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The antihypertensive and cardioprotective effects of (−)-MJ-451, an ATP-sensitive K^+ channel opener

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Abstract

ATP-sensitive K^+ (K_{ATP}) channel openers have been shown to be a potential class of therapeutic agents for the control of cardiovascular diseases, including angina, arrhythmias, and hypertension. In this study, the pharmacological activity of 6-cyano-3*S*,4*R*-dihydro-2,2-dimethyl-2*H*-3-hydroxy-4-[5*S*-(1-hydroxymethyl)-2-oxo-1-pyrrolidinyl]-1-benzopyran ((−)-MJ-451), a synthetic K_{ATP} opener, was evaluated in anesthetized rat models and in isolated rat thoracic rings. Results demonstrated that intravascular injection of (−)-MJ-451 (0.02, 0.05 and 0.1 mg/kg) produced an immediate, dose-related reduction in mean arterial blood pressure in anesthetized spontaneously hypertensive rats (SHR), which persisted for more than 3 h and was not accompanied by reflex tachycardia. The hemodynamic changes were completely abolished by pretreatment with glibenclamide (4 mg/kg, i.v. bolus), a selective K_{ATP} channel blocker. In isolated thoracic aorta, (−)-MJ-451 (10 nM–3 μM) produced a concentration-dependent vasodilator effect on the phenylephrine (0.3 μM)-induced vasoconstriction. Moreover, (−)-MJ-451 relaxed the thoracic aorta contracted by low (5, 20 and 30 mM), but not high (40 and 60 mM) concentrations of extracellular potassium. In addition, (−)-MJ-451 showed cardioprotective effects in the rat model of 45-min left coronary artery occlusion followed by 1-h reperfusion. In myocardial ischemia, pretreatment with (−)-MJ-451 (2, 5 and 10 μg/kg, i.v. bolus) significantly reduced the incidence of ventricular fibrillation and the mortality, also reducing the total number of ventricular premature contractions, total duration of ventricular tachycardia and ventricular fibrillation. A significant reduction in infarct size was noted in three (−)-MJ-451 (2, 5 and 10 μg/kg)-treated groups. Also, the cardioprotective effects of (−)-MJ-451 were virtually abolished by pretreating the rats with glibenclamide (4 mg/kg, i.v. bolus). In conclusion, (−)-MJ-451, through opening the K_{ATP} channel, exerted antihypertensive and cardioprotective effects. Therefore, it is suggested that (−)-MJ-451 has potential in the treatment of hypertension or acute myocardial infarction.

Keywords: K_{ATP} channel; Hypertension; Arrhythmias; Myocardial ischemia; Reperfusion

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channel openers have received considerable attention for their potential clinical value. These agents may be used for a variety of illnesses including asthma, hypertension, myocardial ischemia, and arrhythmias (Lawson, 1996). K_{ATP} channels are found in a number of different tissues where they have distinct physiological functions. In the normal heart, K_{ATP} channels are blocked by intracellular ATP binding, and are activated during ischemia (lowered intracellular ATP levels). Activating these channels will lead to K^+ outward flow and to membrane potential hyperpolarization, which results in a reduction of intracellular calcium by blocking both voltage-regulated calcium channels and intracellular calcium release and thereby decrease contraction and so conserve ATP (Yokoshiki et al., 1998). Many effects, including vasorelaxation, bronchodilatation, or uterine smooth muscle relaxation, follow K_{ATP} channel activation (Lawson, 1996). These effects can be blocked by glibenclamide, a well-known specific K_{ATP} channel blocker

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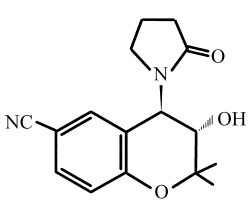
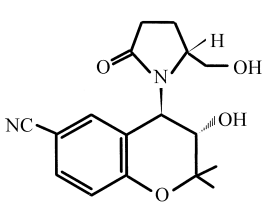
which is used as a clinical oral antidiabetic sulfonylurea (Quayle et al., 1997). Therefore, this protective function is thought to be a special property of these K_{ATP} channels.

There is general agreement that K_{ATP} channel openers afford cardioprotection in myocardial ischemia. In in vitro perfused rat heart submitted to transient global ischemia, administration of K_{ATP} channel openers before ischemia significantly improves the post-ischemic recovery of contractile function, increases the time to ischemic contracture, enhances reflow and reduces necrosis (Grover, 1997). In vivo studies showed that, when given prior to ischemia, the K_{ATP} channel openers, pinacidil, cromakalim, and aprikalim, reduce infarct size (Auchampach et al., 1991; Grover et al., 1990). Open K_{ATP} channels lead to hyperpolarized membranes and shortened action potential durations, which have been suggested to be the mechanisms underlying their cardioprotective actions (Cole, 1993).

