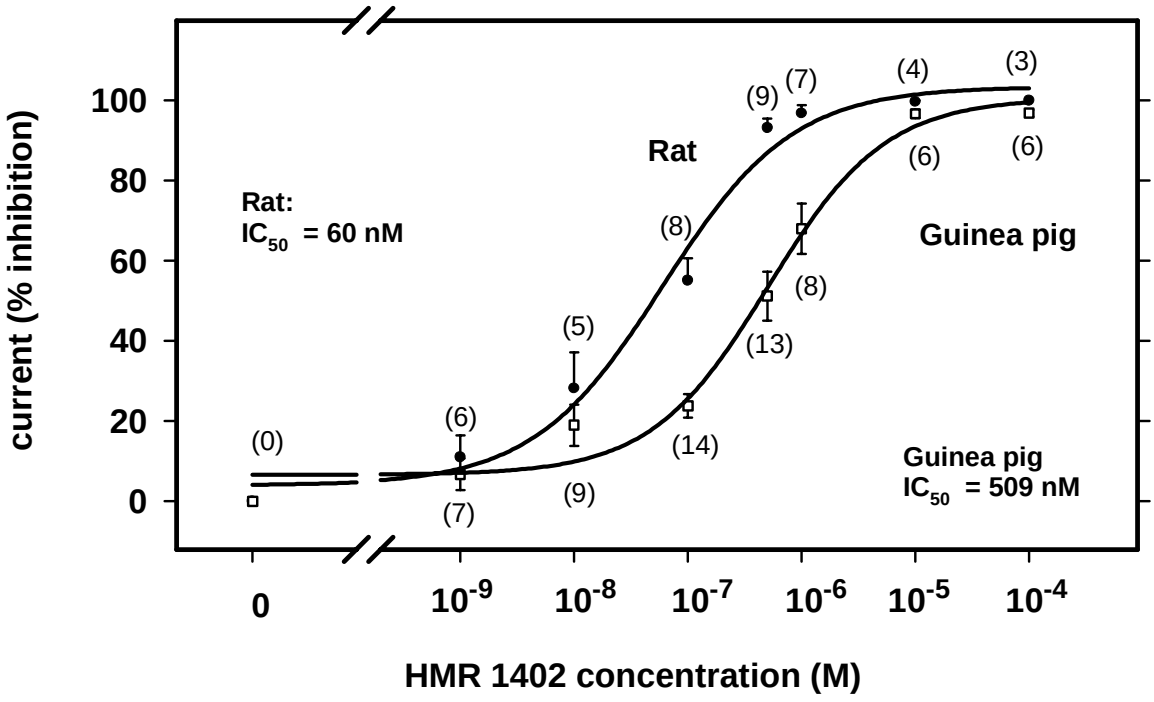

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Figure 7

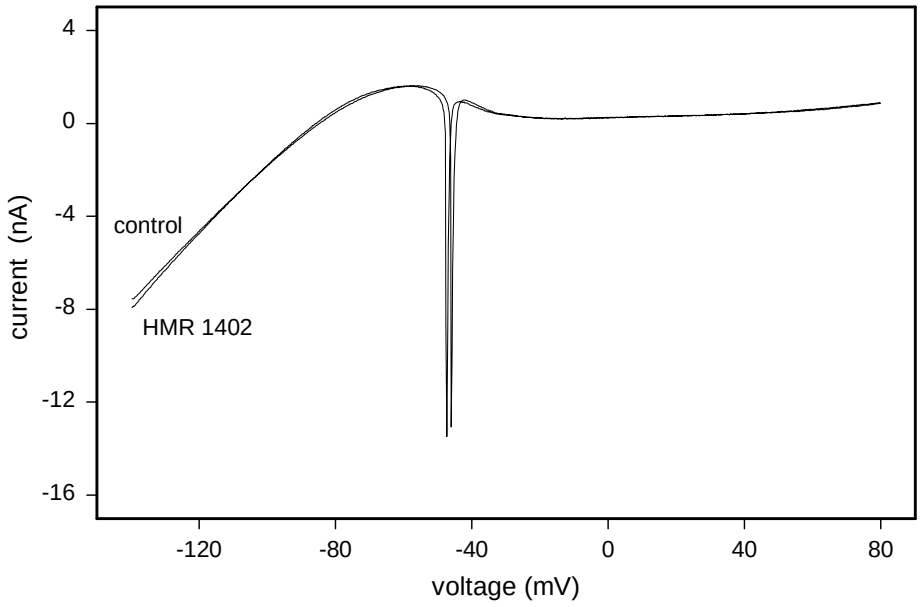

