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European Journal of Pharmacology 294 (1995) 271–280

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A comparison between the effects of BMS-180448, a novel K^+ channel opener, and cromakalim in rat and dog

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Received 18 May 1995; revised 14 August 1995; accepted 22 August 1995

Abstract

BMS-180448 [(3*S*-trans)-*N*-(4-chlorophenyl)-*N'*-cyano-*N''*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl) guanidine] is a structural analog of cromakalim, which was found to similarly decrease ischemic injury, but was 18- to 100-fold less potent as a vasodilator. In the present study, the vascular and cardiac effects of cromakalim and BMS-180448 were evaluated in both in vitro and in vivo preparations. Cromakalim evoked a concentration-dependent relaxation to a K^+-induced contracture in rat aorta. BMS-180448 behaved in a similar fashion but was 18-fold less potent than cromakalim. Measurements of ischemic damage made in isolated perfused rat hearts demonstrated that cromakalim and BMS-180448 were equipotent as cardioprotective agents; time to contracture was increased with an EC_{25} value of 4.8 and 4.7 μM, respectively, and lactate dehydrogenase levels were significantly reduced compared to those in the presence of vehicle. In vivo electrophysiologic studies in anesthetized dogs were conducted at basic cycle lengths of 400, 333, and 286 ms, and showed that BMS-180448 caused no significant effect on electrophysiologic parameters with the exception of decreasing atrial effective refractory periods by $12 \pm 3\%$ and $17 \pm 4\%$ at 3 and 10 mg/kg, respectively. There was also a significant drop in mean blood pressure of $18 \pm 5\%$ and $33 \pm 4\%$ at these doses. In contrast, cromakalim was shown to produce shortening of atrial to His conduction time ($20 \pm 7\%$; basic cycle length = 286 ms), atrial effective refractory period ($34 \pm 3\%$; basic cycle length = 400 ms), ventricular effective refractory period ($14 \pm 2\%$; basic cycle length = 400 ms), wavelength ($13 \pm 3\%$; basic cycle length = 400 ms), PR-interval ($14 \pm 3\%$; basic cycle length = 333 ms) and mean blood pressure ($65 \pm 3\%$; basic cycle length = 400 ms) at a dose of 0.3 mg/kg. No supraventricular or ventricular arrhythmias were observed for either compound tested. Based on the reduced cardiac electrophysiologic and vascular effects of BMS-180448, we suggest that BMS-180448 should provide cardioprotective efficacy similar to cromakalim with reduced risk of hypotension or arrhythmias.

Keywords: Vasorelaxation; Heart; ATP-sensitive K^+ channel; His bundle; Conduction; Refractory period; Electrophysiology

1. Introduction

BMS-180448 [(3*S*-trans)-*N*-(4-chlorophenyl)-*N'*-cyano-*N''*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl) guanidine] (Fig. 1) has recently been described as a novel ATP-sensitive K^+ channel opener with relatively selective cardioprotective activity (Atwal et al., 1993; Grover et al., 1995; Grover and Parham, 1994). Unlike classical ATP-sensitive K^+ channel openers, BMS-180448 has been shown to have less vascular activity in relation to its cardioprotective activity (Grover et al., 1995). BMS-180448 was shown to retain glyburide-reversible cardioprotective effects of cromakalim, but BMS-180448 was 100-fold less potent as a vasodilator (Atwal et al., 1993; Grover et al., 1995). Reversal of the cardioprotective effects of the ATP-sensitive K^+ channel openers by glyburide distinguishes the activity of these compounds relative to agents that block other channels, e.g., Ca^{2+} channel blockers, Na^+ channel blockers, and calmodulin inhibitors (Grover et al., 1990a). Due to the hypotensive nature of cromakalim, it had to be administered into a coronary artery in order to observe cardioprotective efficacy without significant hemodynamic alterations (Grover et al., 1990a,b), whereas BMS-180448 has been

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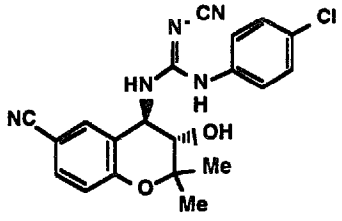
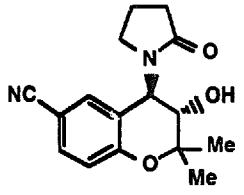
SSDI 0014-2999(95)00543-9



BMS -180448



Cromakalim

Fig. 1. Chemical structures of BMS-180448, [(3*S-trans*)-*N*-(4-chlorophenyl)-*N'*-cyano-*N''*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)guanidine], and cromakalim are shown.

shown to be efficacious following intravenous administration implying an enhanced window of efficacy (Atwal et al., 1993; Grover et al., 1995; Grover and Parham, 1994). However, the electrophysiologic effects of BMS-180448 have only been described in part (D'Alonzo et al., 1994). It was important to fully characterize the electrophysiologic effects of BMS-180448 as Yao and Gross (1994) have recently suggested that electrophysiologic changes do not correlate with cardioprotective activity. Thus, it would be possible to maintain cardioprotection with less electrophysiologic consequences. Previous reports with BMS-180448 demonstrated effects consistent with opening of single cardiac ATP-sensitive K^+ channels; however, it did so only under ischemia-like or hypoxic conditions (D'Alonzo et al., 1994; Grover et al., 1995). In contrast, cromakalim can open ATP-sensitive K^+ channels under nonischemic conditions (Liu et al., 1988; McCullough et al., 1990; Sanguinetti et al., 1988), although ischemia also augments ATP-sensitive K^+ channel opening with cromakalim (D'Alonzo et al., 1992). The electrophysiologic effects of the ATP-sensitive K^+ channel openers, specifically, their ability to hyperpolarize membranes and shorten action potential durations, have been suggested to be the underlying mechanism by which they exert their respective hemodynamic and cardioprotective actions (Cole, 1993; Smallwood and Steinberg, 1988). Furthermore, ATP-sensitive K^+ channel openers, such as cromakalim, pinacidil, and nicorandil, have varied effects on myocardial conduction and refractoriness (Spinelli et al., 1990). Thus, in the present study, we wanted to compare the effects of BMS-180448 on the cardiac electrophysiologic vascular and antiischemic activities to those of cromakalim.

