Cardioprotective Effects of KR-30450, a Novel K^+_{ATP} Opener, and Its Major Metabolite KR-30818 on Isolated Rat Hearts

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ABSTRACT—The cardiac effects of KR-30450 ((−)-(2*R*)-2-([1,3]-dioxolan-2-yl)-2-methyl-4-(2-oxopyrrolidin-1-yl)-6-nitro-2*H*-1-benzopyran), a newly synthesized potassium channel activator, and its major metabolite KR-30818 ((−)-(2*R*)-2-hydroxymethyl-2-methyl-4-(2-oxopyrrolidin-1-yl)-6-nitro-2*H*-1-benzopyran) were compared with those of lemakalim, a prototype of this class, in isolated globally ischemic rat hearts. KR-30450 and KR-30818 significantly improved reperfusion cardiac function (LVDP, left ventricular developed pressure; double product, LVDP × heart rate/1000), their potency being 5.2-fold and 0.7-fold greater than lemakalim (ED_{50} for recovering predrug double product: 0.10, 0.80 and 0.54 μM, respectively). KR-30450 and KR-30818 significantly attenuated reperfusion contracture and lactate dehydrogenase release with potency greater than and equal to lemakalim, respectively. They significantly increased time to contracture (TTC) during ischemia in a dose-dependent manner with a greater potency than lemakalim (EC_{25} for increasing TTC: 1.2, 2.1 and 3.2 μM, respectively). The protective effects of three compounds on the measured parameters were reversed by glyburide, a selective K^+_{ATP} blocker. In non-ischemic hearts, KR-30450 and lemakalim exerted weak negative inotropism at high concentrations and KR-30818 had no effects, whereas the three compounds significantly increased coronary flow at doses studied. Glyburide completely reversed preischemic cardiodepressant effects of these compounds but not their effects on coronary flow. In conclusion, KR-30450, a recently developed K^+_{ATP} opener, exerted more potent cardioprotective effects than lemakalim, and its major metabolite KR-30818 may play a significant role in its action in vivo.

Keywords: KR-30450 (SKP-450), KR-30818 (SKP-818), Lemakalim, Potassium channel activator, Cardioprotection

It is known that compounds that exert modulatory effects on ATP-dependent potassium channels may be potentially useful for the treatment of various types of diseases (1–3). Over the last several years, this class of compounds has been one of the research targets for which most active studies have been conducted. Sulfonylureas are well-known as effective agents for the treatment of insulin-independent diabetes due to their blockade of K^+_{ATP}. Recent research activities toward potassium channel modulators have been mainly focused on K^+_{ATP} openers, since a racemic mixture cromakalim and its active (−)optical isomer lemakalim were identified as benzopyran K^+_{ATP} openers that exert potent vasorelaxant and antihypertensive activities via a specific mechanism (4). K^+_{ATP} openers are known to relax smooth muscle by reducing intracellular calcium concentration via hyperpolarization of cell membrane followed by the blockade of voltage-dependent calcium channel and intracellular calcium release (5). Thus, it was originally thought that their main clinical application was in the treatment of hypertension.

Recently, it was recognized that K^+_{ATP} in cardiac muscle may be activated during ischemia and contribute to preservation of intracellular ATP, raising the possibility that K^+_{ATP} openers may be applicable to the treatment of ischemic heart disease (6–9). This possibility has been

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supported by recent reports that K^+_{ATP} openers of various structures synthesized by many institutes possess cardioprotective activities in several experimental models of ischemic heart disease.

(−)KR-30450 (SKP-450), ((−)-(2*R*)-2-([1,3]-dioxolan-2-yl)-2-methyl-4-(2-oxopyrrolidin-1-yl)-6-nitro-2*H*-1-benzopyran, was newly synthesized as a benzopyran derivative at the Korea Research Institute of Chemical Technology (KRICT, Taejeon, Korea) (Fig. 1). It was shown that KR-30450 exerts potent vasorelaxant effects on rat aorta and antihypertensive effects in various types of hypertensive rats including spontaneous hypertensive, DOCA/salt-hypertensive and renal hypertensive rats (10, 11). Recent studies using the patch clamp technique showed that KR-30450 antagonized the inhibitory effect of ATP on the ATP-sensitive potassium channel activity in single rat ventricular myocytes probably via its action on the ATP-binding unit, thereby enhancing the channel openings (12). These findings suggest that the mode of action of KR-30450 on the ATP-sensitive potassium channel is distinct from those of lemakalim, pinacidil and nicorandil in that lemakalim and pinacidil may activate the channel by interacting with the transducer unit either in the phosphorylated state or in the nucleotide diphosphates-bound state, whereas nicorandil may activate the channel only by interacting with the nucleotide diphosphate bound but not with the phosphorylated transducer unit (12, 13). Pharmacokinetic studies have shown that ^{14}C-KR-30450 mainly undergoes metabolic changes to KR-30818 (SKP-818), ((−)-(2*R*)-2-hydroxy-methyl-2-methyl-4-(2-oxopyrrolidin-1-yl)-6-nitro-2*H*-1-benzopyran, immediately after oral and intravenous administration to rats and dogs (personal communication with Dr. D.H. Kim, Korea Institute of Science and Technology (KIST), Seoul, Korea), indicating that KR-30818 is one of the main active metabolites mediating the activities of the parent compound. In the present study, we evaluated the cardioprotective activities of KR-30450 and KR-30818 in globally ischemic isolated rat hearts and compared the results with those of lemakalim.

