0022-3565/02/3031-132–140$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 303, No. 1
36988/1006668
Printed in U.S.A.
Copyright © 2002 by The American Society for Pharmacology and Experimental Therapeutics
JPET 303:132–140, 2002

The Journal of PHARMACOLOGY And Experimental Therapeutics

In Vivo Characterization of the Mitochondrial Selective K_{ATP} Opener (3*R*)-*trans*-4-((4-Chlorophenyl)-*N*-(1*H*-imidazol-2-ylmethyl)dimethyl-2*H*-1-benzopyran-6-carbonitril Monohydrochloride (BMS-191095): Cardioprotective, Hemodynamic, and Electrophysiological Effects

GARY J. GROVER, ALBERT J. D'ALONZO, RAYMOND B. DARBENZIO, CHARLES S. PARHAM, THOMAS A. HESS, and MOHINDER S. BATHALA

Metabolic and Cardiovascular Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Pennington, New Jersey

Received April 17, 2002; accepted May 16, 2002

ABSTRACT

Recent studies have shown the importance of mitochondrial ATP-sensitive potassium channels (K_{ATP}) in cardioprotection, and studies in vitro have shown that the benzopyran analog (3*R*)-*trans*-4-((4-chlorophenyl)-*N*-(1*H*-imidazol-2-ylmethyl)dimethyl-2*H*-1-benzopyran-6-carbonitril monohydrochloride (BMS-191095) is a selective mitochondrial K_{ATP} opener with cardioprotective activity. The goal of this study was to show selective cardioprotection for BMS-191095 in vivo without hemodynamic or cardiac electrophysiological effects expected for nonselective K_{ATP} openers. BMS-191095 reduced infarct size in anesthetized dogs (90-min ischemia + 5-h reperfusion) in a dose-dependent manner (ED_{25} = 0.4 mg/kg i.v.) with efficacious plasma concentrations of 0.3 to 1.0 μM, which were consistent with potency in vitro. None of the doses of BMS-191095 tested caused any effect on peripheral or coronary hemodynamic status. Further studies in dogs showed no effects of BMS-191095 on cardiac conduction or action potential configuration within the cardioprotective dose range. In a programmed electrical stimulation model, BMS-191095 showed no proarrhythmic effects, which is consistent with its lack of effects on cardiac electrophysiological status. BMS-191095 is a potent and efficacious cardioprotectant that is devoid of hemodynamic and cardiac electrophysiological side effects of first generation K_{ATP} openers, which open both sarcolemmal and mitochondrial K_{ATP}. Selective opening or activation of mitochondrial K_{ATP} seems to be a potentially effective strategy for developing well tolerated and efficacious K_{ATP} openers.

Pharmacological K_{ATP} activation is associated with cardioprotection and may simulate some aspects of ischemic preconditioning (Auchampach et al., 1991; Gross and Auchampach, 1992; Grover et al., 1994; Armstrong et al., 1995). Numerous K_{ATP} subtypes exist and seem to be differentially expressed in various tissues (Inoue et al., 1991; Atwal et al., 1993; Grover et al., 1995a; Chutkow et al., 1996; Inagaki et al., 1996). Pharmacological data suggest that mitochondrial K_{ATP} activation is critical for cardioprotection and that this channel is distinct from sarcolemmal channels (Garlid et al., 1996, 1997; Liu et al., 1998; Nakai et al., 2001). Clear pharmacological separation between smooth muscle relaxation and cardioprotection has been reported for several K_{ATP} openers such as BMS-180448 and BMS-191095 (Atwal et al., 1993; Grover et al., 1995b, 2001; Rovnyak et al., 1997) (chemical structures shown in Fig. 1). BMS-191095, in particular, is very selective with no vasorelaxant activity while retaining the cardioprotective efficacy of nonselective agents such as cromakalim or pinacidil (Grover et al., 2001). In addition, there are no electrophysiological effects in isolated rat or guinea pig hearts, suggesting a lack of effect on cardiac sarcolemmal K_{ATP}. Further evidence for selectivity was the lack of effect of BMS-191095 on whole cell myocyte K_{ATP} current (Grover et al., 2001). Interestingly, the protective effects of this compound were completely abolished by glyburide and 5-HD.

Recent data suggest the importance of mitochondrial K_{ATP} in mediating cardioprotection (Garlid et al., 1997; Liu et al., 1998), and these studies have been primarily

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.102.036988.

ABBREVIATIONS: K_{ATP}, ATP-sensitive potassium channel; BMS-191095, (3*R*)-*trans*-4-((4-chlorophenyl)-*N*-(1*H*-imidazol-2-ylmethyl)dimethyl-2*H*-1-benzopyran-6-carbonitril monohydrochloride; 5-HD, sodium 5-hydroxydecanoate; LCX, left circumflex coronary artery; ET, excitation threshold; APD, action potential duration; CT, conduction time; CV, conduction velocity; ERP, effective refractory period; RVOT, right ventricular outflow tract; LAD, left anterior descending coronary artery; BCL, basic cell length; PES, programmed electrical stimulation.

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

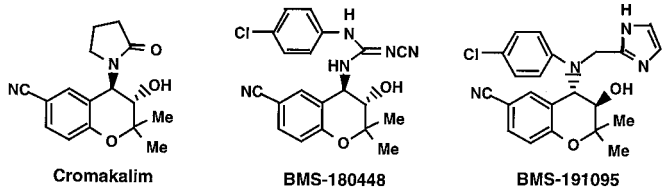

Fig. 1. Chemical structures of BMS-180448, cromakalim, and BMS-191095.

done using diazoxide. Although diazoxide opens cardiac mitochondrial K_{ATP} with 1000-fold selectivity compared with cardiac sarcolemmal channels, it is a potent sarcolemmal K_{ATP} opener in vascular smooth muscle (Garlid et al., 1997). Diazoxide, therefore, will significantly reduce arterial blood pressure well before cardioprotective doses are achieved. Recently published data show that BMS-191095 selectively opens mitochondrial K_{ATP} without affecting sarcolemmal channels in vascular smooth muscle, heart or pancreatic β-cells (Grover et al., 2001). BMS-191095 would be expected to be devoid of vasodilator and proarrhythmic activity unlike nonselective agents, and such activity is contraindicated during acute myocardial ischemia (Chi et al., 1990; Belin et al., 1996; Grover et al., 2001). This study with BMS-191095 was done in vitro or in isolated hearts and smooth muscle ex vivo (Grover et al., 2001). Although previous work on related K_{ATP} openers show that the in vitro and in vivo potencies correlate well (D'Alonzo et al., 1995a; Grover et al., 1995a, 1996), the goal of this study was to compare the cardioprotective, electrophysiological, and hemodynamic properties of BMS-191095 in vivo. This was done in canine models of ischemia and reperfusion as well as proarrhythmia models. Our data showed that BMS-191095 reduced infarct size in a dose-dependent manner while being devoid of cardiac electrophysiological and hemodynamic effects. No proarrhythmogenic activity was observed, which is consistent with selectivity for mitochondrial channels.