Cromakalim, containing a benzopyran ring system, is the prototype K_{ATP} channel opener (Hamilton and Weston, 1989). However, the potent vasodilatation of K_{ATP} opening produces significant side effects such as reflex tachycardia, edema, headache, and flushing (Atwal, 1994). Therefore, additional work is needed to find new K_{ATP} openers with improved clinical potential for treating various diseases. The benzopyran derivative, 6-cyano-3*S*,4*R*-dihydro-2,2-dimethyl-2*H*-3-hydroxy-4-[5*S*-(1-hydroxymethyl)-2-oxo-1-pyrrolidinyl]-1-benzopyran ((−)-MJ-451) showed promise (Fig. 1). (−)-MJ-451 exhibits antihypertensive effects without reflex tachycardia. In addition, (−)-MJ-451 acts as a cardioselective K_{ATP} opener because the doses effective for preventing myocardial ischemia–reperfusion insult do not produce significant hemodynamic changes. The present study thus demonstrated both the antihypertensive effect of (−)-MJ-451 in anesthetized spontaneously hypertensive rats (SHR) and cardioprotective action on ischemia–reperfusion injury in Sprague–Dawley rats and the possible mechanism of these effects.



Cromakalim



(-)-MJ-451

Fig. 1. The chemical structures of cromakalim and (−)-MJ-451.

2. Materials and methods

The study was conducted in strict compliance with the European Community guidelines for the Care and Use of Laboratory Animals.

2.1. Measurement of blood pressure in SHR

The Wistar strain of SHR developed through selective breeding techniques by Okamoto and Aoki (1963) was chosen for this study. The close resemblance of hemodynamics in human essential hypertension and in the SHR justifies the widespread investigations with this experimental model (Udenfriend, 1972). Male SHR of body weight 250–350 g (age: 12–16 weeks) were used in this study. The rats, whose stock originated from the Charles River Breeding Laboratories in Japan, were purchased from the National Animal Center of the National Science Council, Taiwan, caged individually in clear plastic cages, and kept in an environmentally controlled room maintained at 23°C, relative humidity of 55%, and a light-dark cycle of 12/12 h. Under general anesthesia (urethane 0.6 g/kg and chloral hydrate 0.4 g/kg, i.p.), catheters were placed in the left femoral artery for monitoring of blood pressure and in the left femoral vein for the administration of drugs. Via a pressure transducer (Statham P23ID, Gould, USA), blood pressure and heart rate were recorded continuously on a biotechnometer (RS 3400, Gould, USA). Body temperature was maintained at 37 ± 0.5°C with a heating pad and monitor with a rectal thermometer. Measurements of mean arterial blood pressure and heart rate were made in groups treated with (−)-MJ-451 and cromakalim (0.01, 0.02, 0.05 and 0.1 mg/kg, i.v. bolus) 5, 30, 60, 120 and 180 min after the drug was given to SHR.

2.2. Isolated rat aortic vessels preparation

Male normotensive Sprague–Dawley rats weighing 270–350 g were used. The animals were anaesthetized with urethane 0.6 g/kg and chloral hydrate 0.4 g/kg, i.p. The thoracic aorta was isolated and excess fat and connective tissue were removed. Vessels were cut into rings about 3–4 mm in length and mounted in organ baths containing Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.5; and glucose, 11. The tissue bath solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Two stainless steel hooks were inserted into the lumen of the rings in an organ bath containing 20 ml of Krebs solution. One hook was fixed while the other was con-

nected to a force-displacement transducer (FT03, Grass, USA). Aorta rings were equilibrated in the medium for 90 min with three changes of Krebs solution. Each ring was progressively stretched to the optimal point on its length-tension curve as determined from the active tension developed in response to phenylephrine (1 μM) and maintained under optimal resting tension (2 g) until experimental procedures were initiated. Vasocontractions were recorded isometrically via a force-displacement transducer connected to a Grass Model 7D polygraph. During the experiment, aorta rings were precontracted with phenylephrine (0.3 μM) or potassium (5–60 mM). When this contraction reached steady state, various concentrations of cromakalim (1 nM–0.3 μM), or (−)-MJ-451 (10 nM–3 μM) were added into the tissue bath cumulatively to produce a concentration–response curve.

2.3. Experiments for myocardial ischemia / reperfusion

Male Sprague–Dawley rats weighing 250–300 g were anesthetized with intraperitoneal pentobarbital (50 mg/kg). Tracheotomy was performed and an intubating cannula was connected to a rodent ventilator (New England, USA). The animals were ventilated artificially with room air. The respiratory rate was synchonised with the rat's spontaneous rate (60–80 strokes/min, 1 ml/100 g). Arterial blood pH and blood gases were maintained within normal physiological limits (pH: 7.35–7.45; P_{CO_2}: 30–35 mm Hg; P_{O_2}: 85–100 mm Hg) by adjusting the respiratory rate and tidal volume. The left femoral artery and vein were cannulated for the measurement of arterial blood pressure and heart rate via a Statham pressure tranducer (Gould, USA) and a Biotechnometer (RS 3400, Gould, USA) and for the administration of drugs, respectively. Electrocardiograms (ECG) were recorded from standard lead II limb leads, with a positive electrode connected to the left hind leg, a negative electrode to the right foreleg and a ground electrode to the left foreleg. An oscilloscope ECG monitor (DSO 420, Gould, USA) was used to display the ECG continuously throughout the experiment. All signals, including the ECG and hemodynamic data, were recorded on a biotechnometer (RS 3400, Gould, USA).

By way of a left thoracotomy through the fourth intercostal space, the heart was exposed by opening the pericardial sac. A 6/0 silk suture on a tapered crochet hook was passed around the left main coronary artery close to its origin. The thread was then knotted as an occluder. Another thick cotton thread was put through the knot as a releaser to loosen the knot by pulling the thread to produce reperfusion. The left coronary artery was occluded for 45 min followed by 1 h of reperfusion.