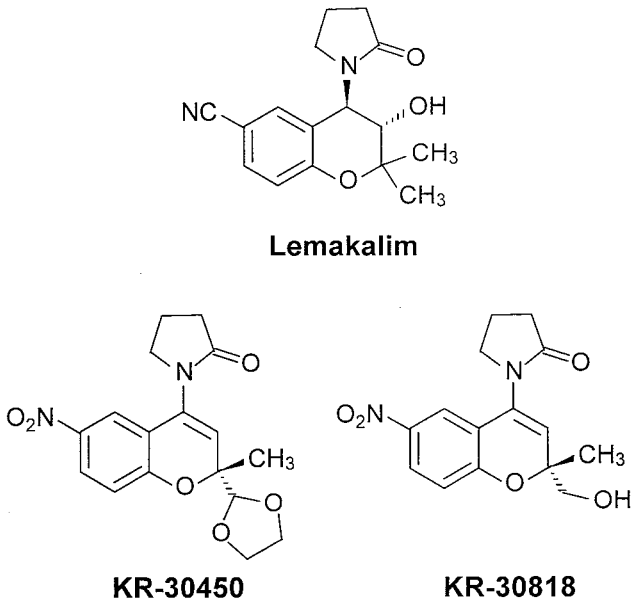

Fig. 1. Chemical structures of KR-30450, its major metabolite KR-30818 and lemakalim.

MATERIALS AND METHODS

Chemicals

KR-30450, its major metabolite KR-30818 and lemakalim were synthesized at Bioorganic Division of KRICT. Glyburide was purchased from Sigma Chemical (St. Louis, MO, USA). These compounds were dissolved in 100% dimethyl sulfoxide (DMSO) and diluted with Modified Krebs-Henseleit bicarbonate buffer as required (the final concentration of DMSO: 0.04%). Sodium pentobarbital was purchased from Hanlim Pharmaceutical Co. (Seoul, Korea).

Isolated rat heart studies

For all in vitro studies, isolated rat hearts were used according to the published methods after some modification (14, 15). Male Sprague-Dawley rats weighing 300−450 g were anesthetized with sodium pentobarbital (100 mg/kg, i.p.). The tail vein was injected with heparin (1,000 U/kg) and then the trachea was intubated. While rats were mechanically ventilated with a rodent ventilator (Model 7025; Ugo Basile, Comerio-Varese, Italy), their hearts were perfused *in situ* with oxygenated modified Krebs-Henseleit bicarbonate buffer (described herein) by retrograde aortic cannulation. The hearts were then excised and moved to a Langendorff apparatus (Hugo Sachs Electronik, March-Hugstetten, Germany), where they were perfused with oxygenated modified Krebs-Henseleit bicarbonate buffer containing 116 mM NaCl, 24.9 mM $NaHCO_3$, 4.7 mM KCl, 1.1 mM $MgSO_4$, 1.17 mM KH_2PO_4, 2.52 mM $CaCl_2$, 8.32 mM glucose and 2.0 mM pyruvate at a constant perfusion pressure (75 mmHg). A water-filled latex balloon attached to a metal cannula was placed in the left ventricle through the pulmonary vein and connected to an Isotec pressure transducer (Hugo Sachs Electronik) for measurement of left ventricular pressure (LVP). The hearts were allowed to equilibrate for 15 min, at which time left ventricular end-diastolic pressure (EDP) was adjusted to 10 mmHg, and this balloon volume was maintained throughout the experiment. Then, baseline contractile function, heart rate (HR) and coronary flow (CF) (extracorporeal electromagnetic flow

probe; Narco Bio-Systems, Houston, TX, USA) were measured (16–19). Cardiac contractile function was calculated by subtracting LVEDP from LV peak systolic pressure (LVSP), yielding developed pressure (LVDP). The double product (DP), another important parameter for assessing cardiac performance, was calculated by multiplying HR by LVDP (20). Throughout the experiment, all these parameters were measured or calculated before and 10 min after pretreatment with each compound and 30 min after the onset of reperfusion with buffer (see below). Data on reperfusion DP were further expressed as the percentage of the respective predrug DP values, and the log curve fitting was applied to the concentration-response curve to calculate EC_{50} defined as the concentration of drug causing 50% recovery of predrug DP. Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37℃ buffer, which was allowed to accumulate in a chamber.