Materials and Methods

Canine Model of Ischemia and Reperfusion

Mongrel dogs of either sex (15–24 kg) were anesthetized with i.v. sodium pentobarbital (30 mg/kg), and a catheter was placed into the right femoral artery for later collection of blood samples for blood gases and reference blood flow analysis. This technique has been described previously (Grover et al., 1995a). Another catheter was placed into the right femoral vein for supplementation of anesthesia and drug infusion. A Mikrotip catheter pressure transducer (Millar, Houston, TX) was placed into the left femoral artery and was advanced into the aortic arch for the measurement of arterial blood pressure. An endotracheal tube was placed into the trachea and the animals were artificially respired, and this was maintained such that eucapnia and normoxia were observed throughout the study.

A left thoracotomy was performed at the 5th intercostal space and the heart was exposed. The left circumflex coronary artery (LCX) was isolated proximal to its first branch, and a silk suture was placed around it for later occlusion. A catheter was placed into the left atrial appendage for dye (area at risk measurement) and radioactive microsphere injection.

The animals were allowed to stabilize for 5 to 10 min at which time an arterial blood sample was removed anaerobically for measurement of blood gases using a Radiometer (ABL4, Copenhagen, Denmark) blood gas analyzer. The blood gases were adjusted to normoxic and eucapnic levels by adjustment of the ventilator. Arterial blood pressure and heart rate were measured at baseline once the eucapnia was achieved. At this time myocardial blood flow was measured using radioactive microspheres (^{113}Sn, ^{57}Co, ^{85}Sr, or ^{46}Sc; 15 $\pm$ 3 μm; PerkinElmer Life Sciences, Boston, MA). Before coronary occlusion, the animals were divided into five groups: 1) vehicle-treated animals (i.v., saline, $n = 6$) starting 10 min before LCX occlusion and given over a total of 25 min; 2) BMS-191095-treated animals (8 μg/kg/min, $n = 6$); 3) BMS-191095-treated animals (25 μg/kg/min i.v., $n = 6$); 4) BMS-191095-treated animals (80 μg/kg/min i.v., $n = 6$); 4) BMS-191095-treated animals (139 μg/kg/min i.v., $n = 6$); and 5) BMS-191095-treated animals (139 μg/kg/min i.v.) + 150 μg/kg/min 5-HD (150 μg/kg/min, intracoronary, $n = 6$). BMS-191095 or vehicle was started 10 min preocclusion and the total dose given as 0.2, 0.6, 2.0, or 3.5 mg/kg (over a total of 25 min). 5-HD was given directly into the LCX starting 10 min before ischemia and for an additional 10 min into ischemia for a total dose of 3 mg/kg. After the first 10 min of drug infusion, the LCX was completely occluded for a total of 90 min. Myocardial blood flow was again measured 40 min after the initiation of LCX occlusion and in this model is a measure of collateral blood flow into the ischemic region. At 90 min after occlusion, the LCX was reperfused. After 1 h of reperfusion, regional myocardial blood flow was again measured.

The reperfusion was continued for a total of 5 h at which time the LCX was cannulated and perfused at the animals' existing pressure with Ringer's lactate for determination of the area at risk. Patent blue violet dye (1 mg/kg of a 10-mg/ml solution) was injected into the left atrial catheter and the heart was quickly excised and the atria trimmed. The ventricles were cut transversely into 0.5-cm slices. The borders of the area at risk (no stain) were delineated and separated and the slices were incubated at 37°C for 30 min in a 1% solution of 2,3,5-triphenyl tetrazolium chloride in phosphate-buffered saline. This agent stains viable tissue red, whereas infarcted tissue is not stained and becomes white or gray in color. The myocardial area at risk and infarct areas of interest were measured using computerized planimetric techniques. The tracings were digitized using Applescan (Apple Computer Inc., Cupertino, CA) and then area was determined on a Macintosh IIcx computer using Macdraft software (Innovative Data Design, Concord, CA). The infarct size was expressed as a percentage of the left ventricular area at risk. Myocardial blood flow was calculated by taking myocardial pieces from the subepicardial and subendocardial halves of the ischemic and nonischemic regions (four pieces from each area) of the left ventricular free wall. The center of the ischemic region was used for the ischemic regional blood flow determinations. The radioactivity in the tissue pieces as well as the reference blood samples were determined in an Autogamma 8000 gamma counter (Beckman Coulter, Inc., Irvine, CA), and tissue flows were calculated from these counts. Reference blood withdrawal rate was 9 ml/min.

A separate study was done to ascertain plasma concentrations of BMS-191095 at times relevant to ischemic protection. Conscious mongrel dogs of either sex (15–24 kg) were infused i.v. with a total dose of 0.2 mg/kg ($n = 3$) or 0.6 ($n = 3$) mg/kg BMS-191095. The right cephalic vein of each dog was cannulated for drug infusion and the left cephalic vein was cannulated for blood withdrawal. The animals were restrained using a sling. This infusion was continued for a total of 25 min. Blood was withdrawn from the dogs at 10, 25, and 40 min after initiation of drug infusion. The 40-min time represented a point in which BMS-191095 was washing out for 15 min. It also is equivalent to 30 min into the ischemic interval for the infarct size studies, and thus is a relevant time in terms of cardioprotective efficacy. Venous blood was collected into sterile Vacutainer tubes containing EDTA. The tubes were centrifuged at 20°C at 2700 rpm for 17 min. The plasma was transferred to 5-ml opaque plastic test tube, sealed, and frozen. All

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

plasma samples were assayed for BMS-191095 concentrations by a validated liquid chromatography/mass spectroscopy method. The lower limit of quantification of the analytical method was 0.75 ng/ml BMS-191095 in plasma.

Electrophysiological and Arrhythmia Models in Dogs

Electrophysiological Characterization in Dogs. Male mongrel dogs (15–25 kg, $n = 13$) were anesthetized with dial urethane (0.35 ml/kg i.v.) and were intubated and mechanically ventilated (model 613; Harvard Apparatus, South Natick, MA) with room air sufficient to maintain eucapnia. The right femoral artery and vein were cannulated to measure systemic blood pressure and to infuse drug, respectively. A lead II ECG was continuously monitored. The arterial catheter was connected to a pressure transducer (model P23XL; Spectromed, Oxnard, CA) and associated amplifier and chart recorder (TA 4000; Gould, Cleveland, OH) to monitor arterial pressure. A left thoracotomy was performed at the 5th intercostal space. Stimulating and recording electrodes were placed on the left atrium and left ventricle such that the heart could be paced from the atrium and stimuli administered (model PGEN; N.B. Datyner, Stoney Brook, NY, and voltage to current converter; SIS, Princeton, NJ) to the ventricle while simultaneously monitoring ventricular electrogram. The left carotid artery was isolated and a quadrapolar catheter was inserted into the vessel and positioned to record the His-bundle electrogram. All waveforms were displayed on a chart recorder (TA4000; Gould).

Using the method of premature stimulation, refractory periods were determined at a BCL of 400, 333, and 286 ms. Using the appropriate rising edge of the QRS complex (+QRS) of the ECG as a trigger (S1), premature stimuli (S2) were introduced at approximately every 7 to 10 beats to determine the following parameters. 1) ET: the minimum current (mA) required to evoke extrasystoles in response to an S2 placed approximately 70% of the cycle length from an S1. The time between the onset of S1 and the onset of S2 is the S1-S2 interval (ms). 2) Ventricular ERP: the maximum S1-S2 (S1 = +QRS) interval at which no extrasystoles were generated at a constant current twice ET and expressed in milliseconds. 3) T wave amplitude was measured from the chart recordings as the height of the T wave in millimeters from the isoelectric level of the ECG. 4) APD90 was measured in milliseconds at the 90% repolarization level from the plateau region of the monophasic action potential.