After the surgical procedures, the animals were randomly assigned to one of the following protocols: (I) control group: rats received an equal volume of vehicle, dimethyl sulfoxide (DMSO; 0.1%), 15 min before occlusion; (II) (−)-MJ-451-treated groups: rats were injected with (−)-MJ-451 (0.5, 2, 5 or 10 μg/kg, i.v. bolus) 15 min before occlusion; (III) cromakalim-treated group: rats were injected with cromakalim 5 μg/kg, i.v. bolus) 15 min before occlusion; (IV) glibenclamide/(−)-MJ-451 group: rats were pretreated with glibenclamide (4 mg/kg, i.v. bolus), a selective K_{ATP} channel blocker (Fosset et al., 1988), 20 min before (−)-MJ-451 (10 μg/kg) injection in the pre-ischemic period. Following drug administration, the left coronary artery was occluded for 45 min to produce a zone of regional left ventricular ischemia. Regional cyanosis, hypotension and S–T segment elevation signified ischemia. Reperfusion began when the knot was released after 45 min of ischemia. Blood pressure, heart rate, and ECG were monitored continuously throughout the experimental period.

Most ventricular arrhythmias occurred within 30 min of ischemia after the start of occlusion. The frequency of arrhythmias was determined from ECG recordings including the total number of ventricular premature contractions and the incidence of ventricular premature contractions, ventricular tachycardia, and ventricular fibrillation during ischemia. The total duration of ventricular tachycardia and ventricular fibrillation and mortality for each experimental group were also recorded. We defined ventricular premature contractions as discrete and identifiable premature QRS complexes (premature in relation to the P wave), ventricular tachycardia as a run of six or more consecutive ventricular premature beats and ventricular fibrillation as signals for lacking identifiable individual QRS deflections and for which a rate could no longer be determined.

After all measurements were completed, the risk area was stained by reocclusion of the coronary artery and injection of 0.4–0.5 ml methylene blue (3 %) into the venous catheter. After 3 min, the heart was excised and the atria were removed. The entire ventricular area immersed in Krebs solution containing 95% oxygen (4°C) was sectioned into 2–3-mm slices from the apex to the base. The slices were incubated with nitroblue tetrazolium (0.1%) at 37°C for 15 min. This solution stained the normal myocardium purple and necrotic tissue appeared pale. The areas of risk and infarct were then determined by computer-aided planimetry.

2.4. Drugs

(−)-MJ-451 was used as a chiral synthetic intermediate in our preparation of a series of tetracyclic rigid analogs of cromakalim and was prepared from the readily available 6-cyano-2,2-dimethyl-2*H*-1-benzopyran and (*S*)-(+)-5-(hydroxymethyl)-2-pyrrolidinone in four steps. The synthesized (−)-MJ-451 (melting point: 164–165°C) was characterized by ^{1}H nuclear magnetic resonance (400 MHz, $CDCl_3$, 1.15 (s, 3H), 1.38 (s, 3H), 2.02 (br, 2H), 2.30–2.36 (m, 2H), 2.46–2.56 (m, 1H), 3.47 (m, 2H), 3.92 (d, 1H),

4.33 (br, 1H), 4.55 (br, 1H), 5.55 (br, 1H), 6.82 (d, 1H), 7.23 (s, 1H), 7.37 (d, 1H)), mass spectrometry (EI, 70eV, m/z 317($M^+ + 1$), 298, 283(base)), and by IR (KBr, 3300, 2950, 2225, 1680, 1490 cm^{-1}) $[\alpha]_D^{25} = -53.5°$ ($c = 1$, MeOH). We purchased methylene blue and nitroblue tetrazolium from Sigma Chem., USA and dissolved them in distilled water. Glibenclamide and cromakalim were obtained from RBI and Biomol Chem (USA), respectively, and dissolved in 0.1% DMSO.

2.5. Statistical analyses

All data are reported as group means and standard error of the mean (S.E.M.). The different incidence of arrhythmias and the differences in mortality between the control and treatment groups were subjected to chi-square test. The other parameters were compared by analysis of variance (ANOVA). If this analysis indicated significant differences between group means, the control group was then compared with each of the treatment groups by means of the Newman–Keuls method. A probability value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Hemodynamic effects of (−)-MJ-451 in SHR

The blood pressure and heart rate changes produced by (−)-MJ-451 are shown in Figs. 2A and 3. After intravenous administration, the action of (−)-MJ-451 began immediately. (−)-MJ-451 (0.01, 0.02, 0.05 and 0.1 mg/kg) produced a dose-dependent reduction in mean arterial blood pressure, which reached its maximum around 5 min after administration. Except with 0.01 mg/kg (−)-MJ-451, the depressor effects persisted for more than 3 h. No significant change of heart rate accompanied this hypotensive effect. In Fig. 2B, treating SHR with cromakalim (0.01, 0.02, 0.05 and 0.1 mg/kg) also produced immediate depressor effects dose-dependently, which is similar to the