2. Materials and methods

2.1. Determination of in vitro vasodilator activity

Male Wistar Kyoto rats (275–300 g) were euthanized by CO_2 inhalation. The thoracic aorta was quickly removed and placed in physiological salt solution (20–22°C) of the following composition, in mM: 118.4 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.9 $CaCl_2$, 25.0 $NaHCO_3$, and 10.1 glucose. The aorta was cleaned of fat and loose adventitia and subsequently cut into rings of approximately 3 mm in width. The endothelium was removed and the rings then mounted for isometric force recording in 10 ml organ chambers. Force was measured using Grass FT.03 force transducers and recorded on Grass Model 7D polygraphs. All solutions were gassed with 95% O_2-5% CO_2 such that the pH was 7.4. Experiments were conducted at 37°C.

Aortic rings were periodically stimulated with 25 mM K^+ and gradually stretched over a 2 h equilibration period to a preload of 2 g. The rings ($n = 4$) were then contracted with 0.1 μM phenylephrine. The removal of the endothelium was confirmed by the absence of relaxation to 1 μM acetylcholine. The tissues were subsequently washed and allowed to recover for approximately 1 h. Rings ($n = 4$) were then made to contract with 25 mM K^+ (approx. EC_{60} concentration); which produced a well maintained contracture. Drug or vehicle (0.05–0.28% dimethylsulfoxide) was then introduced into the bath in a cumulative fashion. Upon completion of the concentration-response determination, the rings were washed with Ca^{2+}-free physiological salt solution (prepared by omission of $CaCl_2$ and addition of 1.0 mM EGTA) to fully relax the tissues. IC_{50} values were determined as the concentration of drug that reduced force by 50% of the maximal relaxation attained in the Ca^{2+}-free solution. Data are reported as means ± S.E.M.

2.2. Cardioprotective effects in isolated rat hearts

Male Sprague-Dawley rats (400–500 g) were anesthetized using 100 mg/kg Na^+ pentobarbital (i.p.). The trachea was intubated and then the jugular vein was injected with heparin (1000 U/kg). While rats were mechanically ventilated, their hearts were perfused in situ via retrograde cannulation of the aorta. The hearts were then excised and quickly moved to a Langendorff apparatus where they were perfused with oxygenated (95% O_2-5% CO_2) Krebs-Henseleit solution containing (in mM): 112 NaCl, 25 $NaHCO_3$, 5 KCl, 1.2 $MgSO_4$, 1 KH_2PO_4, 1.2 $CaCl_2$, 11.5 glucose and 2 pyruvate at a constant perfusion pressure (85 mm Hg). A water-filled latex balloon attached to a metal cannula was then inserted into the left ventricle and connected to a Statham pressure transducer for measurement of left

ventricular pressure. The hearts were allowed to equilibrate for 15 min, at which time end-diastolic pressure was adjusted to 5 mm Hg and this balloon volume was maintained for the duration of the experiment. Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37°C Krebs-Henseleit solution which was allowed to accumulate in a stoppered, heated chamber.

After equilibration, the hearts were subjected to one of several treatments. Hearts were treated with vehicle (0.04% dimethylsulfoxide, $n = 8$), 1, 3, 10 and 30 μM BMS-180448 ($n = 4$ per dose), 1, 3, 10 and 30 μM cromakalim ($n = 4$ per dose), 10 μM BMS-180448 + 0.3 μM glyburide ($n = 4$), 10 μM cromakalim + 0.3 μM glyburide ($n = 4$). The respective drugs, which were included in the perfusate, were given for 10 min. Following this time, the hearts were subjected to 25 min global ischemia and 30 min reperfusion. Ischemia was initiated by completely shutting off perfusate flow. At the end of the reperfusion period, contractile function, coronary flow, and lactate dehydrogenase release were measured. The respective drugs were given only before global ischemia and were not given during reperfusion. Severity of ischemia was determined using the time to contracture (from the onset of ischemia to the onset of contracture), the recovery of contractile function at 30 min into reperfusion, and the amount of lactate dehydrogenase release into the reperfusate. Time to contracture was defined as the time (min) during global ischemia in which the first 5 mm Hg increase in end-diastolic pressure was observed and these data were used to calculate cardioprotective potency. Cardioprotective potency was expressed as the EC_{25} which is the concentration causing a 25% increase in time to onset of contracture relative to vehicle treated hearts.

2.3. Electrophysiologic measurements in the anesthetized dog

Animal preparation

Male mongrel dogs (15–20 kg; $n = 20$) were anesthetized with dial urethane (0.35 ml/kg i.v.). Following anesthesia, animals were intubated with a cuffed endotracheal tube, and mechanically ventilated (Model 613; Harvard Apparatus, South Natick, MA) with room air at a volume (0.15 × kg weight in ml) and rate (10-15 breaths/min) sufficient to maintain normocapnic conditions. Normocapnia was assured by continuously monitoring (Model 253; Datex, Wilmington, MA) expired CO_2 and stabilizing CO_2 levels at 35 ± 1 mmHg throughout the experiment. To prevent atelectasis, the animals were allowed to breathe against a positive end-expiratory pressure of 5 cm of H_2O. The right femoral artery and vein were cannulated to measure systemic blood pressure and to infuse drugs, respectively. A lead II electrocardiogram 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 changes in arterial pressure. A left thoracotomy was performed at the fifth intercostal space and the heart suspended in a pericardial cradle. 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 applied (N.B. Datyner, Stony Brook, NY; Model PGEN, and SIS, Princeton NJ; voltage to current converter) to either the atrium or ventricle while simultaneously monitoring the atrial and ventricular electrograms. The ventricular electrode consisted of a patch containing two pairs of bipolar electrodes with each pole separated by 1 cm and an inter-electrode distance of 2 cm. The patch was placed on the anterior wall of the left ventricle in such a way that the propagation was parallel to the orientation of the fibers. The left carotid artery was also isolated, a quadripolar catheter inserted into the vessel, and positioned to record the His-bundle electrogram. All waveforms were displayed on a chart recorder (TA 4000; Gould, Cleveland, Ohio) as well as a digital oscilloscope (Model DL1200; Yokogawa, Japan).