Conduction times and associated parameters were also measured from the His-bundle electrogram at BCLs of 400, 333, and 286 ms. 1) Atrial-His bundle conduction time was measured from the onset of atrial deflection to the onset of His-bundle deflection in milliseconds. 2) His-bundle ventricular conduction time was measured from the onset of His-bundle deflection to the onset of the ventricular deflection in milliseconds. 3) AV was measured as the duration from the onset of atrial deflection to the onset of the ventricular deflection in milliseconds. 4) ventricular CT was measured as the time from S2 to the onset of the ventricular deflection in milliseconds. 5) Ventricular CV was measured as the interelectrode distance (2 cm) divided by ventricular CT and expressed in centimeters per second. 6) Wavelength was measured as the product of ventricular CV and ventricular ERP and expressed in millimeters.

After the above-described parameters were measured, BMS-191095 was administered over 5 min in cumulative i.v. doses of 0.3, 1, 3, and 10 mg/kg every 20 min and each animal served as its own control.

Effect in a PES Model. Seventeen fasted (12 h) mongrel dogs (16–25 kg) of either sex were anesthetized with thiopental (12 mg/kg i.v.), given atropine (15 mg/kg i.m.), and intubated. The animal was connected to an inhalational anesthesia machine (Narkovet Deluxe; North American Drager, Telford, PA), and anesthesia was maintained with isoflurane (1–2%) delivered with oxygen (100%) at 4 to 10 respirations/min to maintain normocapnia. An ECG harness was connected to the dog, and a standard lead II ECG (VSM Monitor; Physio Control, Redmond, WA) was monitored.

By using standard aseptic techniques, a left thoracotomy was performed at the 5th intercostal space. The heart was suspended in a pericardial cradle, and the LAD was dissected and a silk suture (00) was placed around it. Collateral blood vessels from the LCX were ligated. An aneurysm clamp was placed on the LAD and was completely occluded for 90 min. A 20-gauge needle was placed on top of the LAD and the suture tied securely around both the vessel and needle. The needle was carefully withdrawn and the suture left in place. This produced a critical stenosis, so that the LAD was partially occluded during reperfusion. This technique has been shown to reduce the incidence of mortality during reperfusion (Manning and Hearse, 1984).

At 60 min after the LAD occlusion, the inspired oxygen level was gradually reduced by mixing with nitrous oxide (1:10). Thus, at the time of reperfusion (90 min) the inspired oxygen level was 10%. Once the aneurysm clamp was removed, reperfusion began. The LCX ligatures were removed and the animal was allowed to stabilize for 2 min. At this time, the oxygen level was gradually increased to 50%, and the ligature around the LAD producing the critical stenosis was removed. The thoracotomy was closed using standard surgical technique. Keflin (1 g i.v.) was given prophylactically after closure of the chest.

The dog was returned to a postoperative cage and allowed to recover from the anesthesia. After recovery, the dog was returned to its cage. Only if necessary and the animal seemed in pain, postoperative analgesia was given and consisted of administration of butorphanol, buprenorphine, or oxymorphone. In addition, antibiotics were administered as needed after recovery.

Six to eight days after LAD occlusion, the animal was brought to the laboratory and anesthetized with dial urethane (0.4 ml/kg i.v.). Respiration was maintained (model 613; Harvard Apparatus) with room air at a volume of 200 to 300 ml at a rate of 10 to 15 breaths/min to maintain eucapnia. Eucapnia was assured by continuously monitoring (model 253; Datex, Wilmington, MA) expired CO_2 before starting the experiment. The right femoral artery and vein were cannulated for measuring systemic blood pressure and drug infusion, respectively. A Lead II ECG was continuously monitored. The animal was instrumented with an arterial catheter connected to a pressure transducer (model P23XL; Spectromed, Oxnard, CA) and associated amplifier and chart recorder (TA 4000; Gould) to measure arterial blood pressure. A standard Lead II ECG recording configuration was used to monitor the ECG waveform and heart rate. ECG and pressure waveforms were recorded and analyzed online with a digital acquisition system (model HD-4; Po-ne-mah, Storrs, CT). The chest was reopened and electrodes placed on the left atrial appendage (bipolar surface electrode for pacing), into the septum (a bipolar plunge electrode placed directly over the RVOT for stimulation, into the base of the left ventricle (bipolar plunge electrode placed in the normal zone of the myocardium), and into the infarcted region (bipolar plunge electrode placed into the ischemic zone). Each electrode lead was connected in tandem to an isolated voltage to current converter (SIS) and programmable stimulator (PGEN; N.B. Datyner). An esophageal temperature probe was used to monitor and heating pads were used to maintain core temperature at 38 ± 1°C. A banjo temperature probe (model 427; YSI Inc, Yellow Springs, OH) was sutured to the epicardial surface to measure and control temperature in the thoracic cavity between 38 ± 1°C via a feedback device (model 73ATA; YSI Inc.). The dog was allowed to stabilize for 15 min. After stabilization, control reading of ECG parameters, arterial blood pressure and heart rate were obtained, and electrophysiological measurements of ET, and ERP were obtained from the ischemic and nonischemic zones, and RVOT sites.

Electrophysiological measurements were made before drug administration and between dosing (immediately after the infusion period). Determinations of the ET and ERP were made at an atrial

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

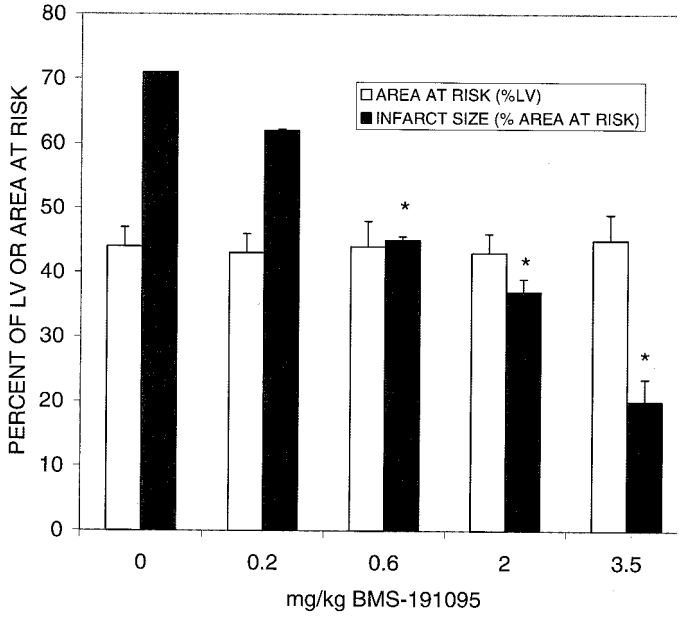

Fig. 2. Effect of increasing doses of BMS-191095 on infarct size expressed as a percentage of the area at risk (AR). BMS-191095 reduced infarct size in a dose-dependent manner starting at the 0.6-mg/kg dose. The area at risk (expressed as percentage of LV) was similar for all groups.

pacing rate of 150 beats/min (2.5 Hz), when possible, with single extrastimuli of 4-ms duration at 3 times the diastolic threshold current. Extrastimuli of 4-ms duration were introduced through the plunge electrodes to evoke extrasystolic beats and to determine ET, ERT, QT-interval, ventricular CT, CV, APD, and wavelength.