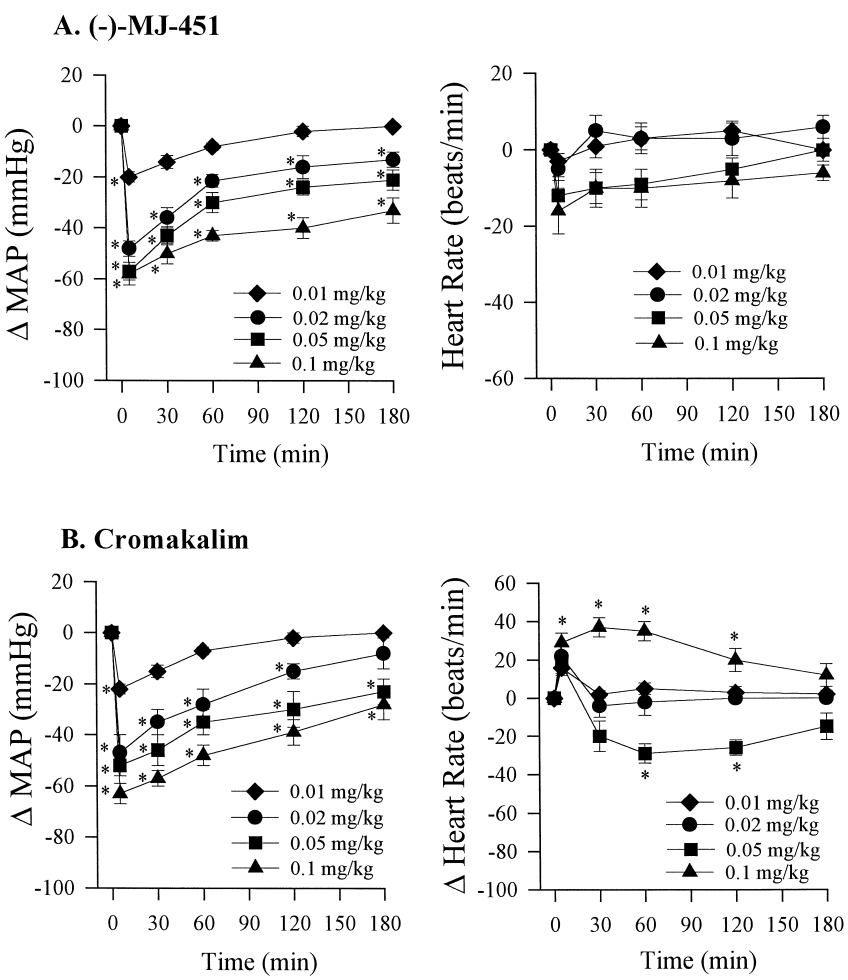


Fig. 2. Dose–response curves for changes in mean arterial blood pressure (MAP) and heart rate of SHR following intravenous administration of (−)-MJ-451 (0.02, 0.05 and 0.1 mg/kg). Data are expressed as means ± S.E.M. of eight observations. The predrug values for mean arterial blood pressure and heart rate are 156 ± 2.1 mm Hg and 312 ± 12 beats/min, respectively.

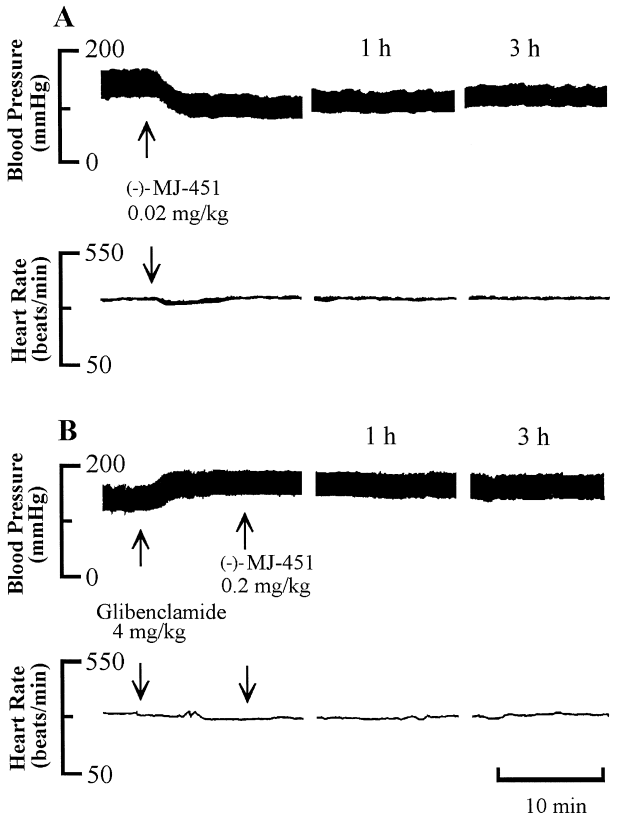


Fig. 3. Traces of blood pressure and heart rate of SHR treated with (−)-MJ-451 (A) or glibenclamide followed by (−)-MJ-451 (B). The predrug values for mean arterial blood pressure and heart rate are 156 ± 2.1 mm Hg and 312 ± 12 beats/min, respectively.