Electrophysiologic determinations

Using the method of premature stimulation, refractory periods were determined at basic cycle lengths of 400, 333, and 286 ms. Using the appropriate rising phase of either the atrial electrogram or QRS complex

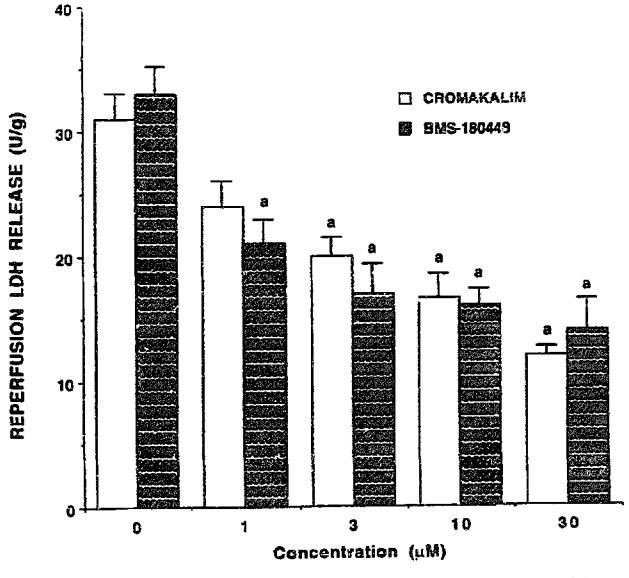


Fig. 2. Effects of cromakalim or BMS-180448 on release of lactate dehydrogenase (LDH) following 25 min of occlusion and 30 min of reperfusion. Both cromakalim and BMS-180448 significantly ($^a P < 0.05$) reduced LDH levels (an index of myocardial damage) compared to vehicle-treated hearts. Both compounds were equipotent in limiting LDH release.

of the electrocardiogram as a trigger (S1), premature stimuli (S2) were introduced at approximately every seven to ten beats to determine the following parameters.

(A) *Excitation threshold* was the minimum current in milliamperes (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).

(B) *Atrial effective refractory period* was the longest S1-S2 (where S1 = rising phase of the atrial electrogram) interval failing to elicit an atrial deflection in the atrial electrogram at a constant current twice excitation threshold.

(C) *Ventricular effective refractory period* was the maximum S1-S2 (where S1 = rising phase of the QRS) interval at which no extrasystoles were generated at a constant current twice excitation threshold.

Conduction times and associated parameters were measured from the His-bundle electrogram at basic cycle lengths of 400, 333, and 286 ms.

(A) *Atrial to His conduction time* was measured from the onset of atrial deflection to the onset of His-bundle deflection in ms.

(B) *His to ventricular conduction time* was measured from the onset of His-bundle deflection to the onset of the ventricular deflection in ms.

(C) *Ventricular conduction time* was measured as the time from S2 to the onset of the ventricular deflection in ms. These measurements were taken at twice the excitation threshold with pulses placed at 70% of the basic cycle length.

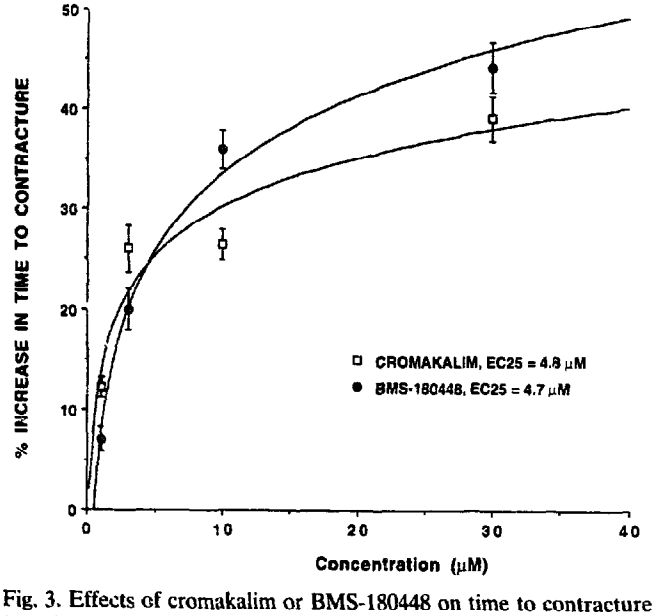


Fig. 3. Effects of cromakalim or BMS-180448 on time to contracture (TTC) during 25 min of occlusion. Both cromakalim and BMS-180448 dose dependently increased TTC compared to vehicle treated hearts. Again, both compounds were equipotent in elevating

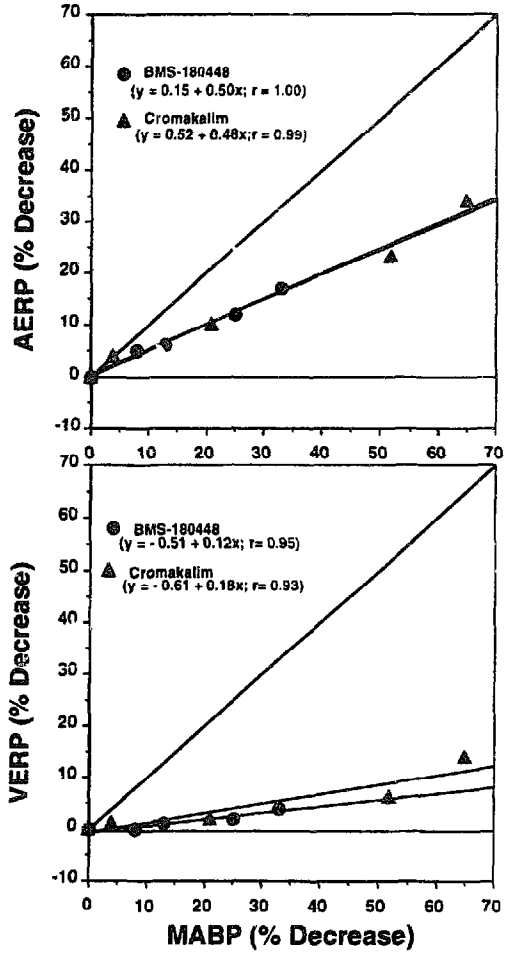


Fig. 4. Relationship between the percent change in atrial (AERP) or ventricular refractory period (VERP) and mean arterial blood pressure (MABP) in the anesthetized dog. A linear relation was fit to the points and are described in parentheses below each treatment along with correlation coefficients (r). Slopes for AERP and MABP were similar for BMS-180448 (0.50) and cromakalim (0.48) treatment groups. VERP vs. MABP slopes were slightly less for BMS-180448 (0.12) then for cromakalim (0.18). These data demonstrate the separation of electrophysiologic and hemodynamic effects of these compounds with a slightly lesser effect of BMS-180448 on VERP and MABP relationship. Additional points were obtained from data not presented in the tables (see text).