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Figure 8

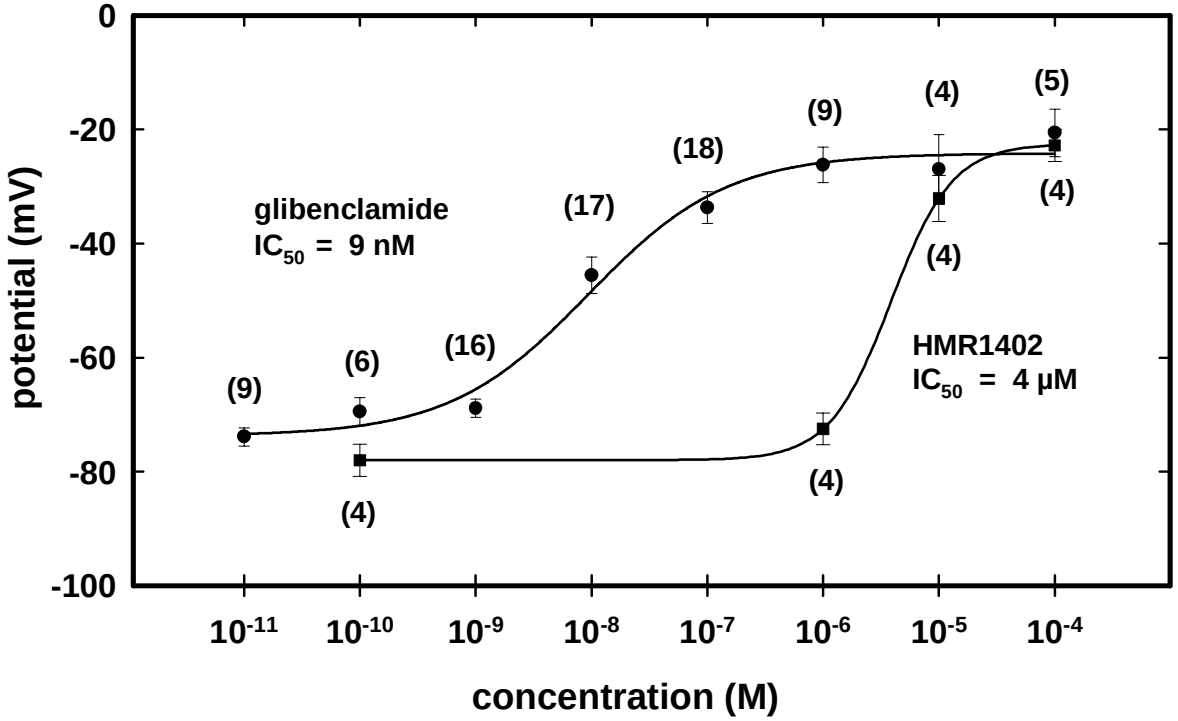

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Figure 9a

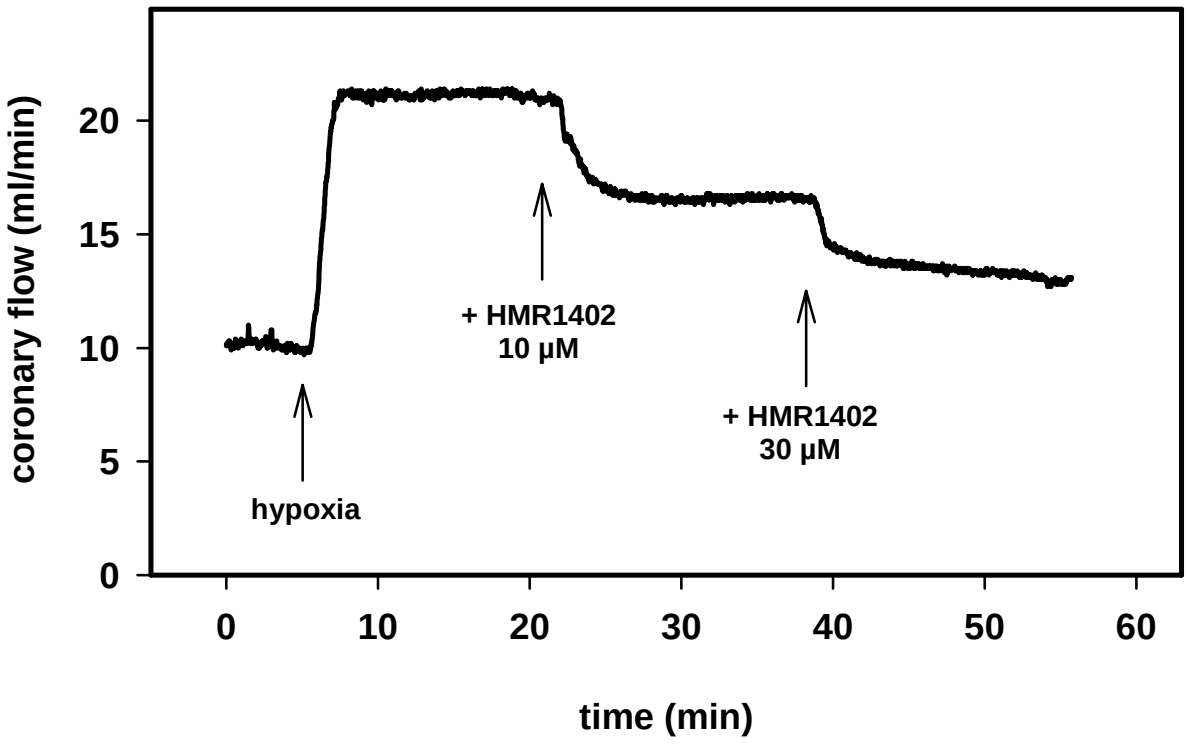