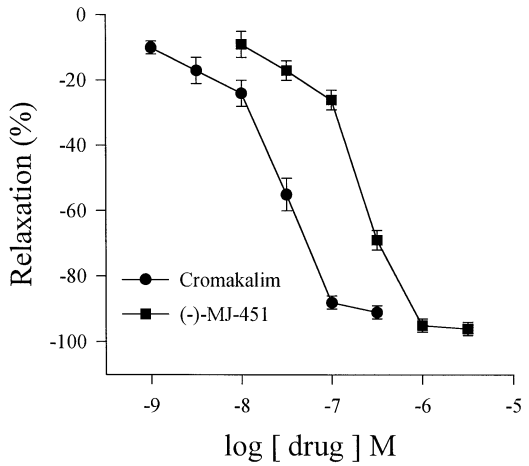


Fig. 4. Comparison of the vasorelaxant effects of (−)-MJ-451 and cromakalim in thoracic aorta precontracted with phenylephrine (0.3 μM). Values are presented as means ± S.E.M. ($n = 8$).

effect of (−)-MJ-451 at the same doses. However, the depressor effect of cromakalim elicited reflex tachycardia. After transient tachycardia, the heart rate of rats treated with the two lower doses (0.01 and 0.02 mg/kg) of cromakalim then gradually decreased and returned to its baseline. The 0.05 mg/kg dose of cromakalim showed a bradycardiac effect after 60–90 min administration. At the highest dose (0.1 mg/kg), the reflex tachycardia persisted for 2 h. In addition, as shown in Fig. 3B, the depressor and bradycardiac actions of (−)-MJ-451 (0.2 mg/kg) were blocked by pretreatment with glibenclamide (4 mg/kg, i.v. bolus).

3.2. Vasorelaxant effects of (−)-MJ-451

In rat thoracic aorta, phenylephrine (0.3 μM) caused a phasic contraction, then a tonic contraction that peristed for at least 30 min. Adding (−)-MJ-451 (10 nM–3 μM) and cromakalim (1 nM–0.3 μM) caused vasorelaxation of the phenylephrine (0.3 μM)-induced vasocontraction in a concentration-dependent manner (Fig. 4). (−)-MJ-451 and cromakalim produced their maximum effect at 1 μM and 0.3 μM, respectively. The EC50 of (−)-MJ-451 and cromakalim against phenylephrine (0.3 μM)-induced vasocontraction were about 0.19 and 0.02 μM, respectively.

The vasorelaxant effects of (−)-MJ-451 (1 μM) and cromakalim (1 μM) against high K^+ (5–60 mM)-induced vasocontraction of thoracic aorta are shown in Fig. 5. Tension of aorta was developed after the addition of various concentrations of K^+ solution. This vasocontraction was associated with membrane depolarization induced by high extracellular K^+ concentrations, leading to calcium influx via voltage-dependent calcium channels. Addition of (−)-MJ-451 reduced dramatically the vasocontraction induced by 5, 20 and 30 mM K^+ solution. This vasodilatation by (−)-MJ-451 decreased as the extracellu-

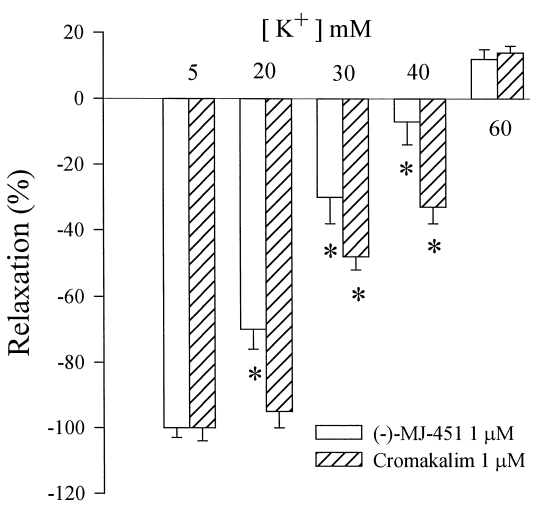


Fig. 5. The vasorelaxant effects of (−)-MJ-451 and cromakalim in thoracic aorta precontracted with various concentrations of K^+ (5–60 mM). Values are presented as means ± S.E.M. ($n = 8$), * $P < 0.05$: compared with the vasorelaxation with (−)-MJ-451 or cromakalim on 5 mM K^+-induced contraction.

Table 1
Hemodynamic effects of (−)-MJ-451 (i.v. bolus) during ischemic and reperfusion periods in Sprague–Dawley rats