In the first series of studies using the isolated rat heart model of ischemia and reperfusion, rat hearts were prepared as already described and were pretreated with a) vehicle (0.04% DMSO, n=10 per group); b) KR-30450 (0.1–3 μM, n=5–8 for each group); c) KR-30818 (0.3–3 μM, n=5–7 for each group); or d) lemakalim (0.3–3 μM, n=6–8 for each group). The structures of KR-30450, KR-30818 and lemakalim are shown in Fig. 1. The hearts were pretreated 10 min with the respective drug or vehicle before onset of global ischemia; test agents were administered directly into the oxygenator of the Langendorff apparatus immediately above the aortic root in a retrograde fashion as solutions in the perfusate. We then rendered the hearts globally ischemic by completely shutting off the perfusate for 25 min. Severity of ischemia was determined as the time (min) to contracture during global ischemia (TTC) in which the first 5 mmHg increase in EDP was observed. From these determinations, the EC_{25} for TTC, which was defined as the concentration of drug causing a 25% increase in TTC as compared with vehi-

Table 1. Effect of lemakalim with and without glyburide on cardiac function and coronary flow before and after global ischemia

		Before ischemia		After ischemia	n
		Predrug	10 min Postdrug	30 min Postreperfusion	
LVDP (mmHg)					
Vehicle		95.5±4.4	94.1±5.6	22.9±2.7[a]	10
Glyburide	1 μM	93.8±5.1	83.0±3.7	20.8±4.2[a]	4
Lemakalim	0.3 μM	94.0±5.3	86.0±6.7	29.8±6.5[a]	8
	1 μM	93.8±4.8	97.2±11	76.8±4.6[a,b]	6
	3 μM	93.0±6.3	84.5±6.9	74.2±6.8[a,b]	6
Lemakalim	3 μM				
+ Glyburide	1 μM	87.8±7.8	97.8±5.5	8.0±1.8[a,b]	4
Heart rate (beats/min)					
Vehicle		252±5	233±5	243±8	10
Glyburide	1 μM	237±3	205±7[a,b]	208±13[a,b]	4
Lemakalim	0.3 μM	249±6	235±7	238±9	8
	1 μM	264±9	234±7	239±9[a]	6
	3 μM	267±8	252±8	252±7	6
Lemakalim	3 μM				
+ Glyburide	1 μM	256±9	247±9	223±9[a]	4
Coronary flow (ml/min)					
Vehicle		19.1±1.1	19.6±1.9	12.1±1.3[a]	10
Glyburide	1 μM	16.3±0.6	14.1±0.6[a,b]	10.9±1.6[a]	4
Lemakalim	0.3 μM	17.8±1.3	22.8±1.3[a]	11.1±0.4[a]	8
	1 μM	19.0±1.0	23.1±1.4[a]	13.4±1.6[a]	6
	3 μM	17.6±1.0	23.2±1.3[a]	12.0±1.3[a]	6
Lemakalim	3 μM				
+ Glyburide	1 μM	19.0±1.6	21.9±2.1	13.8±1.5[a]	4

All values are means±S.E.M. [a]Significantly different from its respective predrug value ($P<0.05$). [b]Significantly different from its respective vehicle group values ($P<0.05$).

cle-treated heart, was calculated from the log curve fit to the concentration-response curve (16). Then the hearts were reperfused and, 30 min later, contractile function (LVDP, DP) and cumulative reperfusion lactate dehydrogenase (LDH) release were measured. LDH was measured as a sensitive index for loss of cell viability (21) with a kit supplied by Boerhinger Mannheim based on the technique of Wroblewski and LaDue (22).

In the second series of studies, we determined the possible mechanism for the antiischemic activity of the test agents. Rat hearts were prepared as above and pretreated with a) vehicle (0.04% DMSO, n=10, same group as in preceding study); b) glyburide, a selective inhibitor of K^+_{ATP} (20, 21) (1 μM, n=4 per group); c) KR-30450 (3 μM) + glyburide (1 μM) (n=5); d) KR-30818 (3 μM) + glyburide (1 μM) (n=5); or e) lemakalim (3 μM) + glyburide (1 μM) (n=5). Then, rat hearts were subjected to global ischemia and reperfusion as described above with the measurement of parameters mentioned already.

Statistics

All values are expressed as means±S.E.M. Data were analyzed by the unpaired Student's *t*-test between two groups and one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons. All statistical differences were determined at $P<0.05$ level.