After the above-mentioned parameters were measured, a PES protocol using three extrastimuli was applied through the RVOT electrode in an attempt to elicit reentrant or ventricular tachyarrhythmias (ventricular tachyarrhythmia or ventricular fibrillation). This protocol has been published previously (D'Alonzo et al., 1995b). Only animals that were noninducible (failure to elicit ventricular tachyarrhythmia) in the control state were used in this study. BMS-191095 was administered at a fixed time interval of 5 min in cumulative i.v. doses of 0.1 to 3 mg/kg every 20 min. Electrophysiological parameters were measured and inducibility determined systematically before drug administration and after each dose. After the last dose of BMS-191095, the LCX was completely occluded, and the survival of the animals was monitored for the next 2 h. Animals either died from this protocol (ventricular fibrillation) or were euthanized.

After the last protocol, the heart was excised, ventricles cross-sectioned into 1-cm slices, and stained with 2,3,5-triphenyltetrazolium chloride to distinguish normal from infarcted tissue. The sections were dissected into right ventricle, left ventricle, and infarcted areas. From this, infarct size was calculated as a percentage of wet weight of left ventricle.

Drug Preparation

Dial urethane was prepared as follows: 40% urethane (Sigma-Aldrich), 20% 5,5 diallyl barbituric acid (Sigma-Aldrich), and 40% of ethyl urea (Aldrich Chemical Co., Milwaukee, WI). The chemicals were placed in a graduated cylinder and distilled water added to achieve a proper concentration. The solution was transferred to 100-ml amber bottles, stored at room temperature, and protected from light. Hearts were stained with 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich) prepared as a 1% solution in phosphate-buffered saline, pH 7.4 (Sigma-Aldrich). BMS-191095 was prepared (20 mg/ml) fresh on the day of use in polyethylene glycol 400 (Fisher Scientific, Fair Lawn, NJ).

Statistics

Comparisons between treatments for infarct size, hemodynamics, and regional myocardial blood flow electrophysiological parameters were done using a factorial analysis of variance. For the analysis of variance studies, a Newman-Keuls post hoc test was used. Differences were deemed significant if $p < 0.05$. Inducibility differences were determined using a Fisher's exact test. All values were expressed as mean ± S.E.M.

Results

Effect of BMS-191095 on Infarct Size in Dogs. The infarct size and area at risk data for BMS-191095 and vehicle-treated animals are shown in Fig. 2. The left ventricular area at risk (shown as percentage of left ventricle) was similar for all groups, indicating comparable anatomy. Infarct size expressed as a percentage of the area at risk was approximately 70% in vehicle-treated animals and was significantly reduced by BMS-191095 in a dose-dependent manner, with the lowest efficacious dose being approximately 0.6 mg/kg. The infarct size in the 3.5 mg/kg BMS-191095-treated group was reduced by approximately 70% relative to vehicle-treated animals. The infarct size

TABLE 1
Effect of BMS-191095 on arterial blood pressure and heart rate before and after myocardial ischemia
All values are expressed as mean ± S.E.

	Control	Drug	LCX Occlusion (40 min)	Hours Into Reperfusion		
				1	3	5
Mean Arterial Blood Pressure (mm Hg)						
Vehicle	118 ± 7	117 ± 7	91 ± 11	81 ± 4[a]	82 ± 5[a]	73 ± 5[a]
BMS (0.2 mg/kg)	113 ± 5	117 ± 6	98 ± 5	91 ± 5[a]	90 ± 3[a]	80 ± 3[a]
BMS (0.6 mg/kg)	113 ± 2	116 ± 4	100 ± 4	82 ± 4[a]	83 ± 4[a]	77 ± 2[a]
BMS (2.0 mg/kg)	123 ± 4	120 ± 3	109 ± 4	94 ± 4[a]	98 ± 8[a]	83 ± 4[a]
BMS (3.5 mg/kg)	122 ± 6	119 ± 5	108 ± 4	90 ± 5[a]	98 ± 5[a]	83 ± 4[a]
BMS + 5-HD[b]	119 ± 7	117 ± 8	107 ± 5	88 ± 4[a]	93 ± 4[a]	85 ± 5[a]
Heart Rate (beats/min)						
Vehicle	156 ± 10	158 ± 10	160 ± 6	153 ± 7	159 ± 4	161 ± 5
BMS (0.2 mg/kg)	162 ± 5	163 ± 6	165 ± 5	168 ± 5	160 ± 6	159 ± 4
BMS (0.6 mg/kg)	165 ± 8	166 ± 9	151 ± 6	150 ± 7	158 ± 4	166 ± 6
BMS (2.0 mg/kg)	156 ± 8	158 ± 9	152 ± 8	144 ± 9	150 ± 3	154 ± 3
BMS (3.5 mg/kg)	161 ± 7	162 ± 7	161 ± 8	151 ± 4	158 ± 7	164 ± 5

[a] Significantly different from its respective preischemic control value ($p < 0.05$).
[b] 8.5 mg/kg BMS-191095 + 3 mg/kg 5-HD (intracoronary).

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

TABLE 2
Effect of BMS-191095 on regional myocardial blood flow before and after myocardial ischemia
All values are mean ± S.E.M. Occ, 40 min into occlusion; Reper, 1 hr into reperfusion; Preocc, preocclusion.

	Occluded Region			Nonoccluded Region		
	Preocc	Occ	Reper	Preocc	Occ	Reper
Subepicardium (ml/min/100 g)						
Vehicle	101 ± 7	7 ± 3[a]	98 ± 8	115 ± 7	93 ± 15	86 ±15
BMS (0.2 mg/kg)	106 ± 5	4 ± 2[a]	79 ± 14	123 ± 11	84 ± 13	96 ±10
BMS (0.6 mg/kg)	104 ± 9	6 ± 2[a]	76 ± 14	126 ± 14	85 ± 9	88 ± 6
BMS (2.0 mg/kg)	105 ± 9	7 ± 3[a]	86 ± 10	114 ± 10	87 ± 13	88 ± 9
BMS (3.5 mg/kg)	103 ± 8	13 ± 4[a]	105 ± 14	115 ± 13	89 ± 9	97 ± 3
BMS + 5-HD[b]	107 ± 7	7 ± 3[a]	101 ± 11	111 ± 12	92 ± 10	99 ± 8
Subendocardium (ml/min/100 g)						
Vehicle	114 ± 8	2 ± 1[a]	54 ± 14[a]	120 ± 13	98 ± 17	105 ± 12
BMS (0.2 mg/kg)	121 ± 14	1 ± 1[a]	60 ± 16[a]	123 ± 11	88 ± 13	96 ± 10
BMS (0.6 mg/kg)	113 ± 5	1 ± 1[a]	70 ± 15[a]	121 ± 7	92 ± 11	93 ± 6
BMS (2.0 mg/kg)	117 ± 6	2 ± 1[a]	54 ± 12[a]	124 ± 9	94 ± 17	90 ± 11
BMS (3.5 mg/kg)	117 ± 11	2 ± 2[a]	93 ± 11[c]	122 ± 16	89 ± 9	103 ± 3
BMS + 5-HD	116 ± 11	2 ± 2[a]	61 ± 14[a]	119 ± 14	93 ± 10	98 ± 7