Group	Baseline	After drug (20 min)	Ischemia (min)			Reperfusion (min)		
			5	10	30	10	30	60
Mean arterial blood pressure (mm Hg)								
Control	97.0 ± 3.9	97.0 ± 3.0	79.6 ± 6.0	83.5 ± 6.5	87.7 ± 4.9	86.5 ± 3.7	86.0 ± 3.3	87.6 ± 3.2
Cromakalim	95.8 ± 4.0	72.6 ± 2.3[a]	55.7 ± 3.0[a]	67.3 ± 5.1	78.5 ± 4.3	80.8 ± 3.6	78.1 ± 5.6	80.3 ± 3.4
(−)-MJ-451 (μg/kg)								
0.5	96.7 ± 6.7	96.5 ± 5.0	87.0 ± 4.0	83.7 ± 8.4	80.3 ± 2.2	82.3 ± 4.0	83.0 ± 4.0	80.9 ± 4.2
2	93.5 ± 5.6	92.5 ± 4.7	88.0 ± 3.0	86.5 ± 2.8	88.0 ± 5.5	91.1 ± 4.9	91.6 ± 5.5	90.7 ± 5.8
5	97.5 ± 5.3	97.1 ± 3.2	90.0 ± 2.5	90.3 ± 3.0	88.7 ± 4.5	88.5 ± 5.8	86.0 ± 5.0	87.6 ± 5.6
10	95.2 ± 6.6	95.1 ± 4.8	84.3 ± 5.4	83.0 ± 5.4	89.5 ± 6.0	93.8 ± 5.0	94.1 ± 6.5	89.7 ± 6.8
Heart rate (beats / min)								
Control	395.2 ± 9.0	391.2 ± 6.0	382.3 ± 9.8	378.6 ± 8.9	390.9 ± 7.3	390.7 ± 7.5	387.0 ± 8.3	386.4 ± 7.2
Cromakalim	399.3 ± 3.6	393.5 ± 6.3	389.0 ± 3.6	401.3 ± 3.1	396.5 ± 4.2	402.4 ± 3.6	401.0 ± 3.4	395.5 ± 4.6
(−)-MJ-451 (μg/kg)								
0.5	397.5 ± 7.5	401.0 ± 6.0	402.5 ± 7.5	404.0 ± 6.0	392.5 ± 2.5	383.0 ± 9.0	387.5 ± 8.5	385.0 ± 7.5
2	412.5 ± 8.5	410.0 ± 5.0	415.5 ± 6.5	409.0 ± 4.5	403.5 ± 5.5	405.0 ± 7.0	397.5 ± 7.5	399.0 ± 6.0
5	406.5 ± 3.5	405.0 ± 4.0	410.5 ± 4.5	408.0 ± 6.5	410.5 ± 3.5	404.0 ± 5.0	390.5 ± 5.5	391.0 ± 4.2
10	414.0 ± 5.5	408.0 ± 5.0	412.0 ± 5.5	407.0 ± 5.6	414.5 ± 6.5	414.0 ± 5.0	410.5 ± 6.5	405.0 ± 7.2
Rate–pressure product / 1000 (mm Hg × beats / min)								
Control	53.9 ± 2.0	53.5 ± 2.2	46.6 ± 4.0	47.4 ± 2.2	47.9 ± 3.5	48.4 ± 1.3	46.9 ± 2.6	47.6 ± 3.1
Cromakalim	54.0 ± 3.1	48.2 ± 2.1	41.6 ± 3.4	42.8 ± 2.5	45.2 ± 3.1	46.0 ± 1.9	45.8 ± 3.0	47.7 ± 2.0
(−)-MJ-451 (μg/kg)								
0.5	55.1 ± 6.5	55.1 ± 5.5	49.2 ± 4.7	48.6 ± 6.8	43.2 ± 4.2	39.0 ± 3.0	43.6 ± 5.2	41.2 ± 5.2
2	56.8 ± 4.1	57.9 ± 4.4	52.9 ± 5.4	52.6 ± 6.0	50.7 ± 5.2	53.0 ± 6.4	53.5 ± 5.5	53.7 ± 7.2
5	57.2 ± 3.4	57.4 ± 4.1	52.0 ± 4.9	52.8 ± 4.6	51.6 ± 4.3	49.2 ± 5.2	47.6 ± 5.1	47.1 ± 5.5
10	56.9 ± 3.8	56.7 ± 4.8	51.1 ± 6.0	44.5 ± 3.9	49.8 ± 4.4	53.4 ± 3.9	53.8 ± 3.8	50.3 ± 4.4

Cromakalim: 5 μg/kg. Values are expressed as means ± S.E.M.
[a] $P < 0.05$ vs. the control group.

lar K^+ concentrations were increased. When the extracellular K^+ concentration was 40 mM, (−)-MJ-451 did not produce significant vasorelaxation. However, in the presence of 60 mM K^+, (−)-MJ-451 elicited slight vasoconstriction. In addition, the vasoreactivity to cromakalim influenced by high K^+ was similar to that with (−)-MJ-451 (Fig. 5).

3.3. (−)-MJ-451 and myocardial ischemia–reperfusion

3.3.1. The hemodynamic changes of (−)-MJ-451 in myocardial ischemia–reperfusion

The hemodynamic data are summarized in Table 1. When the coronary artery was occluded, mean arterial blood pressure of all groups immediately (< 1 min)

Table 2
Effects of pretreatment with (−)-MJ-451 (i.v. bolus) on the onset and incidence of ventricular arrhythmias and mortality following left coronary artery occlusion in Sprague–Dawley rats

Group	*N*	VPC		VT		VF		Mortality (%)
		Onset (min)	Incidence (%)	Onset (min)	Incidence (%)	Onset (min)	Incidence (%)	
Control	17	5.3 ± 0.2	100	6.2 ± 0.4	94.1	7.4 ± 0.3	94.1	58.8
Cromakalim 5 μg/kg	7	8.2 ± 0.9[a]	100	8.3 ± 0.8	100	8.9 ± 1.3	43	14[a]
(−)-MJ-451								
0.5 μg/kg	10	4.8 ± 0.2	100	5.5 ± 0.2	100	7.4 ± 0.5	100	60
2 μg/kg	10	6.5 ± 0.6	60	7.5 ± 1.3	40	–	0[a]	0[a]
5 μg/kg	10	7.3 ± 1.2[a]	100	7.8 ± 1.2	60	–	0[a]	0[a]
10 μg/kg	10	7.2 ± 1.2	80	8.9 ± 1.8	40	–	0[a]	0[a]
Glibenclamide (4 mg/kg)	6	5.0 ± 0.6	100	5.3 ± 0.3	100	5.8 ± 0.1	100	50
+(−)-MJ-451 (10 μg/kg)								

N: number of rats; VPC: ventricular premature contractions; VT: ventricular tachycardia; VF: ventricular fibrillation. Values are expressed as means ± S.E.M.
[a] $P < 0.05$ vs. the control group.

dropped by about 15–20 mm Hg, then increased gradually. There were no significant differences in mean arterial blood pressure, heart rate or rate-pressure product, an indirect index of cardiac oxygen consumption, between the control and (−)-MJ-451-treated groups throughout the experiment. In the cromakalim group, mean arterial blood pressure was significantly lower in the pre-occlusion period and during 5 min of ischemia than in the control group.