RESULTS

The effects of KR-30450 and its major metabolite KR-30818 on cardiac contractile function, heart rate and their cardioprotective activity were compared with those of

Table 2. Effect of KR-30450 with and without glyburide on cardiac function and coronary flow before and after global ischemia

		Before ischemia		After ischemia	n
		Predrug	10 min Postdrug	30 min Postreperfusion	
LVDP (mmHg)					
Vehicle		95.5±4.4	94.1±5.6	22.9±2.7[a]	10
Glyburide	1 μM	93.8±5.1	83.1±3.7	20.8±4.2[a]	4
KR-30450	0.1 μM	95.6±7.1	102.6±9.5	48.8±5.2[a,b]	5
	0.3 μM	93.3±5.0	90.9±7.9	79.6±3.3[a,b]	8
	1 μM	93.7±6.2	79.5±5.0	95.5±7.2[b]	6
	3 μM	90.3±5.1	73.3±10.3[a,b]	97.8±10.0[b]	6
KR-30450	3 μM				
+ Glyburide	1 μM	88.8±8.5	88.0±8.9	8.8±1.6[a,b]	5
Heart rate (beats/min)					
Vehicle		252±5	233±5	243±8	10
Glyburide	1 μM	237±3	205±7[a,b]	208±13[a,b]	4
KR-30450	0.1 μM	238±7	234±10	230±12	5
	0.3 μM	236±9	234±9	229±12	8
	1 μM	258±9	242±6	245±12	6
	3 μM	263±4	240±5[a]	245±8[a]	6
KR-30450	3 μM				
+ Glyburide	1 μM	271±8	255±6[a]	246±10[a]	5
Coronary flow (ml/min)					
Vehicle		19.1±1.1	19.6±1.9	12.1±1.3[a]	10
Glyburide	1 μM	16.3±0.6	14.1±0.6[a,b]	10.9±1.6[a]	4
KR-30450	0.1 μM	18.2±1.1	22.4±1.1[a]	11.3±1.8[a]	5
	0.3 μM	18.3±1.5	22.3±0.8[a]	13.4±1.7[a]	8
	1 μM	16.0±1.4	22.3±1.5[a]	15.3±1.3	6
	3 μM	16.9±1.4	25.3±1.5[a,b]	16.6±1.3[b]	6
KR-30450	3 μM				
+ Glyburide	1 μM	19.5±1.9	22.4±1.4	12.5±1.2	5

All values are means±S.E.M. [a]Significantly different from its respective predrug value ($P<0.05$). [b]Significantly different from its respective vehicle group values ($P<0.05$).

lemakalim in isolated perfused rat hearts. Data on contractile function, heart rate and coronary flow are shown in Tables 1–3. The contractile function, HR and CF were similar for all groups before drug administration. In non-ischemic hearts, KR-30450 and lemakalim had a tendency to exert weak negative inotropic effects (LVDP) only at high concentration (3 μM), but KR-30818 did not display any effects on the contractile function at the concentrations used. KR-30450, KR-30818 and lemakalim did not affect both preischemic and reperfusion HR significantly as in the vehicle-treated group. KR-30450, KR-30818 and lemakalim significantly increased nonischemic CF at all concentrations in a dose-independent manner. However, these compounds did not have significant effects on reperfusion CF at these concentrations as compared with the vehicle-treated group except for KR-30450 at high concentration (3 μM), at which reperfusion CF recovered almost up to the predrug level with significant difference from reperfusion CF in the vehicle-treated group.

In the vehicle-treated group, cardiac contractile function (LVDP) was significantly depressed after 30-min reperfusion, indicating severe ischemic/reperfusion injury. KR-30450, KR-30818 and lemakalim significantly improved reperfusion cardiac function in a concentration-dependent manner as shown in reperfusion LVDP and DP, beginning at 0.1, 1.0 and 1.0 μM, respectively (ED_{50} for recovering predurg DP: 0.10, 0.80 and 0.54 μM, respectively), indicating marked cardioprotection (Fig. 2). Reperfusion DP in vehicle-treated rats was 23.3±2.8% of predrug DP. During reperfusion, HR was slightly decreased in the vehicle-treated rats; this tendency was not significantly changed by any of these compounds. Data on reperfusion EDP and LDH release are shown in Fig. 3. Severe contracture (increase in EDP) was observed in the vehicle-treated hearts, which was significantly attenuated by KR-30450, KR-30818 and lemakalim in a concentration-related manner (the order of potency: KR-

Table 3. Effect of KR-30818 with and without glyburide on cardiac function and coronary flow before and after global ischemia