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Figure 9b

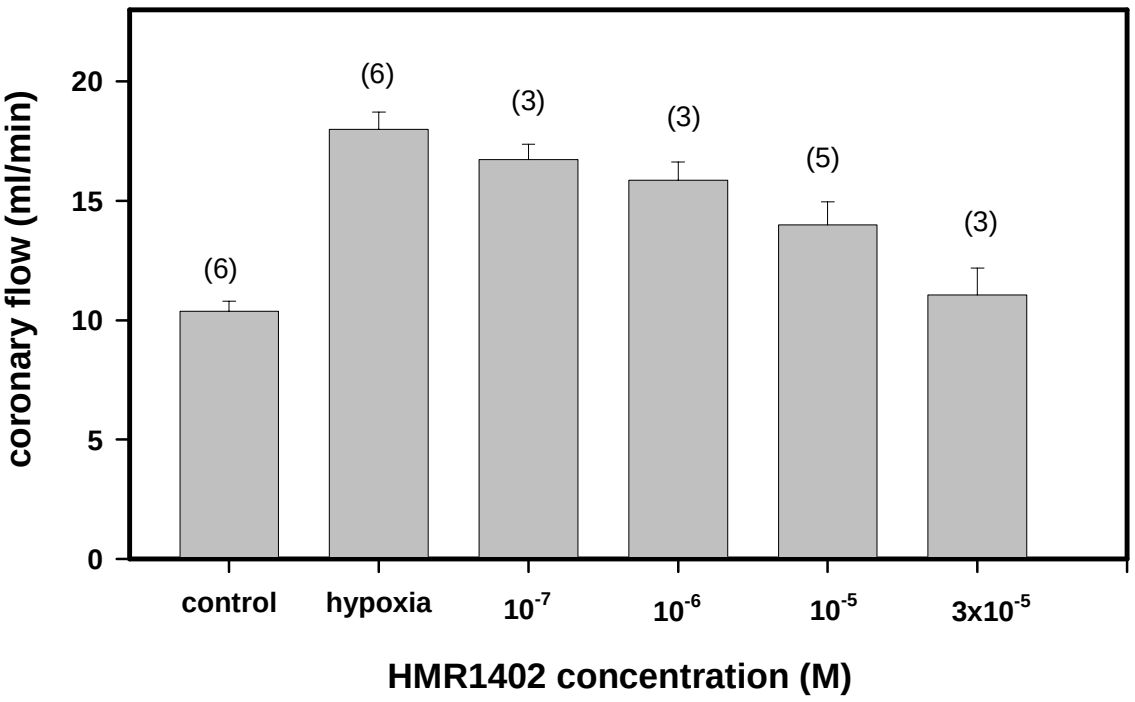

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Figure 9c