(D) *Ventricular conduction velocity* was measured as the inter-electrode distance (2 cm) divided by ventricular conduction time and is expressed in cm/s.

(E) *Wavelength* was measured as the product of ventricular conduction velocity and ventricular effective refractory period and is expressed in mm.

After the above parameters were measured, vehicle ($n = 7$; polyethylene glycol; volume equivalents), BMS-180448 ($n = 7$; 0.3, 1, 3, and 10 mg/kg) or cromakalim ($n = 6$; 0.01, 0.03, 0.1, and 0.3 mg/kg) were administered over 5 min in cumulative i.v. doses every 20 min. Thus, each animal served as its own control. All experiments in this study conformed with the Guide for the Care and Use of Laboratory Animals published by the

US National Institutes of Health (NIH publication No. 85-23, revised 1985).

2.4. Drug preparation

For in vitro studies, compounds were dissolved in dimethylsulfoxide (Mallinkrodt; Paris, Kentucky) to yield a final concentration of 0.04%–0.28%. For the in vivo studies, dial urethane was prepared as follows: 40% urethane (Sigma Chemical Company, Saint Louis, MO), 20% of 5,5-diallyl barbituric acid (Sigma), and 40% of ethyl urea (Aldrich Chemical Company, 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. BMS-180448 (20 mg/ml) and cromakalim (0.4 mg/ml) were prepared fresh on the day of use in 100% polyethylene glycol (Fisher Scientific, Fair Lawn, NJ).

2.5. Statistics

Differences between treatment groups for the in vitro studies were determined using an unpaired Student's *t*-test. For the in vivo studies a three-way analysis of variance was performed followed by a LSD test to determine significant differences at the $P < 0.05$ level. All values are reported as means ± S.E.M.

3. Results

3.1. Comparison of vasodilator versus cardioprotective effects of BMS-180448 and cromakalim

Contracture responses elicited by 25 mM K^+ were well maintained in time-matched controls exposed to the same volumes of vehicle; force changed by −6 ± 9%, during the time course of the experiment. Cromakalim evoked a concentration-dependent relaxation

Table 1
Effect of volume equivalents (Veq) of vehicle (PEG400) on electrophysiologic parameters and blood pressure in the anesthetized dog measured at basic cycle lengths (BCL) of 400, 333 and 286 ms

Dose (Veq/kg i.v.)	A-H (ms)	H-V (ms)	AERP (ms)	VERP (ms)	VCT (ms)	VCV (cm/s)	WL (mm)	PR (ms)	QT (ms)	MABP (mm Hg)
BCL = 400 ms										
Dose 0	63 ± 3 (6)	41 ± 4 (6)	111 ± 3 (6)	154 ± 1 (6)	53 ± 1 (6)	37 ± 1 (6)	58 ± 1 (6)	115 ± 4 (5)	195 ± 10 (5)	103 ± 4 (6)
Dose 1	−2 ± 3 (6)	−2 ± 2 (6)	2 ± 3 (6)	−1 ± 1 (6)	−1 ± 1 (6)	1 ± 1 (6)	0 ± 2 (6)	−4 ± 1 (5)	1 ± 1 (5)	−7 ± 3 (6)
Dose 2	3 ± 5 (6)	−1 ± 1 (6)	−6 ± 6 (6)	0 ± 1 (6)	−1 ± 1 (6)	1 ± 1 (6)	1 ± 2 (6)	10 ± 2 (5)	3 ± 1 (5)	−11 ± 2 (6)
Dose 3	−1 ± 2 (6)	2 ± 2 (6)	−7 ± 6 (6)	−2 ± 1 (6)	1 ± 1 (6)	−1 ± 1 (6)	−1 ± 1 (6)	1 ± 3 (5)	0 ± 1 (5)	−11 ± 2 (6)
BCL = 333 ms										
Dose 0	68 ± 3 (7)	41 ± 3 (7)	103 ± 7 (7)	143 ± 1 (7)	53 ± 1 (7)	38 ± 1 (7)	54 ± 1 (6)	120 ± 3 (6)	183 ± 3 (6)	102 ± 5 (7)
Dose 1	−2 ± 2 (7)	−2 ± 2 (7)	−1 ± 6 (7)	1 ± 1 (7)	−1 ± 1 (7)	0 ± 1 (7)	1 ± 1 (6)	−1 ± 1 (6)	1 ± 2 (6)	−9 ± 3 (7)
Dose 2	−2 ± 4 (7)	−1 ± 2 (7)	−2 ± 6 (7)	0 ± 1 (7)	0 ± 1 (7)	1 ± 1 (7)	0 ± 1 (6)	−2 ± 1 (6)	1 ± 1 (6)	−13 ± 2 (7)
Dose 3	1 ± 3 (7)	−2 ± 2 (7)	−7 ± 6 (7)	0 ± 1 (7)	2 ± 1 (7)	−3 ± 1 (7)	−2 ± 1 (6)	−1 ± 1 (6)	2 ± 1 (6)	−12 ± 2 (7)
BCL = 286 ms										
Dose 0	71 ± 4 (7)	40 ± 3 (7)	104 ± 6 (7)	134 ± 1 [b] (7)	54 ± 1 (7)	37 ± 1 (7)	52 ± 2 [b] (6)	122 ± 3 (6)	168 ± 9 [b] (6)	99 ± 6 (7)
Dose 1	2 ± 4 (7)	−1 ± 2 (7)	−2 ± 5 (7)	2 ± 4 (7)	0 ± 1 (7)	−1 ± 1 (7)	−1 ± 1 (6)	13 ± 1 (6)	−1 ± 1 (6)	−9 ± 3 (7)
Dose 2	−1 ± 2 (7)	4 ± 3 (7)	−4 ± 4 (7)	−1 ± 2 (7)	−1 ± 1 (7)	0 ± 1 (7)	−1 ± 1 (6)	2 ± 1 (6)	−2 ± 1 (6)	−11 ± 3 (7)
Dose 3	2 ± 2 (7)	1 ± 2 (7)	−6 ± 6 (7)	2 ± 2 (7)	0 ± 1 (7)	0 ± 1 (7)	−2 ± 1 (6)	4 ± 1 (6)	−1 ± 2 (6)	−11 ± 3 (7)

Parameters included atrial to His conduction time (A-H); His to ventricular conduction time (H-V); atrial effective refractory period (AERP); ventricular effective refractory period (VERP); ventricular conduction time (VCT); ventricular conduction velocity (VCV); wavelength (WL); PR-interval (PR); QT-interval (QT) and mean arterial blood pressure (MABP). [a] Significantly different ($P < 0.05$) from corresponding control (Dose = 0.0 mg/kg) value. [b] Significantly different ($P < 0.05$) from corresponding BCL = 400 value. [c] Significantly different ($P < 0.05$) from corresponding vehicle value. *n* values are given in parentheses; values at dose = 0.0 mg/kg are given in absolute ± S.E.M., all other values are expressed as percent change ± S.E.M.

of K^+-induced force with an IC_{50} of 0.1 ± 0.07 μM. BMS-180448 behaved in a similar fashion but was 18-fold less potent (IC_{50} of 1.8 ± 0.5 μM) than cromakalim.