		Before ischemia		After ischemia	n
		Predrug	10 min Postdrug	30 min Postreperfusion	
LVDP (mmHg)					
Vehicle		95.5±4.4	94.1±5.6	22.9±2.7[a]	10
Glyburide	1 μM	93.5±5.1	83.1±3.7	20.8±4.2[a]	4
KR-30818	0.3 μM	93.5±4.7	93.5±6.4	14.0±5.8[a]	6
	1 μM	93.7±5.8	105.0±7.7	58.3±5.6[a,b]	7
	3 μM	92.4±5.3	91.6±8.3	76.0±11.5[a,b]	5
KR-30818	3 μM				
+ Glyburide	1 μM	89.5±6.6	100.5±5.9	9.0±3.7[a,b]	4
Heart rate (beats/min)					
Vehicle		252±5	233±5	242.8±8	10
Glyburide	1 μM	237±3	205±7[a,b]	208±13[a,b]	4
KR-30818	0.3 μM	256±5	242±6	232±8	6
	1 μM	244±3	223±5[a]	223±9[a]	7
	3 μM	248±9	238±7	235±8	5
KR-30818	3 μM				
+ Glyburide	1 μM	260±8	244±9[a]	217±8[a,b]	4
Coronary flow (ml/min)					
Vehicle		19.1±1.1	19.6±1.9	12.1±1.3[a]	10
Glyburide	1 μM	16.3±0.6	14.1±0.6[a,b]	10.9±1.6[a]	4
KR-30818	0.3 μM	18.0±1.6	25.6±0.6[a,b]	12.4±1.0[a]	6
	1 μM	17.9±0.9	24.4±0.7[a,b]	12.3±1.5[a]	7
	3 μM	18.8±1.8	25.5±1.6[a,b]	13.8±2.6[a]	5
KR-30818	3 μM				
+ Glyburide	1 μM	18.8±1.6	25.9±1.0[a,b]	11.1±1.2[a]	4

All values are means±S.E.M. [a]Significantly different from its respective predrug value ($P<0.05$). [b]Significantly different from its respective vehicle group values ($P<0.05$).

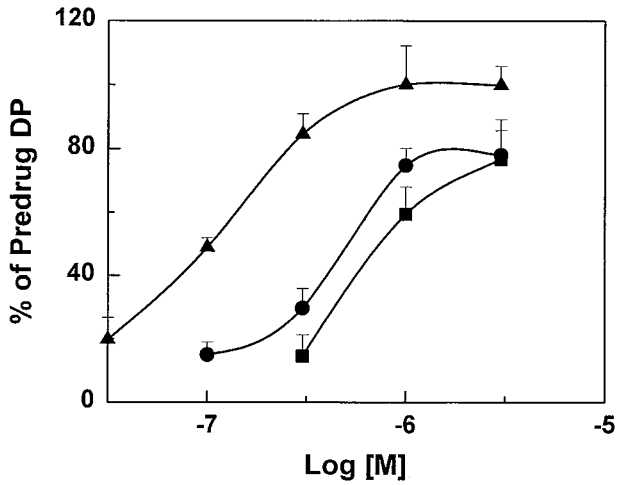

Fig. 2. Effect of KR-30450, KR-30818 and lemakalim on reperfusion double product (DP) in isolated rat heart subjected to 25-min global ischemia followed by 30-min reperfusion. Each value is the mean percentage of the respective predrug DP±S.E.M. (n=5–8). Reperfusion DP was 23.3±2.8% of predrug DP in the vehicle-treated group (n=10). Lemakalim (circle), KR-30450 (triangle) and KR-30818 (square). The concentration-response curve to KR-30450 was shifted to the left compared with that of lemakalim.