[a] Significantly different from its respective preischemic value ($p < 0.05$).
[b] 8.5 mg/kg BMS + 3 mg/kg 5-HD.
[c] Significantly different from its respective vehicle-treated group value ($p < 0.05$).

data were also calculated as a percentage of reduction from the respective vehicle-treated group. From these data we calculated an ED_{25} for infarct size reduction. ED_{25} was defined as the dose causing 25% reduction in infarct size from vehicle-treated group values and 25% reduction was chosen as a minimal effective dose. The ED_{25} for BMS-191095 was found to be 0.4 mg/kg. 5-HD abolished the protective effect of 3.5 mg/kg BMS-191095 such that infarct size was 66 ± 6% of area at risk, which is not significantly different from vehicle-treated animals (Fig. 2)

Hemodynamic data for BMS-191095 and vehicle-treated hearts are shown in Table 1. Baseline values for arterial blood pressure and heart rate were similar for all groups. Ischemia tended to lower blood pressure and this was continued during reperfusion. BMS-191095 did not affect blood pressure at any time point measured during the experiment. Heart rate was not affected by either ischemia or by reperfusion. BMS-191095 had no effect on heart rate at any of the doses given. No differences in reperfusion ectopy were observed between any of the groups (data not shown).

Regional myocardial blood flow values are shown in Table 2. Baseline myocardial blood flows were similar for both groups in all regions in which flow was measured. Occlusion of the LCX significantly reduced blood flow into the ischemic bed, particularly into the subendocardial region. Reperfusion blood flow was significantly reduced compared with preischemic baseline values in the subendocardium, whereas it returned to baseline in the subepicardium in vehicle-treated hearts. No differences in ischemic regional blood flow during ischemia were observed between drug- and vehicle-treated animals. Reperfusion blood flow was significantly improved by BMS-191095 at the high dose in the subendocardial region of the formerly ischemic zone. This improved reperfusion blood flow was completely abolished by 5-HD. Nonoccluded region blood flow values did not change at any time during the study.

Additional studies were done to determine plasma concentrations of BMS-191095 for the 0.2- and 0.6-mg/kg doses. The data show (Fig. 3) that the 0.2-mg/kg dose, which failed to reduce infarct size, showed <0.4 μM at 25 min, whereas a 1 μM concentration was achieved for 0.6 mg/kg. This is consistent with the cardioprotective potency values reported in vitro previously (Grover et al., 2001).

Electrophysiological Characterization of BMS-191095. BMS-191095 had no significant effects on His-bundle conduction (Table 3). Also, there were no significant changes in ventricular ERP, CT, CV, wavelength, or APD90 with BMS-191095 treatment (Table 4). No changes in AERP were observed (data not shown). There were no rate-dependent changes in ventricular CT or CV observed in these studies. As expected, decreases in ventricular ERP and wavelength were rate-dependent. There were no significant changes in wavelength in both treatment groups, and any decreases were associated with decreases in BCL and not administration of compound. The data in Tables 3 and 4 are expressed as percentages of change from predrug values.

No electrocardiographic changes in PR-interval, QT-in-

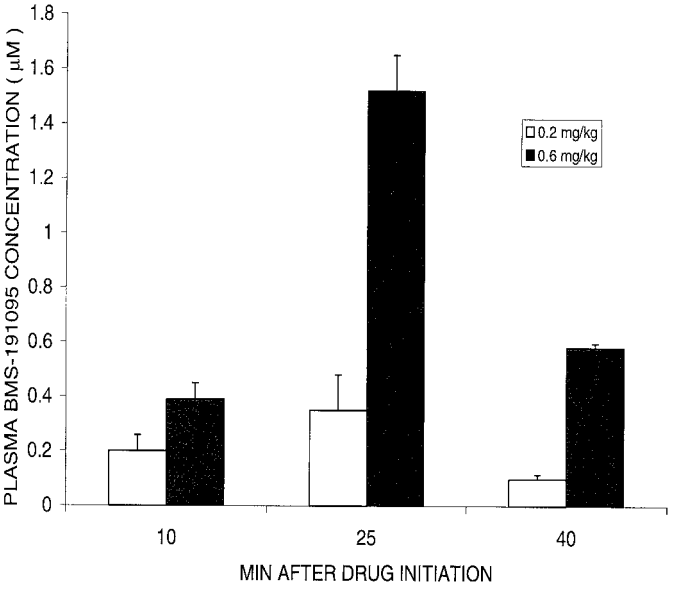

Fig. 3. Plasma BMS-191095 concentrations within the cardioprotective range (0.6 mg/kg) and below the cardioprotective range (0.2 mg/kg). Plasma concentrations were between 0.4 and 1 μM at the relevant cardioprotective dose. This agrees well with the cardioprotective potency calculated in vitro.

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

TABLE 3
Effect of BMS-191095 on His-bundle conduction intervals (millisecond)

Dose	A-H Interval		H-V Interval		A-V Interval	
	Absolute	Δ%	Absolute	Δ%	Absolute	Δ%
mg/kg i.v.						
BCL 400 ms						
0.0	74 ± 8		58 ± 2		132 ± 8	
0.3	74 ± 8	0 ± 4	57 ± 2	−2 ± 0	131 ± 6	0 ± 2
(5)						
1.0	72 ± 5	−2 ± 5	57 ± 2	−3 ± 1	130 ± 4	−4 ± 2
3.0	71 ± 8	−4 ± 6	57 ± 2	−3 ± 1	127 ± 4	−3 ± 4
10.0	70 ± 5	−4 ± 8	59 ± 2	1 ± 1	128 ± 4	−2 ± 5
BCL 333 ms						
0.0	75 ± 6		57 ± 2		132 ± 6	
0.3	76 ± 7	1 ± 3	57 ± 2	0 ± 2	133 ± 8	1 ± 2
1.0	75 ± 5	0 ± 3	58 ± 2	2 ± 1	131 ± 5	−1 ± 2
3.0	76 ± 5	2 ± 5	56 ± 2	−2 ± 2	132 ± 5	0 ± 3
10.0	75 ± 7	2 ± 8	59 ± 1	3 ± 3	134 ± 5	2 ± 5
BCL 286 ms						
0.0	77 ± 7		57 ± 2		135 ± 5	
0.3	80 ± 7	4 ± 1	57 ± 2	1 ± 1	137 ± 6	2 ± 1
1.0	77 ± 7	1 ± 1	57 ± 2	−1 ± 1	134 ± 6	0 ± 1
3.0	82 ± 7	7 ± 3	56 ± 1	−2 ± 1	138 ± 7	3 ± 2
10.0	83 ± 7	8 ± 7	58 ± 1	1 ± 2	141 ± 7	5 ± 4

terval, or T-wave amplitude were observed with BMS-191095 treatment (Table 5). Decreases in QT-interval were rate-dependent, but not associated with BMS-191095 treatment. No hemodynamic changes were observed for any dose of BMS-191095 used (data not shown). Percentage of change was calculated from predrug values.