The relative cardioprotective potencies of BMS-180448 and cromakalim were similar. Lactate dehydrogenase released during reperfusion was significantly reduced by BMS-180448 and cromakalim (Fig. 2). Hearts treated with vehicle released 33 ± 3 U/g of lactate dehydrogenase. At 10 μM, both cromakalim (17 ± 2 U/g) and BMS-180448 (16 ± 1 U/g) significantly reduced lactate dehydrogenase levels. This effect was completely blocked by glyburide (0.3 μM). Values for cromakalim + glyburide and BMS-180448 + glyburide were 34 ± 4 and 34 ± 3 U/g, respectively. Another index of myocardial protection is given by the effects of these agents on time to contracture (Fig. 3). Vehicle hearts had a time to contracture of 17.5 ± 1.0 min. At 10 μM, both cromakalim (22.2 ± 0.3 min) and BMS-180448 (23.7 ± 0.3 min) demonstrated equivalent potencies in time to contracture with EC_{25} values of 4.8 and 4.7 μM, respectively. Effects on time to contracture with these agents were completely blocked by glyburide. Values for cromakalim + glyburide and BMS-180448 + glyburide were 15.2 ± 0.1 and 16.4 ± 0.2 min, respectively, at 10 μM.

3.2. Effects on His bundle conduction and atrial effective refractory period

Since the lower doses of BMS-180448 and cromakalim (0.3 and 0.01 mg/kg, respectively) did not have significant effects on any of the studied parameters, they were not reported in the tables. However, these values were used in the construction of Fig. 4 (see below). Vehicle treatment produced no affect on His conduction (Table 1). The variation in the n values were a result of increased intrinsic heart rates that

Table 2
Effect of BMS-180448 on electrophysiologic parameters and blood pressure in the anesthetized dog measured at basic cycle lengths (BCL) of 400, 333 and 286 ms

Dose (mg/kg i.v.)	A-H (ms)	H-V (ms)	AERP (ms)	VERP (ms)	VCT (ms)	VCV (cm/s)	WL (mm)	PR (ms)	QT (ms)	MABP (mm Hg)
BCL = 400 ms										
0.0	68 ± 3 (6)	43 ± 4 (6)	97 ± 8 (5)	149 ± 7 (6)	52 ± 3 (6)	39 ± 2 (6)	58 ± 2 (6)	115 ± 3 (6)	184 ± 9 (6)	109 ± 9 (6)
1.0	2 ± 3 (6)	-2 ± 2 (6)	-6 ± 2 [c] (5)	-1 ± 1 (6)	-1 ± 1 (6)	1 ± 1 (6)	0 ± 1 (6)	-1 ± 2 (6)	-1 ± 1 (6)	-13 ± 4 (6)
3.0	2 ± 3 (6)	-3 ± 2 (6)	-12 ± 3 [c] (5)	-2 ± 1 (6)	0 ± 1 (6)	0 ± 1 (6)	-1 ± 1 (6)	0 ± 2 (6)	0 ± 1 (6)	-25 ± 4 [a] (6)
10.0	-5 ± 4 (6)	-2 ± 2 (6)	-17 ± 4 [c] (5)	-4 ± 1 (6)	1 ± 2 (6)	-1 ± 2 (6)	-5 ± 2 (6)	-1 ± 3 (6)	-2 ± 1 (6)	-33 ± 4 [a,c] (6)
BCL = 333 ms										
0.0	73 ± 3 (7)	43 ± 3 (7)	97 ± 8 (5)	141 ± 6 (7)	52 ± 3 (7)	39 ± 2 (7)	54 ± 1 (7)	123 ± 3 (5)	185 ± 6 (5)	108 ± 8 (7)
1.0	0 ± 4 (7)	-4 ± 1 (7)	-9 ± 1 [c] (5)	0 ± 1 (7)	0 ± 1 (7)	1 ± 1 (7)	0 ± 1 (7)	-2 ± 3 (5)	-1 ± 1 (5)	-10 ± 4 (7)
3.0	0 ± 3 (7)	0 ± 2 (7)	-12 ± 2 [c] (5)	-1 ± 1 (7)	2 ± 1 (7)	-1 ± 1 (7)	-2 ± 1 (7)	-3 ± 3 (5)	-1 ± 1 (5)	-18 ± 5 [a] (7)
10.0	-4 ± 4 (7)	-2 ± 2 (7)	-16 ± 4 [c] (5)	-3 ± 1 (6)	0 ± 2 (6)	-1 ± 2 (6)	-4 ± 2 (6)	-6 ± 4 (5)	1 ± 1 (5)	-28 ± 6 [a] (7)
BCL = 286 ms										
0.0	75 ± 4 (7)	42 ± 3 (7)	98 ± 9 (5)	133 ± 5 [b] (7)	52 ± 3 (7)	39 ± 2 (7)	51 ± 2 [b] (7)	116 ± 3 (7)	168 ± 8 (7)	106 ± 8 (7)
1.0	1 ± 3 (7)	-1 ± 2 (7)	-7 ± 1 [c] (5)	1 ± 1 (7)	1 ± 1 (7)	-1 ± 1 (7)	-1 ± 2 (7)	0 ± 2 (7)	-5 ± 2 (7)	-8 ± 4 (7)
3.0	4 ± 5 (7)	-2 ± 2 (7)	-12 ± 2 [c] (5)	0 ± 1 (7)	0 ± 2 (7)	0 ± 2 (7)	1 ± 2 (7)	0 ± 2 (7)	-4 ± 2 (7)	-19 ± 5 [a] (7)
10.0	0 ± 5 (7)	-2 ± 2 (7)	-17 ± 4 [c] (5)	-2 ± 1 (6)	1 ± 1 (6)	0 ± 1 (6)	-2 ± 2 (6)	-3 ± 2 [c] (7)	-2 ± 2 (7)	-25 ± 6 [a] (7)