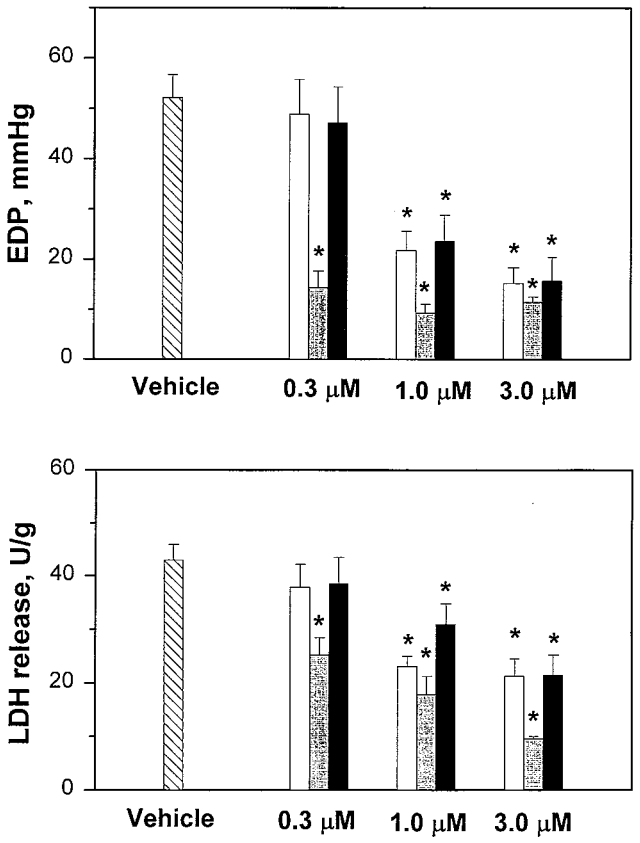

Fig. 3. Effect of KR-30450 (n=8 for 0.3 μM and n=6 for 1 and 3 μM), KR-30818 (n=6, 7 and 5 for 0.3, 1 and 3 μM, respectively), lemakalim (n=8 for 0.3 μM and n=6 for 1 and 3 μM) and vehicle (n=10) on left ventricular end-diastolic pressure (EDP) and lactate dehydrogenase (LDH) release during reperfusion in isolated rat heart subjected to 25-min global ischemia followed by 30-min reperfusion. Vehicle (hatched), lemakalim (open), KR-30450 (shaded) and KR-30818 (solid bar). Three compounds significantly reduced reperfusion EDP and LDH release as compared with the vehicle (*$P<0.05$).

30450>lemakalim=KR-30818). Significant LDH release was observed in the reperfusion coronary effluent of vehicle-treated hearts, and reperfusion LDH release was also significantly reduced by those compounds with the same order of potency as that for EDP. TTC was 21.4±1.0 min for the vehicle-treated rats, and this was significantly increased in a dose-dependent manner by KR-30450, KR-30818 and lemakalim (EC_{25} for increasing TTC: 1.2, 2.1 and 3.2 μM, respectively), further indicating their antiischemic activities (Fig. 4).

To test whether KR-30450 and its major metabolite protect ischemic rat heart by activating K^+_{ATP} as for lemakalim, we examined the effect of the K^+_{ATP} blocker glyburide on the cardioprotective activity of KR-30450, KR-30818 and lemakalim (Tables 1–3, Figs. 4 and 5). Glyburide (1 μM) did not affect preischemic and reperfusion cardiac contractility, whereas it significantly reduced preischemic and reperfusion heart rate as compared with vehicle controls. Although glyburide significantly reduced preischemic CF, it had no effects on reperfusion CF as compared with the vehicle group. Concomitant treatment with glyburide not only reversed the protective effects of KR-30450, KR-30818 and lemakalim on reperfusion LVDP, DP, EDP, LDH release and TTC but also decreased reperfusion LVDP relative to vehicle controls.

We also determined whether the cardiodepressant tendency of KR-30450 and lemakalim at rather higher concentrations was related to the activation of K^+_{ATP}. As shown in Tables 1–3, glyburide completely reversed the slight decrease in preischemic LVDP by KR-30450 and lemakalim, whereas it increased the preischemic LVDP relative to the predrug level in rats treated with KR-30818, where no effects on preischemic LVDP were observed (Tables 1–3).

DISCUSSION

In this study, we determined the antiischemic activities of newly synthesized K^+_{ATP} opener KR-30450 and its major active metabolite KR-30450 in isolated perfused rat heart model of myocardial ischemia and compared the results with those of lemakalim, a prototype of this class. It has been reported that the opening of K^+_{ATP} may play a role as an endogenous protective mechanism for ischemic myocardium since it was known to be involved in part in the