PES Model. BMS-191095 produced no significant changes in ventricular ERP (148 ± 4 and 140 ± 4 ms), APD (152 ± 4 and 153 ± 4 ms), conduction time (56 ± 3 and 52 ± 3 ms), or wavelength (55 ± 3 and 56 ± 3 mm) relative to control values in either the normal or ischemic regions of the myocardium, respectively (Table 6). There was also no change in QT-intervals from a control value of 178 ± 4 ms. To normalize for differences in heart rate, some of the animals were tested at their intrinsic rate. In the BMS-191095 treatment group, none (0%) of the animals converted to an inducible state. After final ligation of the LCX, BMS-191095 did not cause any mortality. Historical data with vehicle show approximately 70 to 80% survival using this protocol. BMS-191095 caused no hemodynamic effects at any dose tested (data not shown).

Discussion

Pharmacological activation of K_{ATP} is associated with cardioprotection in numerous models of ischemia and reperfusion (McPherson et al., 1993; Yao and Gross, 1994; Mizimura et al., 1995; Liu et al., 1998). The pharmacological cardioprotection is seen in these studies with distinct K_{ATP} opener chemotypes. The cardioprotective effects K_{ATP} openers are abolished by glyburide and 5-HD. Although the broad class of K_{ATP} openers exert cardioprotective effects, there is some diversity in terms of the pharmacological profiles of K_{ATP} openers with some being potent vasodilators, some having effects on pancreatic β-cells, and others having no effect on cardiac action po-

TABLE 4
Effect of BMS-191095 on ventricular refractory period (VERP, milliseconds), conduction time (VCT, milliseconds), conduction velocity (VCV, millimeters per second), wavelength, and monophasic APDs at 90% repolarization (APD90, milliseconds)

Dose	VERP		VCT		VCV		Wavelength
	Absolute	% Δ	Absolute	% Δ	Absolute	% Δ	Absolute
mg/kg i.v.							
BCL 400 ms							
0.0	150 ± 1		53 ± 2		38 ± 1		57 ± 2
0.3	150 ± 1	0 ± 0	52 ± 2	−2 ± 2	39 ± 1	2 ± 2	58 ± 2
1.0	149 ± 1	−1 ± 1	52 ± 2	−2 ± 2	39 ± 2	2 ± 3	58 ± 2
3.0	148 ± 1	−1 ± 1	52 ± 2	−1 ± 2	39 ± 1	1 ± 2	57 ± 2
10.0	150 ± 1	0 ± 1	52 ± 2	−1 ± 2	38 ± 1	1 ± 2	57 ± 2
BCL 333 ms							
0.0	140 ± 1		51 ± 2		39 ± 1		55 ± 1
0.3	139 ± 1	0 ± 1	51 ± 2	0 ± 1	39 ± 1	1 ± 1	55 ± 2
1.0	140 ± 1	1 ± 1	51 ± 2	−1 ± 1	40 ± 2	1 ± 2	55 ± 2
3.0	141 ± 1	1 ± 0	51 ± 2	−1 ± 2	40 ± 2	2 ± 2	56 ± 2
10.0	139 ± 1	−1 ± 0	52 ± 2	1 ± 2	39 ± 1	0 ± 2	54 ± 2
BCL 286 ms							
0.0	130 ± 1		52 ± 2		38 ± 1		50 ± 1
0.3	132 ± 1	2 ± 0	51 ± 2	−2 ± 1	39 ± 1	2 ± 1	52 ± 2
1.0	133 ± 1	2 ± 1	51 ± 2	−3 ± 1	40 ± 2	3 ± 2	53 ± 2
3.0	131 ± 1	1 ± 1	51 ± 2	−3 ± 2	40 ± 2	3 ± 2	52 ± 2
10.0	132 ± 1	2 ± 1	52 ± 2	−2 ± 1	39 ± 1	2 ± 1	52 ± 2

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

TABLE 5
Effect of BMS-191095 on PR-interval (ms), QT-interval (ms), and T wave amplitude (min)

Dose	PR-Interval		QT-Interval		T Wave	
	Absolute	% Δ	Absolute	% Δ	Absolute	% Δ
mg/kg i.v.						
BCL 400 ms						
0.0	117 ± 6		191 ± 3		5.1 ± 0.4	
0.3	116 ± 6	0 ± 2	188 ± 4	−2 ± 1	4.6 ± 0.3	−9 ± 3
1.0	113 ± 5	−4 ± 2	186 ± 3	−4 ± 2	5.3 ± 0.3	6 ± 7
3.0	113 ± 5	−3 ± 2	185 ± 3	−3 ± 1	4.7 ± 0.3	−7 ± 2
10.0	112 ± 2	−4 ± 3	188 ± 3	−1 ± 1	4.9 ± 0.4	−3 ± 4
BCL 333 ms						
0.0	116 ± 6		177 ± 1		4.7 ± 0.6	
0.3	115 ± 6	−1 ± 1	177 ± 2	−1 ± 1	4.3 ± 0.5	−3 ± 2
1.0	113 ± 6	−2 ± 1	176 ± 1	−1 ± 1	4.6 ± 0.4	−1 ± 5
3.0	113 ± 6	−2 ± 2	176 ± 2	−1 ± 1	4.8 ± 0.4	3 ± 4
10.0	114 ± 5	−1 ± 3	176 ± 2	−1 ± 1	4.6 ± 0.6	−1 ± 8
BCL 286 ms						
0.0	115 ± 6		170 ± 2		5.0 ± 0.9	
0.3	115 ± 6	0 ± 1	167 ± 1	−1 ± 1	4.9 ± 0.7	4 ± 11
1.0	116 ± 7	0 ± 1	166 ± 1	−2 ± 1	4.9 ± 0.7	5 ± 15
3.0	116 ± 6	1 ± 2	166 ± 2	−2 ± 1	4.9 ± 0.7	5 ± 13
10.0	120 ± 6	5 ± 4	167 ± 2	−1 ± 1	5.2 ± 0.8	4 ± 15

tential duration (Atwal et al., 1993; Inagaki et al., 1996; Rovnyak et al., 1997). Studies from several laboratories showed a poor correlation between action potential shortening and cardioprotection for compounds and preconditioning, suggesting that sarcolemmal activation may not be important (Yao and Gross, 1994; Grover et al., 1995b; Grover and Sleph, 1995; Hamada et al., 1998). Structure-activity studies using benzopyran and cyanoguanidine analogs showed a clear delineation between vasodilator and APD shortening effects versus cardioprotection (Atwal et al., 1993; Rovnyak et al., 1997). Despite this unusual profile, the cardioprotective effects of these selective agents were completely abolished by K_{ATP} blockers.