Parameters included atrial to His conduction time (A-H); His to ventricular conduction time (H-V); atrial effective refractory period (AERP); ventricular effective refractory period (VERP); ventricular conduction time (VCT); ventricular conduction velocity (VCV); wavelength (WL); PR-interval (PR); QT-interval (QT) and mean arterial blood pressure (MABP). [a] Significantly different ($P < 0.05$) from corresponding control (Dose = 0.0 mg/kg) value. [b] Significantly different ($P < 0.05$) from corresponding BCL = 400 value. [c] Significantly different ($P < 0.05$) from corresponding vehicle value. n values are given in parentheses; values at dose = 0.0 mg/kg are given in absolute ± S.E.M., all other values are expressed as percent change ± S.E.M.

prevented the ability to pace animals or difficulty in obtaining a reliable signal. Similar results on His conduction were obtained with BMS-180448 (Table 2), and no significant changes were seen between vehicle and drug treatment groups. Atrial effective refractory period was slightly, but not significantly reduced (7 ± 6%) with vehicle alone at the 10 mg/kg dose at a basic cycle length of 400 ms. BMS-180448 dose dependently reduced atrial effective refractory period 12 ± 3% and 17 ± 4% at the 3 and 10 mg/kg doses, respectively, and at a basic cycle length of 400 ms. However, these changes were not significantly different from control (Dose 0) values, and were independent of changes in basic cycle length. Cromakalim (Table 3) decreased atrial to His conduction time intervals. These changes (−20 ± 7% at basic cycle length = 286 ms) were not significant from vehicle or control values. Cromakalim significantly reduced atrial effective refractory period at a basic cycle length of 400 ms 23 ± 2% and 34 ± 3% at 0.1 and 0.3 mg/kg, respectively. These changes were slightly diminished at shorter basic cycle length.

3.3. Effects on ventricular refractoriness, conduction and wavelength

Changes in ventricular electrical properties: ventricular effective refractory period, ventricular conduction time, ventricular conduction velocity, or wavelength were not affected with either vehicle (Table 1) or BMS-180448 (Table 2) treatment. There were no rate-dependent changes in ventricular conduction time or ventricular conduction velocity observed in these studies. However, decreases in predrug ventricular effective refractory period and wavelength values were rate-dependent. Decreases in ventricular effective refractory period were seen with increasing rate in all groups before administration of compound. Significant changes in wavelength in vehicle and BMS-180448 treatment groups were observed, but only with decreasing cycle length and not administration of compound. Statistically significant decreases in ventricular effective refractory period were seen with cromakalim (0.3 mg/kg; Table 3). These effects were slightly greater at longer

Table 3
Effect of cromakalim on electrophysiologic parameters and blood pressure in the anesthetized dog measured at basic cycle lengths (BCL) of 400, 333 and 286 ms

Dose (mg/kg i.v.)	A-H (ms)	H-V (ms)	AERP (ms)	VERP (ms)	VCT (ms)	VCV (cm/s)	WL (mm)	PR (ms)	QT (ms)	MABP (mm Hg)
BCL = 400 ms										
0.0	75 ± 5 (6)	51 ± 4 (6)	97 ± 3 (6)	146 ± 3 (6)	49 ± 1 (6)	41 ± 0 (6)	60 ± 1 (6)	114 ± 4 (6)	198 ± 9 (6)	106 ± 6 (6)
0.03	−8 ± 8 (5)	−2 ± 2 (5)	−10 ± 1 [c] (5)	−2 ± 1 (5)	−2 ± 2 (5)	2 ± 2 [c] (5)	−1 ± 3 (5)	−9 ± 7 [a] (5)	−6 ± 1 (5)	−21 ± 4 [a] (5)
0.1	−8 ± 10 (5)	−4 ± 5 (5)	−23 ± 2 [a,c] (5)	−6 ± 2 (5)	−1 ± 2 (5)	1 ± 2 [c] (5)	−5 ± 3 (5)	−5 ± 6 (5)	−10 ± 2 [c] (5)	−52 ± 4 [a,c] (5)
0.3	−6 ± 11 (4)	−4 ± 5 (4)	−34 ± 3 [a,c] (4)	−14 ± 2 [a,c] (4)	−1 ± 2 (4)	1 ± 2 [c] (4)	−13 ± 3 [a] (4)	−8 ± 7 [a,c] (4)	−10 ± 3 (4)	−65 ± 3 [a,c] (4)
BCL = 333 ms										
0.0	80 ± 5 (6)	51 ± 4 (6)	98 ± 3 (6)	137 ± 3 [b] (6)	49 ± 0 (6)	41 ± 0 (6)	56 ± 1 (6)	120 ± 3 (6)	184 ± 4 (6)	107 ± 7 (6)
0.03	−13 ± 9 (6)	−2 ± 1 (6)	−5 ± 1 [c] (6)	−1 ± 1 (6)	−5 ± 3 [c] (6)	6 ± 4 [c] (6)	5 ± 3 [c] (6)	−11 ± 4 [a,c] (6)	−4 ± 1 (6)	−17 ± 3 [a] (6)
0.1	−16 ± 9 (6)	−3 ± 5 (6)	−20 ± 4 [a,c] (6)	−3 ± 1 [c] (6)	−1 ± 2 [c] (6)	1 ± 2 [c] (6)	−2 ± 2 (6)	−13 ± 4 [a,c] (6)	−6 ± 1 (6)	−48 ± 3 [a,c] (6)
0.3	−17 ± 9 (6)	1 ± 4 (6)	−32 ± 3 [a,c] (6)	−10 ± 2 [a,c] (6)	1 ± 3 [c] (6)	0 ± 3 [c] (6)	−10 ± 2 [a] (6)	−14 ± 3 [a,c] (6)	−8 ± 2 (6)	−61 ± 1 [a,c] (6)
BCL = 286 ms										
0.0	81 ± 5 (6)	50 ± 4 (6)	93 ± 5 [c] (6)	130 ± 3 [b] (6)	49 ± 0 (6)	40 ± 0 (6)	53 ± 1 [b] (6)	118 ± 2 (6)	159 ± 2 [b] (6)	104 ± 7 (6)
0.03	−13 ± 8 (6)	−7 ± 1 (6)	−7 ± 1 [c] (6)	0 ± 1 (6)	−4 ± 1 [c] (6)	5 ± 2 [c] (6)	5 ± 2 (6)	−7 ± 4 [c] (6)	1 ± 1 (6)	−17 ± 4 [a] (6)
0.1	−16 ± 7 (6)	−10 ± 6 (6)	−17 ± 3 [c] (6)	−5 ± 2 [c] (6)	−3 ± 2 [c] (6)	3 ± 3 [c] (6)	−1 ± 4 (6)	−10 ± 3 [a,c] (6)	1 ± 2 (6)	−47 ± 4 [a,c] (6)
0.3	−20 ± 7 (6)	−3 ± 4 (6)	−28 ± 7 [a,c] (6)	−9 ± 1 [a,c] (6)	0 ± 2 (6)	0 ± 2 (6)	−9 ± 2 [a] (6)	−10 ± 5 [a,c] (6)	−2 ± 2 (6)	−58 ± 2 [a,c] (6)