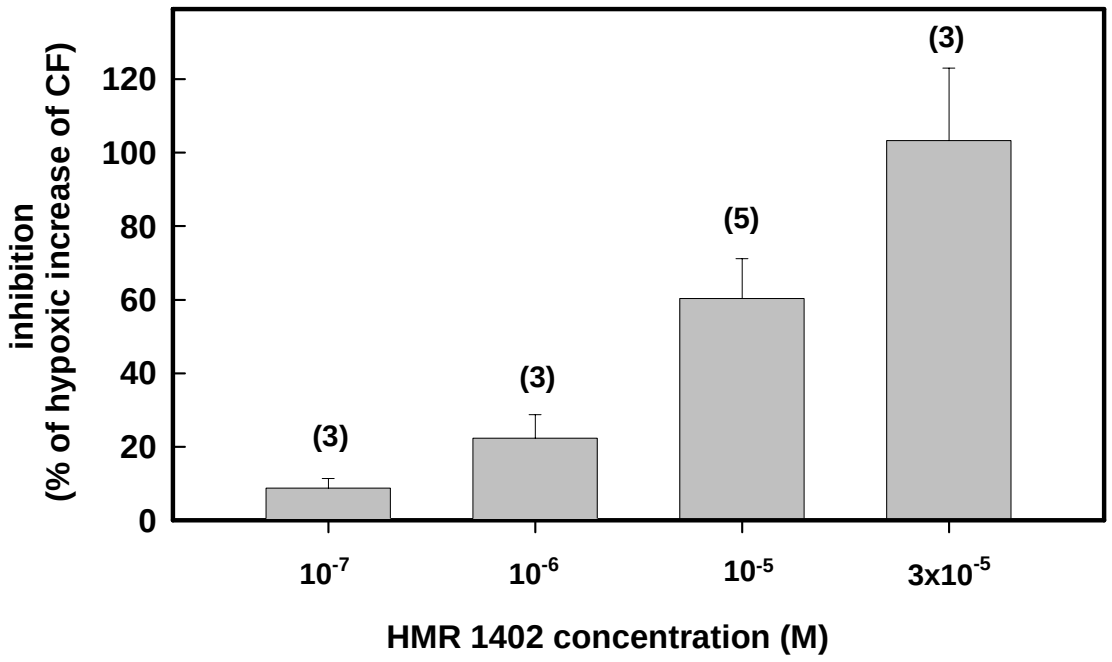

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Figure 10

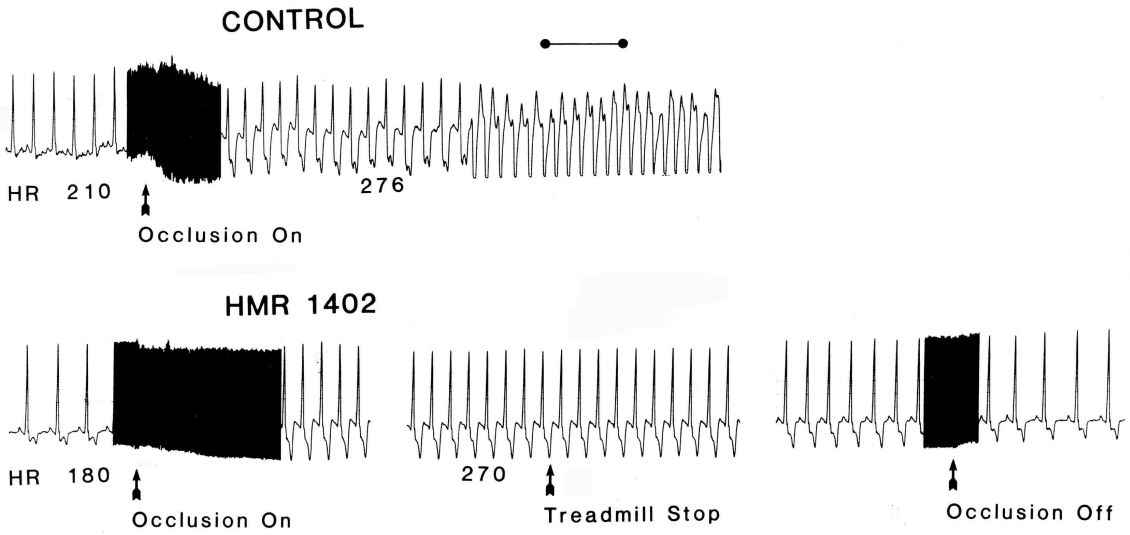