One K_{ATP} opener, diazoxide, is a potent vasodilator despite weak effects on APD shortening (Faivre and Findlay, 1989). Garlid showed that diazoxide is fairly selective for cardiac mitochondrial K_{ATP} compared with cardiac sarcolemmal channels (Garlid et al., 1996). Within the dose range that it specifically opens cardiac mitochondrial K_{ATP}, diazoxide still exerted cardioprotective effects (Garlid et al., 1997). Nonselective K_{ATP} openers such as bimakalim also opened mitochondrial channels (Garlid et al., 1996), although this occurred within the dose range where sarcolemmal activation was observed. Mitochondrial K_{ATP} have been shown to be important in mediating pharmacological cardioprotection and preconditioning (Liu et al., 1998; Nakai et al., 2001)

BMS-191095 was shown to be highly selective for mitochondrial K_{ATP} and has no effects on smooth muscle or pancreatic cells (Grover et al., 2001). Although this compound is of great interest, only in vitro and ex vivo (Langendorff hearts) studies have been reported previously. The goal of the present study was to show efficacy and selectivity of BMS-191095 in vivo. This was done by comparing hemodynamic, electrophysiological, and cardioprotective activity in dogs and correlating this with activity expected for a mitochondrial-specific opener.

BMS-191095 reduced infarct size in a clear, dose-dependent manner. We were able to calculate a potency that was described as the dose causing a 25% reduction in infarct size (ED_{25}). ED_{25} was selected because it is approximately the minimal reduction in infarct size needed to see statistically relevant changes. The ED_{25} value of 0.4 mg/kg probably represents the minimally efficacious dose for this compound. The plasma concentrations for efficacious doses of BMS-191095 were consistent with in vitro potency data showing low micromolar potency (Grover et al., 2001). The cardioprotective effect of the highest dose of BMS-191095 tested was abolished by 5-HD, further confirming the mechanism of protection as being mitochondrial K_{ATP}.

TABLE 6
Effect of BMS-191095 on ventricular ERP, APD, CT, CV, wavelength, and QT-interval of the ECG in the anesthetized postinfarcted dog

Dose	ERP	APD	CT	CV
mg/kg/i.v.	*ms*	*ms*	*ms*	*cm/s*
Normal zone				
Control	148 ± 4	152 ± 4	56 ± 3	37 ± 2
0.3	147 ± 3	151 ± 3	55 ± 3	37 ± 2
1.0	144 ± 3	149 ± 3	54 ± 3	38 ± 2
3.0	145 ± 3	147 ± 3	54 ± 3	37 ± 2
10.0	145 ± 3	150 ± 2	55 ± 3	37 ± 2
Ischemic zone				
Control	140 ± 4	153 ± 4	52 ± 3	40 ± 2
0.3	139 ± 3	151 ± 3	51 ± 3	40 ± 2
1.0	138 ± 5	149 ± 3	51 ± 3	40 ± 2
3.0	139 ± 3	152 ± 4	50 ± 3	41 ± 2
10.0	138 ± 3	152 ± 4	51 ± 3	41 ± 2

QT, QT interval; WL, wavelength.

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

This cardioprotective activity was not associated with any hemodynamic change, distinguishing BMS-191095 from first generation compounds such as cromakalim and diazoxide. The hemodynamic effects of these first generation agents preclude their clinical use due to the toxic effects of hypotension (Belin et al., 1996). Although diazoxide is selective for cardiac mitochondrial K_{ATP} relative to cardiac sarcolemmal channels, its potent vasodilator effect precludes its use as a cardioprotectant clinically. Reperfusion myocardial blood flow was increased in the BMS-191095-treated animals, but this may be related to improved viability and metabolic demand from salvaged tissue rather than a direct vasorelaxant effect. This is confirmed by the loss of the enhanced reperfusion blood flow effect of BMS-191095 by coadministration with 5-HD. 5-HD will not block direct vasodilator effects of K_{ATP} openers (McCullough et al., 1991). This is further confirmed by the lack of blood flow changes in the nonischemic regions. It must be stated that it is possible that some additional actions for BMS-191095 at doses at or above BMS-191095 exist. This may explain the lack of clear dose dependence for these effects at the lower doses.

The degree of cardioprotection seen for BMS-191095 was profound particularly because the 90-min coronary occlusion model is so severe. Ischemic preconditioning is not highly efficacious in dog in this model (Gumina et al., 1999). The 70% reduction in infarct size also compares favorably to the degree of protection for sodium/hydrogen exchange inhibitors seen in the 90-min coronary occlusion model in dogs (Gumina et al., 1999). This degree of protection has not typically been found for other K_{ATP} openers, perhaps due to their lack of selectivity (Grover, 1994) and consequent limitation of dosing.

APD shortening or changes in refractory periods may also be contraindicated due to increased propensity for arrhythmogenesis. Selective openers of mitochondrial K_{ATP} would be expected to be devoid of electrophysiological effects typically seen for nonselective agents (Cole et al., 1991; D'Alonzo et al., 1992; Hiraoka and Furukawa, 1998). Doses of BMS-191095 that were within the cardioprotective range had no effects on atrial and ventricular conduction, ventricular refractory period, or APD configuration. As would be expected of an agent devoid of such electrophysiological changes, no proarrhythmic activity was observed. The lack of effect of BMS-191095 in proarrhythmia models confirms the observations that this compound has no effects on APD and therefore is devoid of cardiac sarcolemmal K_{ATP} activation. BMS-191095 had no effects in the PES model and a model of sudden cardiac death. Therefore, it is possible to retain the cardioprotective effects of K_{ATP} openers while effectively eliminating potentially undesirable proarrhythmic activity.

Identification of the importance of mitochondrial K_{ATP} in the pathogenesis of myocardial ischemia and preconditioning was a critical step forward. First generation K_{ATP} openers are nonselective and shorten APD, relax smooth muscle, and exert cardioprotective effects (Edwards and Weston, 1993; Ashcroft et al., 1996). Structure-activity work clearly showed a separation between smooth muscle relaxant and cardioprotective activities for several chemotypes (Grover et al., 1995). BMS-191095 is completely devoid of vasodilator activity but nevertheless retains cardioprotective activity and has recently been shown to be a selective mitochondrial K_{ATP} opener that not only explains its lack of effect on blood pressure and cardiac APD but also confirms the central role for this channel in cardioprotection. From a clinical viewpoint, mitochondrial selective K_{ATP} openers will have a significantly greater therapeutic window compared with cromakalim or diazoxide due to less hemodynamic or proarrhythmic activity. Also, agents such as BMS-191095 will be potentially useful research tools.

References

Armstrong SC, Liu GS, Downey JM, and Ganote CE (1995) Potassium channels and preconditioning of isolated rabbit cardiomyocytes: Effects of glyburide and pinacidil. *J Mol Cell Cardiol* **27:**1765–1774.

Atwal KS, Grover GJ, Ahmed S, Ferrara FN, Harper TW, Kim KS, Sleph PG, Dzwonczyk S, Russell AD, Moreland S, et al. (1993) Cardioselective anti-ischemic ATP-sensitive potassium channel openers. *J Med Chem* **36:**3971–3974.

Auchampach JA, Maruyama M, Cavero I, and Gross G (1991) The new K^+ channel opener aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes. *J Pharmacol Exp Ther* **259:**961–967.