Parameters included atrial to His conduction time (A-H); His to ventricular conduction time (H-V); atrial effective refractory period (AERP); ventricular effective refractory period (VERP); ventricular conduction time (VCT); ventricular conduction velocity (VCV); wavelength (WL); PR-interval (PR); QT-interval (QT) and mean arterial blood pressure (MABP). [a] Significantly different ($P < 0.05$) from corresponding control (Dose = 0.0 mg/kg) value. [b] Significantly different ($P < 0.05$) from corresponding BCL = 400 value. [c] Significantly different ($P < 0.05$) from corresponding vehicle value. *n* values are given in parentheses; values at dose = 0.0 mg/kg are given in absolute ± S.E.M., all other values are expressed as percent change ± S.E.M.

basic cycle length. Since there were no dose-dependent changes in either ventricular conduction time or ventricular conduction velocity following administration of cromakalim, changes in wavelength paralleled cromakalim's effects on ventricular effective refractory period.

3.4. Effects on PR-interval, QT-interval, and mean blood pressure

Electrocardiographic changes in PR-intervals were not observed with vehicle (Table 1), or BMS-180448 (Table 2) treatment. However, significant decreases in PR-interval did occur with cromakalim treatment (Table 3). Rate-dependent decreases in QT-interval were observed in all groups prior to treatment. Cromakalim reduced PR- and QT-intervals, in particular at basic cycle length of 400 and 333 ms; however only changes in PR-intervals were statistically significant. Significant lowering of blood pressure was observed in all treatment groups (Tables 1–3). Vehicle alone produced an $11 \pm 2\%$ to $13 \pm 2\%$ decrease in mean arterial blood pressure at both the 3 and 10 equivalent doses over the frequencies studied. BMS-180448 produced a significant decrease in mean arterial blood pressure from predrug (109 ± 9 mmHg) values of $25 \pm 4\%$ and $33 \pm 4\%$ at the 3 and 10 mg/kg dose, respectively, at a basic cycle length of 400 ms, but was 120-fold less potent as a hypotensive agent compared to cromakalim (EC_{25} was 0.3 and 3.6 mg/kg for cromakalim and BMS-180448, respectively). Cromakalim produced a significant and dose-dependent decrease in mean arterial blood pressure from both predrug (106 ± 6 mmHg) and corresponding vehicle values of 52% and 65% at the 0.1 and 0.3 mg/kg dose, respectively, at a basic cycle length of 400 ms. Effects on mean arterial blood pressure for BMS-180448 and cromakalim treatment groups were dose- and frequency-dependent. Specifically, mean arterial blood pressure was lowered with increasing doses of compound, but hypotension diminished slightly at shorter basic cycle length.

3.5. Relationship between changes in blood pressure and myocardial refractoriness

Previous studies have indicated that there is a relationship between blood pressure lowering effects of the ATP-sensitive K^+ channel openers and their ability to shorten action potential duration (Smallwood and Steinberg, 1988). We correlated the effects of BMS-180448 and cromakalim with their effects on mean blood pressure and refractoriness (Fig. 4). BMS-180448 and cromakalim had similar relationships between mean arterial blood pressure and atrial effective refractory period with slopes of 0.50 and 0.48, respectively. Relationships between mean arterial blood pressure and ventricular effective refractory period demonstrated that BMS-180448 (0.12) had slightly reduced slopes of approximately one-third compared to that of cromakalim (0.18).

4. Discussion

Vasorelaxant, cardioprotective and electrophysiologic studies of BMS-180448, a new ATP-sensitive K^+ channel opener, and cromakalim were performed in isolated rat heart preparations and anesthetized dogs. BMS-180448 was found to be 18-fold less potent in vitro as a vasodilator than cromakalim. The vasodilatation observed with BMS-180448 was smaller than previously reported (Grover et al., 1995). Differences between this study and previous results may reside in the different strain of rats used as well as a slight modification of the experimental conditions. Nonetheless BMS-180448 was less potent as a vasodilator than cromakalim, and in the whole animal was 120-fold less potent as a vasodilator when compared to cromakalim.

In agreement with our previous results, the cardioprotective effects of BMS-180448, measured as a reduction in lactate dehydrogenase release and an increase in time to contracture, was similar to that of cromakalim (Atwal et al., 1993; Grover et al., 1995). The block of the activities of both compounds by glyburide suggests a similar mechanism of action for their cardioprotective activity.

Electrophysiologically, no significant changes in His-bundle conduction times or refractory periods were observed following BMS-180448 treatment. Therefore, deleterious effects associated with changes in this system would not be expected to occur with BMS-180448. However, there was a dose-dependent decrease in atrial effective refractory period with BMS-180448, that was significantly greater than vehicle, but not from predrug control values. Agents which shorten refractoriness may facilitate re-entrant circuits, and thereby promote arrhythmogenicity. Both cromakalim and pinacidil have been shown to decrease atrial effective refractory period and to promote atrial arrhythmias (Spinelli et al., 1990). However, these effects were not observed with these agents unless blood pressure was lowered by $> 40\%$. BMS-180448 produce smaller decreases in atrial effective refractory period and mean arterial blood pressure relative to cromakalim. Thus, at cardioprotective doses (Atwal et al., 1993; Grover et al., 1995; Grover and Parham, 1994; Grover et al., 1993), i.e., 1–2 mg/kg, BMS-180448 reduced atrial effective refractory period by 12% and mean arterial blood pressure by 25% at a basic cycle length of 400 ms. The changes in these parameters with BMS-180448 are not likely to increase the propensity of BMS-180448 to exacerbate atrial arrhythmias.