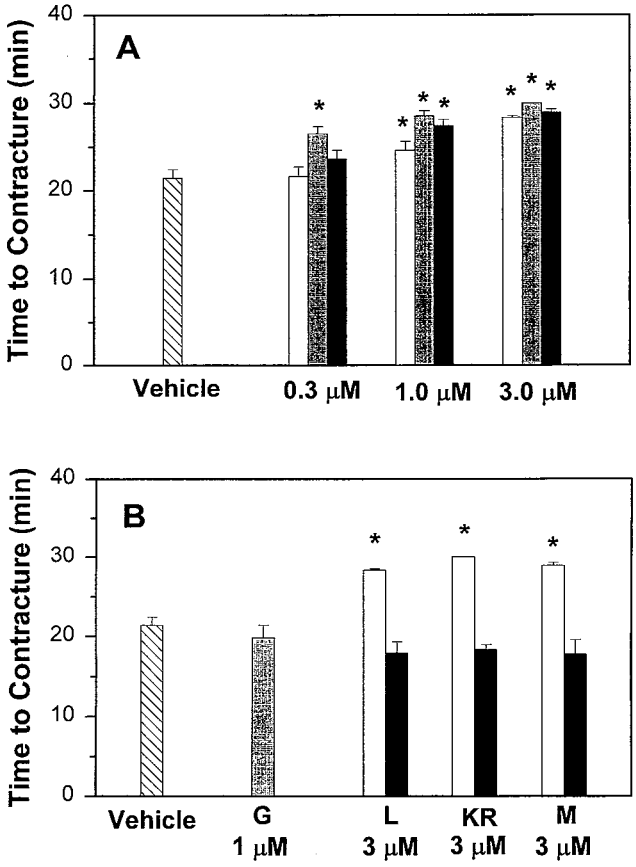

Fig. 4. Effect of a K^+_{ATP} inhibitor glyburide (1 μM, n=4) on protective activities of KR-30450 (n=7 for 0.3 μM and n=4 for 1 and 3 μM), KR-30818 (n=6 for 0.3 and 1 μM and n =5 for 3 μM) and lemakalim (n=9 for 0.3 μM and n=4 for 1 and 3 μM) on time to contracture (TTC) in isolated rat heart subjected to 25-min global ischemia followed by 30-min reperfusion. Three compounds significantly increased TTC in a dose-dependent manner as compared with vehicle (A, *P<0.05), and their effects at a concentration of 3 μM (n=4) were abolished by glyburide (B). A: Vehicle (hatched), lemakalim (open), KR-30450 (shaded) and KR-30818 (solid bar); B: Vehicle (hatched), glyburide alone (G, shaded), lemakalim (L), KR-30450 (KR), KR-30818 (M), without glyburide (open) and with glyburide (solid bar).

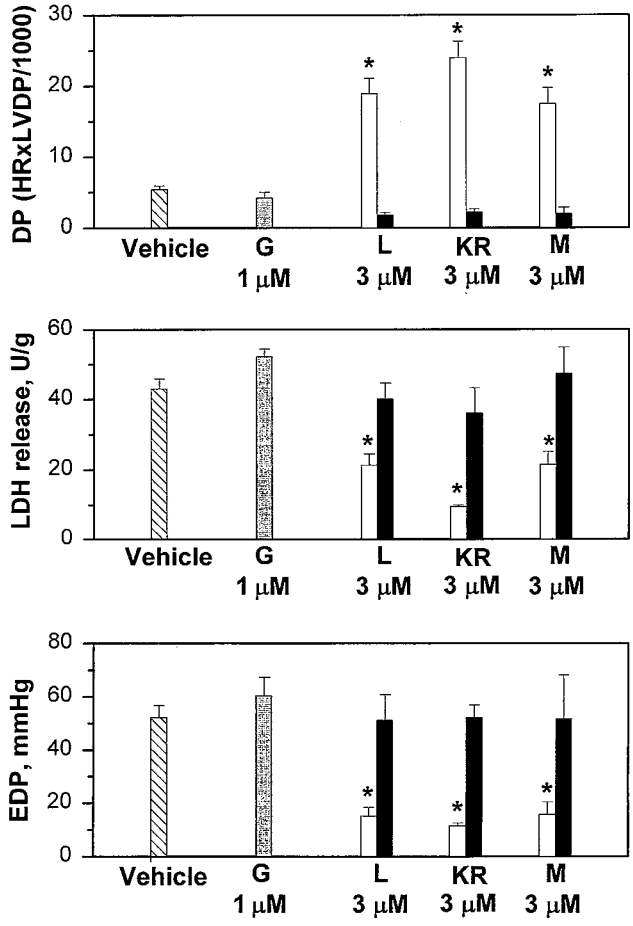

Fig. 5. Effect of a K^+_{ATP} inhibitor glyburide (1 μM, n=4) on cardioprotective effects of KR-30450 (n=4), KR-30818 (n=4) and lemakalim (n=4) at a concentration of 3 μM in isolated rat heart subjected to 25-min global ischemia followed by 30-min reperfusion. Three compounds significantly increased the double product (DP), while significantly reducing left ventricular end-diastolic pressure (EDP) and lactate dehydrogenase (LDH) release during reperfusion as compared with the vehicle (*P<0.05). All these cardioprotective effects were abolished by glyburide. Vehicle (hatched), glyburide alone (G, shaded), lemakalim (L), KR-30450 (KR), KR-30818 (M), without glyburide (open) and with glyburide (solid bar).

cardioprotection caused by a phenomenon called ischemic preconditioning (23). These cell biochemical studies raised as an exciting research area the development of cardioprotective agents targeted at pharmacological modulation of K^+_{ATP} in cardiomyocytes, and currently active studies by different research groups are underway to verify the hypothesis that K^+_{ATP} openers could function as effective chemical preconditioning agents (24).