Belin V, Hodge T, Picaut P, Jordan R, Algate C, Gosselin S, Nohynek G, and Cavero I (1996) The myocardial lesions produced by the potassium channel opener aprikalim in monkeys and rats are prevented by blockade of cardiac-adrenoceptors. *Fund Appl Toxicol* **31:**259–267.

Chi L, Uprichard ACG, and Lucchesi BR (1990) Profibrillatory actions of pinacidil in a conscious canine model of sudden coronary death. *J Cardiovasc Pharmacol* **15:**452–464.

Chutkow WA, Simon MC, Le Beau MM, and Burant CF (1996) Cloning, tissue expression and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle and vascular K_{ATP} channels. *Diabetes* **45:**1439–1445.

Cole WC, McPherson CD, and Sontag D (1991) ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* **69:**571–581.

D'Alonzo AJ, Darbenzio RB, Parham CS, and Grover GJ (1992) Effects of intracoronary cromakalim on postischaemic contractile function and action potential duration. *Cardiovasc Res* **26:**1046–1053.

D'Alonzo AJ, Darbenzio RB, Sewter J, Hess TA, Grover GJ, Sleph PG, Normandin DE, and Lodge NJ (1995a) A comparison between the effects of BMS-180448, a novel K^+ channel opener and cromakalim in rat and dog. *Eur J Pharmacol* **294:**271–280.

D'Alonzo AJ, Grover GJ, Darbenzio RB, Sewter JC, Hess TA, Dzwonczyk S, and Sleph PG (1996) Hemodynamic and cardiac effects of BMS-180448, a novel K^+ ATP opener, in anesthetized dogs and isolated rat hearts. *Pharmacology* **52:**101–112.

D'Alonzo AJ, Sewter JC, Darbenzio RB, and Hess TA (1995b) Effects of dofetilide on electrical dispersion and arrhythmias in post-infarcted anesthetized dogs. *Basic Res Cardiol* **90:**424–434.

Faivre J-F and Findlay I (1989) Effects of tolbutamide, glibenclamide and diazoxide upon action potentials recorded from rat ventricular muscle. *Biochim Biophys Acta* **984:**1–5.

Garlid KD, Paucek P, Yarov-Yarovoy B, Murray HNM, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, and Grover GJ (1997) Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive potassium channels: possible mechanism of cardioprotection. *Circ Res* **81:**1072–1082.

Garlid KD, Paucek P, Yarov-Yarovoy V, Sun X, and Schindler PA (1996) The mitochondrial KATP channel as a receptor for potassium channel openers. *J Biol Chem* **271:**8796–8799.

Gross GJ and Auchampach JA (1992) Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* **70:**223–233.

Grover GJ Protective effects of ATP-sensitive potassium channel openers in experimental myocardial ischemia. *J Cardiovasc Pharmacol* **24 (Suppl 4):**S18S27, 1994.

Grover GJ, D'Alonzo AJ, Hess T, Sleph PG, and Darbenzio RB (1995b) Glyburide-reversible cardioprotective effect of BMS-180448 is independent of action potential shortening. *Cardiovasc Res* **30:**731–738.

Grover GJ, McCullough JR, D'Alonzo AJ, Sargent CA, and Atwal KS (1995a) Cardioprotective profile of the cardiac-selective ATP-sensitive potassium channel opener BMS-180448. *J Cardiovasc Pharmacol* **25:**40–50.

Grover GJ and Sleph PG (1995) Protective effect of KATP openers in ischemic rat hearts treated with a potassium cardioplegic solution. *J Cardiovasc Pharmacol* **265:**695–706.

Grover GJ, D'Alonzo AJ, Garlid KD, Bajgar R, Lodge NJ, Sleph PG, Darbenzio RB, Hess TA, Smith MA, Paucek P, and Atwal KS (2001) Pharmacologic characterization of BMS-191095, a mitochondrial KATP opener with no peripheral vasodilator or cardiac action potential shortening activity. *J Pharmacol Exp Ther* **297:**1184–1192.

Gumina RJ, Buerger E, Eickmeier C, Moore J, Daemmgen J, and Gross GJ (1999) Inhibition of the Na^+/H^+ exchanger confers greater cardioprotection against 90 minutes of myocardial ischemia than ischemic preconditioning in dogs. *Circulation* **100:**2519–2526.

Hamada K, Yamazaki J, and Nagato T (1998) Shortening of action potential duration is not a prerequisite for cardiac protection by ischemic preconditioning or a K_{ATP} channel opener. *J Mol Cell Cardiol* **30:**1369–1379.

Hiraoka M and Furukawa T (1998) Functional modulation of cardiac ATP-sensitive K^+ channels. *News Physiol Sci* **13:**131–137.

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017

Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, and Seino S (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* **16:**1011–1017.

Inoue I, Nagase H, Kishi K, and Higuti T (1991) ATP-sensitive K^+ channel in the mitochondrial inner membrane. *Nature (Lond)* **352:**244–247.

Liu Y, Sato T, O'Rourke B, and Marban E (1998) Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* **97:**2463–2469.

Manning AS and Hearse DJ (1984) Reperfusion-induced arrhythmias: mechanisms and prevention. *J Mol Cell Cardiol* **16:**497–518.

McCullough JR, Normandin DE, Conder ML, Sleph PG, Dzwonczyk S, and Grover GJ (1991) Specific block of the anti-ischemic actions of cromakalim by sodium 5-hydroxydecanoate. *Circ Res* **69:**949–958.

McPherson CD, Pierce GN, and Cole WC (1993) Ischemic cardioprotection by ATP-sensitive potassium channels involves high-energy phosphate preservation. *Am J Physiol* **265:**H1809–H1818.

Mizumura T, Nithipatikom K, and Gross GJ (1995) Bimakalim, an ATP-sensitive potassium channel opener, mimics the effects of ischemic preconditioning to reduce infarct size, adenosine release and neutrophil function in dogs. *Circulation* **92:**1236–1245.

Nakai Y, Horimoto H, Mieno S, and Sasaki S (2001) Mitochondrial ATP-sensitive potassium channel plays a dominant role in ischemic preconditioning of the rabbit heart. *Eur Surg Res* **33:**57–63.

Paucek P, Mironva G, Mahdi F, Beavis AD, Woldegiorgis D, and Garlid KD (1992) Reconstitution and partial purification of the glibenclamide-sensitive ATP-dependent K^+ channel from rat liver and beef heart mitochondria. *J Biol Chem* **36:**26062–26069.

Rovnyak GC, Ahmed SZ, Ding CZ, Dzwonczyk S, Ferrara FN, Humphreys WG, Grover GJ, Santafianos D, Atwal KS, Baird AJ, et al. (1997) Cardioselective anti-ischemic ATP-sensitive potassium channel (K_{ATP}) openers. 5. Identification of 4-(*N*-Aryl)-substituted benzopyran derivatives with high selectivity. *J Med Chem* **40:**24–34.

Yao Z and Gross GJ (1994) Effects of the K_{ATP} opener bimakalim on coronary blood flow, monophasic action potential duration and infarct size in dogs. *Circulation* **89:**1769–1775.

Address correspondence to: Dr. Gary J. Grover, Metabolic and Cardiovascular Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 311 Pennington-Rocky Hill Rd., Pennington, NJ 08534. E-mail: groverg@bms.com

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 9, 2017