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Figure 11

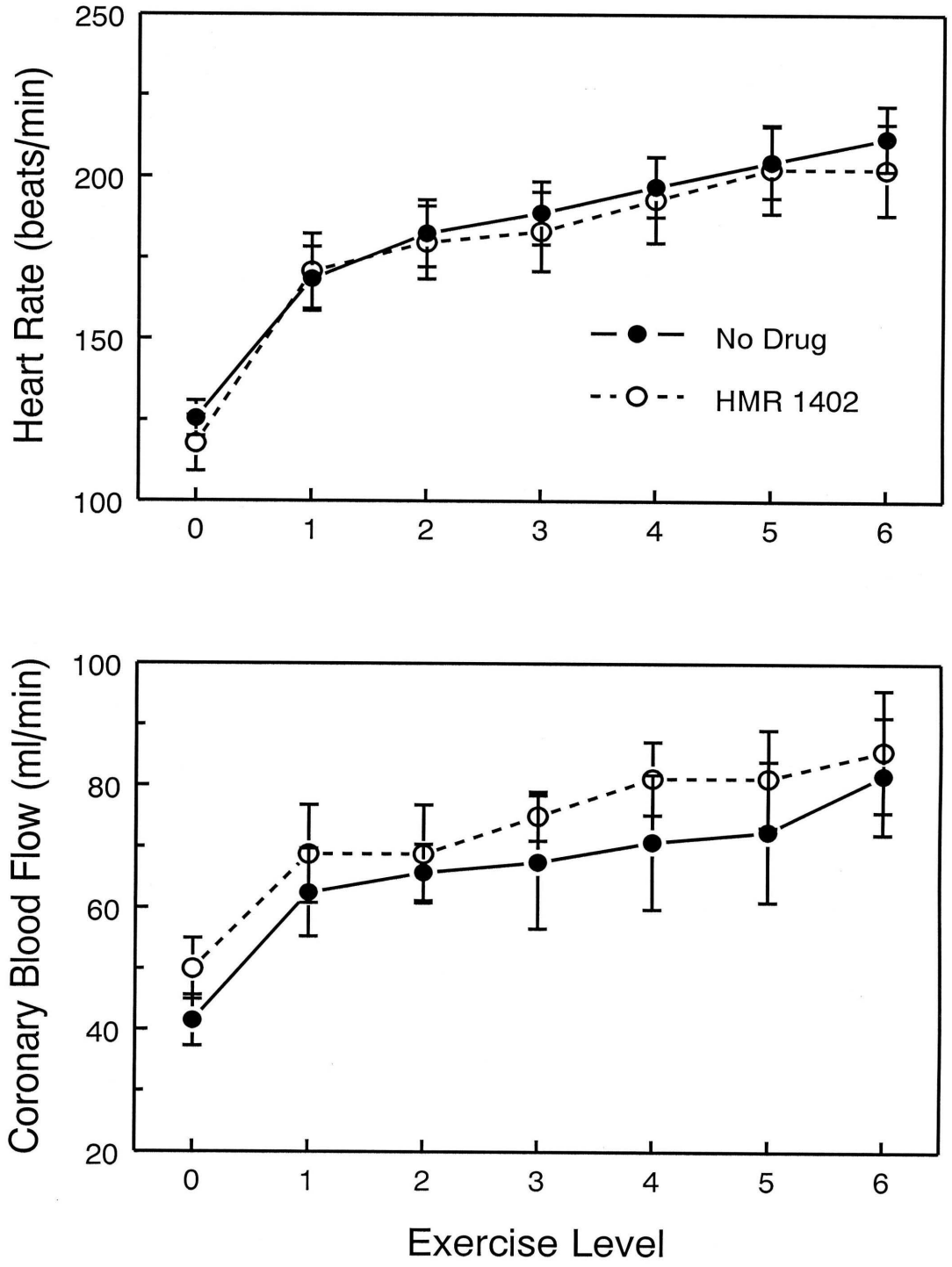

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Figure 12