3.3.2. Effects of (−)-MJ-451 on ischemia-induced arrhythmias

Ventricular arrhythmias occurred immediately after ligation of the left coronary artery. Major arrhythmias occurred between 5 and 20 min post-occlusion, including ventricular premature contractions, ventricular tachycardia, and ventricular fibrillation. (−)-MJ-451 (2, 5 and 10 μg/kg) markedly reduced the incidence of ventricular fibrillation and mortality (Table 2) to zero. Meanwhile, injection of (−)-MJ-451 (2, 5 and 10 μg/kg) reduced the total number of ventricular premature contractions and total duration of ventricular tachycardia during the ischemic period (Fig. 6). These beneficial effects were markedly blocked by pretreatment with glibenclamide. In addition, cromakalim (5 μg/kg) significantly reduced mortality, total duration of ventricular fibrillation during ischemia and infarct size after reperfusion.

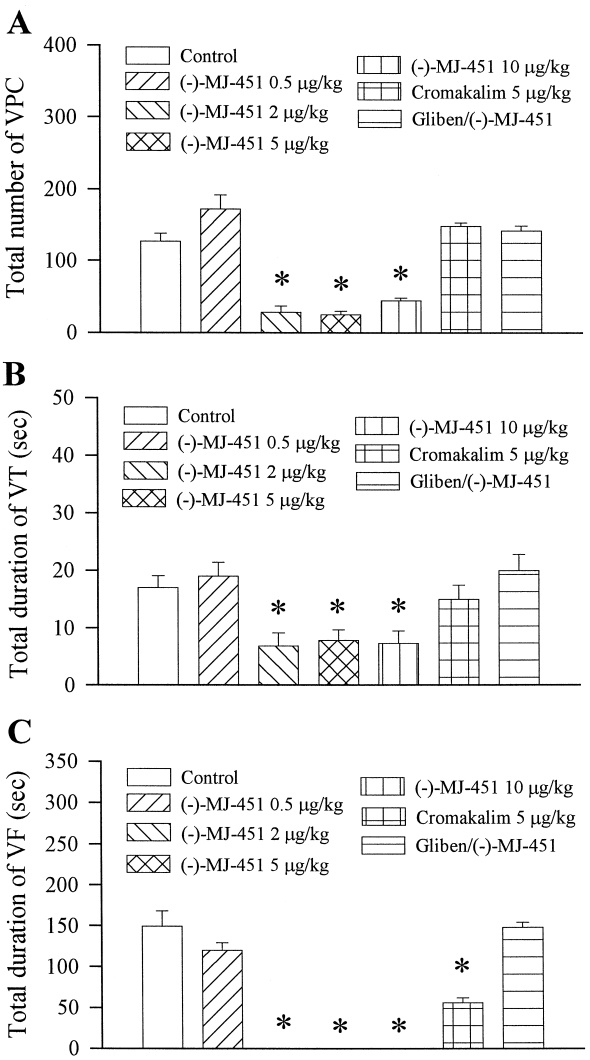


Fig. 6. Effects of (−)-MJ-451 on ventricular arrhythmias in anesthetized Sprague–Dawley rats. The total number of ventricular premature contractions (VPC) (A) and the total duration of ventricular tachycardia (VT) (B) and ventricular fibrillation (VF) (C) are shown. Gliben/(−)-MJ-451 means that glibenclamide (4 mg/kg, i.v. bolus) was given 20 min before (−)-MJ-451 (10 μg/kg, i.v. bolus) injection in pre-ischemic period. Values are expressed as means ± S.E.M., * $P < 0.05$ vs. the control group.

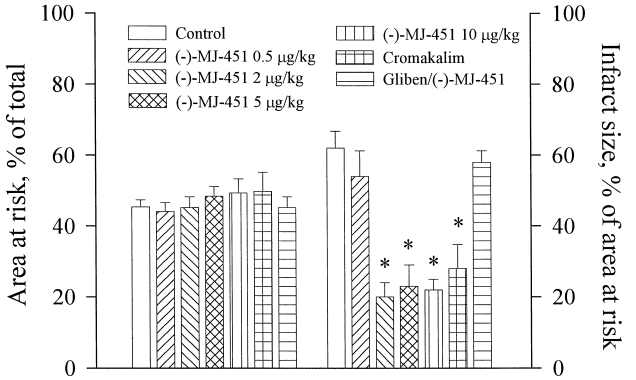


Fig. 7. Effects of (−)-MJ-451 on myocardial infarct size, expressed as a percentage of risk region, in Sprague–Dawley rats undergoing 45 min of left coronary artery occlusion followed by 1 h of reperfusion. Gliben/(−)-MJ-451 means that glibenclamide (4 mg/kg, i.v. bolus) was given 20 min before (−)-MJ-451 (10 μg/kg, i.v. bolus) injection in pre-ischemic period. Values are expressed as means ± S.E.M., * $P < 0.05$ vs. the control group.