It has previously been reported that BMS-180448 was without hemodynamic effects at doses up to 4.2 mg/kg (Grover and Parham, 1994). In those studies, BMS-180448 was administered as a slow infusion over 30 min, and blood pressure effects were taken following the first 15 min of infusion. In contrast, we administered cumulative doses over 5 min and recorded blood pressure 5 min later. Thus, the mode of administration of BMS-180448 can significantly alter the hemodynamic profile of the compound.

In the ventricle, there was no significant change in ventricular effective refractory period, ventricular conduction time, ventricular conduction velocity, or wavelength with BMS-180448 at doses up to 10 mg/kg i.v. Observed changes in ventricular effective refractory period were attributed to changes in cycle length alone. Wavelength (ventricular effective refractory period × ventricular conduction velocity) was used as an index of myocardial vulnerability (Allessie et al., 1977), and served as a measure of proarrhythmic tendency. Specifically, decreases in wavelength have been shown to be an index of potential proarrhythmic activity, in particular, enhancement of re-entrant arrhythmias (Smeets et al., 1986). For example, pinacidil, which shortens action potential durations and decreases ventricular effective refractory period has displayed proarrhythmic activity (Chi et al., 1990). In contrast, some Class Ic antiarrhythmic agents also tend to be proarrhythmic by decreasing conduction velocity without affecting ventricular effective refractory period or action potential duration (DiCarlo et al., 1985). Thus, a combination of a decrease in ventricular effective refractory period and/or ventricular conduction velocity would constitute tendencies towards a reduction in wavelength and consequently proarrhythmic activity. Conversely, increases in wavelength tend to elicit antiarrhythmic activity (Hill et al., 1990). In the present study, BMS-180448 did not significantly alter wavelength. However, cromakalim did significantly decrease wavelength (13%) from control at 0.3 mg/kg, but no arrhythmogenic activity was observed. Nonetheless, the fact that BMS-180448 alone does not decrease wavelength, suggests that it possesses less potential for proarrhythmic activity than cromakalim in the ventricle.

Although the potential for proarrhythmic effects of cromakalim may exist, these effects may not be observed until blood pressure is dramatically reduced. Therefore, it is difficult to determine if the changes in electrophysiologic parameters, which may initiate arrhythmias, are a consequence of a direct effect of the drug on the myocardium or an indirect effect through a decreased perfusion (possibly an onset of ischemia) of the tissue (Himori et al., 1990), a reflexive enhancement of sympathetic tone due to the reduction in blood pressure, a reduction in load (Dean and Lab, 1989; Dean and Lab, 1990), or an indirect release of catecholamines (Zhu et al., 1995). However, it is unlikely that load reduction would contribute to the electrophysiologic changes, since that would increase parameters such as action potential duration and QT-interval. Further studies would be needed to more directly address these issues.

In comparison, cromakalim produced slight decreases in His-bundle conduction times, but more pronounced effects on atrial refractory periods than BMS-180448. These results were consistent to those reported by others (Spinelli et al., 1990). Cromakalim produced significant shortening of the PR-interval in the present study, which was consistent with changes in the His conduction system. In the ventricular mass, cromakalim produced significant shortening of ventricular effective refractory period at higher doses that were more pronounced at slower rates, which accounted for a decrease in wavelength. These data suggest a potential proarrhythmic profile for cromakalim. However, this still remains a controversial issue, since some investigators have shown ATP-sensitive K^+ channel openers to be proarrhythmic while others have observed antiarrhythmic activity of these agents (see review by D'Alonzo and Grover, 1994).

Spinelli et al. (1990) described the relationship between the percent decrease in mean blood pressure and the percent decrease in atrial (slopes of 0.48 for cromakalim in this study vs. 0.46 in their study) or ventricular refractory periods (slopes of 0.18 for cromakalim in this study vs. 0.25 in their study). Our results for cromakalim were similar to those obtained for other ATP-sensitive K^+ channel openers (Spinelli et al., 1990). We did observe that BMS-180448 had a less steep relationship on ventricular effective refractory period and blood pressure, approximately one-third that of cromakalim, suggesting less effects of BMS-180448 on ventricular electrophysiology. Although the slopes for BMS-180448 and cromakalim were similar for changes in mean blood pressure and atrial effective refractory period, the degree of blood pressure lowering with BMS-180448 was much less than that of cromakalim. Thus, the relationship between electrophysiologic changes and mean blood pressure effects as previously described (Smallwood and Steinberg, 1988), do not necessarily apply as indicated by our results with BMS-180448. Yet, the cardioprotective potencies of BMS-180448 and cromakalim were similar. Furthermore, doses of BMS-180448 that have cardioprotective activity should be devoid of electrophysiologic effects.

ATP-sensitive K^+ channel openers are known to hyperpolarize the resting membrane potential, shorten action potential duration, and thus limit Ca^{2+} influx into vascular smooth muscle cells. Since their discovery (Noma, 1983), it has been postulated that this mechanism is responsible for the hypotensive as well as

cardioprotective activity of these compounds (Cole, 1993; Smallwood and Steinberg, 1988). Furthermore, the action of the ATP-sensitive K^+ channel openers are blocked by glyburide, a selective blocker of ATP-sensitive K^+ channel. Classical ATP-sensitive K^+ channel openers may not display cardioprotective activity following intravenous administration due to their severe hypotensive effects, which occur before any cardioprotective activity can be observed (Grover et al., 1990b). Although the cardioprotective effects of BMS-180448 are reversed by glyburide as shown in this study as well as other studies (Atwal et al., 1993; Grover et al., 1995; Grover and Parham, 1994), the electrophysiologic and hemodynamic effects do not appear to correlate with its cardioprotective activity.

In conclusion, BMS-180448 was shown to have no effect on His-bundle conduction times or ventricular electrophysiology, and minimal effects on atrial refractory periods. In addition, there were less vascular dilation and lowering of blood pressure as compared to cromakalim. These data suggest that BMS-180448, when compared to cromakalim, should not have a significant potential to exacerbate either atrial or ventricular arrhythmias based on its electrophysiologic profile, cause less hypotension, yet display similar cardioprotective activity.

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