The results from the present study has shown that KR-30450, a newly synthesized benzopyran derivative, has a potent cardioprotective activity, as evidenced by a significant concentration-dependent improvement in TTC and reperfusion cardiac function (LVDP, DP and EDP) and a marked reduction in reperfusion LDH release. The cardioprotective activity of KR-30450 appeared to be approximately five times as potent as lemakalim, an active (−)isomer of a prototype potassium channel opener cromakalim. Before the present study, there had been no detailed evaluation of the effects of lemakalim on preischemic and postischemic myocardium, although the effects of (±) racemate cromakalim were previously examined by other researchers (25). According to the results from the pharmacokinetic studies using ^{14}C-KR-30450 (personal communication with Dr. D.H. Kim, KIST), KR-30450 mainly undergoes metabolic changes to KR-30818 immediately after oral and intravenous administration to

rats and dogs, indicating that KR-30818 is one of the main active metabolites. In male rats administered with KR-30450 (0.5 and 2 mg/kg, p.o.), KR-30450 reached its maximum plasma concentration within 15–20 min after administration, and 30 min later, it was no longer detected, whereas KR-30818, the major active metabolite, reached its maximum plasma concentration within 10–15 min and decreased exponentially up to over 10 hr after administration. The maximal plasma concentration of KR-30818 was about 3 and 6 times higher than that of the parent compound at doses used. In the present study, we determined the cardioprotective activity of this metabolite in the same ischemic model to understand the importance of this metabolite in mediating the cardioprotective activities of the parent compound when applied in vivo. The present study has also shown that KR-30818 exerts significant cardioprotective effects, although its potency was slightly weaker than that of lemakalim. Thus, these findings indicate that the parent compound KR-30450 may exert its cardioprotective effects in part via KR-30818, one of the main metabolites, when given to intact animals. It is not certain whether the relative potency of (−)KR-30450 and its active major metabolite KR-30818 for cardioprotective effects may be equivalent to that for the structurally related benzopyran derivative lemakalim and its metabolite, as no information on the studies with metabolites of lemakalim is available to date. The cardioprotective activities of KR-30450 and its metabolite KR-30818 may not be directly related to their cardiodepressant effects, since the significant cardioprotective activities were observed for KR-30450 even at the concentrations of 0.1 and 0.3 μM that did not exert any cardiodepressant effects and for KR-30818 that did not exert depressant effects on cardiac function at all concentrations used. It seems likely that the cardioprotective activities of KR-30450, KR-30818 and lemakalim may be in part attributed to their coronary vasorelaxant effects, as all these compounds significantly increased preischemic CF at all concentrations used. However, the findings that cardioprotective effects of these three compounds were concentration-dependent, despite the almost constant increase in CF at these concentrations and that KR-30818 was less potent than the other two compounds although it had a tendency toward a similar or greater coronary vasodilatation may suggest the involvement of other mechanisms in their cardioprotective effects. Out of lemakalim, KR-30450 and KR-30818 that significantly increased nonischemic CF, KR-30450 only showed a dose-dependent increase in reperfusion CF probably via a mechanism related to its potent cardioprotective effects.

We further tested whether the cardioprotective activities of KR-30450 and KR-30818 were related to the activation of the ATP-sensitive potassium channel by using glyburide. Glyburide did not affect the outcome of ischemia when administered alone, yet it completely abolished the cardioprotective effects of KR-30450 and KR-30818 (reperfusion LVDP, DP, EDP, LDH release and TTC) as well as the cardiodepressant effects of KR-30450 observed at higher concentrations (1 and 3 μM), like it acted against the effects of lemakalim. The reversal of the antiischemic effects of KR-30450, KR-30818 and lemakalim by glyburide suggests that these compounds exert their effects by opening potassium channels. However, this finding does not appear to confirm the opening of K^+_{ATP} as the only mechanism involved in their cardioprotective effects, as it was reported that the antiischemic effects of cromakalim, the racemic mixture of lemakalim, occurred at concentrations that were low as compared with concentrations shown to increase outward currents by cardiac ATP-sensitive potassium channels (26, 27). Until recently, the currently known K^+_{ATP} openers possess both hypotensive activity and cardioprotective activity, limiting their application as cardioprotective agent due to hemodynamic depression. However, the recent discovery and development of BMS-180448, which represent the first pharmacological separation of cardiac and vascular effects of K^+_{ATP} openers in relation to their cardioprotective activity, may have opened a new possibility for treating ischemic heart disease by a novel mechanism of activating K^+_{ATP} in hearts (28, 29). The results from the present study and our previous study (10, 11) indicate that KR-30450 may not possess such a selectivity for cardioprotection as BMS-180448.

In conclusion, KR-30450, a recently developed K^+_{ATP} opener with potent antihypertensive and vasorelaxant activities, displays significantly more potent cardioprotective effects than lemakalim, and it may exert these effects in vivo mainly via its major active metabolite KR-30818. This property may add to the value of KR-30450, which is currently under development as a novel antihypertensive agent.

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