All experimental groups had clear areas of infarction as a consequence of 45 min of ischemia followed by 1 h reperfusion. The area at risk was not different among these groups (Fig. 7). A significant reduction in infarct size, expressed as a percentage of the area at risk, was found in three (−)-MJ-451 (2, 5 and 10 μg/kg) and cromakalim-treated groups when compared with the control group ($P < 0.05$) (control: 62.2 ± 4.8%; (−)-MJ-451 0.5 μg/kg: 54.0 ± 7.2%; 2 μg/kg: 20.1 ± 4.0%; 5 μg/kg: 23.2 ± 6.0%; 10 μg/kg: 22.0 ± 3.0%). However, the infarct size of the glibenclamide/(−)-MJ-451 group was 58.3 ± 3.0%, which was not statistically different from that of the control group ($P > 0.05$).

4. Discussion

The present study demonstrated that (−)-MJ-451, a synthetic benzopyran derivative, via opening of the K_{ATP} channel, exerts antihypertensive, and antiarrhythmic actions, especially ventricular fibrillation, the most important arrhythmia should be suppressed under conditions of acute

myocardial ischemia, and reduces the size of the infarct caused by myocardial ischemia–reperfusion. The depressor effect of (−)-MJ-451 on hypertension did not elicit reflex tachycardia, but only slightly decreased heart rate. Furthermore, (−)-MJ-451 had cardioprotective effects without changing hemodynamic parameters, suggesting that the benefits of (−)-MJ-451 on hearts were not achieved by vasodilatation. This indicates that (−)-MJ-451 may possess a cardioselective property.

In the present study, (−)-MJ-451 produced an immediate and marked antihypertensive effect in anesthetized SHR, which could be blocked by the selective K_{ATP} blocker, glibenclamide. In isolated thoracic aorta, (−)-MJ-451 relaxed the smooth muscle contracted by exposure to low, but not to high (> 30 mM) concentrations of extracellular K^+. These results support the idea that the antihypertensive effect of (−)-MJ-451 is mediated via vascular K_{ATP} opening. (−)-MJ-451 may produce hyperpoplarization of the cell membrane, leading to a reduction in cytosolic free Ca^{2+} and vasodilatation. However, the potency of (−)-MJ-451 is ~10 fold less than that of cromakalim for inhibition of vasocontraction by phenylephrine (Fig. 4). Because of a lack for the pharmacokinetic data of (−)-MJ-451, the present results cannot explain why the depressor effect of (−)-MJ-451 is similar to that of cromakalim.

Although it has a pronounced antihypertensive effect, (−)-MJ-451 was shown to be efficacious in our myocardial ischemia model without significantly changing hemodynamic parameters. This can avoid complications caused by its potent vasodilating effects, which may result in underperfusion of the area already at risk. U-89,232 (cromakalim analogue) (Toombs et al., 1992) and BMS-180448 (pinacidil analogue) (Grover et al., 1995b; D'Alonzo et al., 1995), both devoid of vascular effects, also show cardioprotection against ischemia in rat and rabbit models. The beneficial effects on the ischemic heart may be a direct (myocardial) action and this relative selectivity for the ischemic myocardium indicates that (−)-MJ-451 shows promise as a selective cardioprotective agent. It has been suggested that K_{ATP} channel openers act via different receptor subtypes of cardiac and vascular tissues to achieve their effects (Atwal, 1994).

While there is general agreement that K_{ATP} channel openers provide cardioprotection in myocardial ischemia, their mechanism of action is unclear. The electrophysiological effects of the K_{ATP} channel openers, specifically, their ability to hyperpolarize membranes and shorten action potential durations, have been suggested to be the underlying mechanisms of their cardioprotective action (Cole, 1993). In ventricular cells of guinea pig, we also found that (−)-MJ-451 shortened the duration of action potentials, which could be blocked by glybenclamide (data not shown). However, it has been argued that treatment with K_{ATP} channel openers could exert cardioprotective actions but without effect on action potential duration (Yao and Gross, 1994; Grover et al., 1995a). Thus, the concept that the cardioprotective effect of K_{ATP} channel openers was achieved via the activation of sarcolemmal K_{ATP} channels is challenged. A recent study has demonstrated that activation of the mitochondrial K_{ATP} channel, but not the sarcolemmal K_{ATP} channel, by diazoxide could produce a cardioprotective effect against ischemic damage (Liu et al., 1998). Thus, the correlation between action potential duration shortening and cardioprotection is not yet clear and requires further study.

In preclinical studies, K_{ATP} channel openers relaxed coronary conductance arteries and selectively increased coronary blood flow (Longman and Hamilton, 1992). Therefore, these agents showed ability to improve oxygen delivery within ischemic regions. However, in this left coronary artery ligation model, coronary blood flow was discontinued. Previous study showed that the rat heart is deficient in functional collaterals (Johns and Olson, 1954). Therefore, increasing coronary blood flow by K_{ATP} channel openers would not contribute to the cardioprotective effect during myocardial ischemia.

In addition, K_{ATP} channel openers have been shown to reduce the rate of depletion of ATP in ischemic myocardium (McPherson et al., 1993). It has been suggested that reduced hydrolysis of ATP mediates cardioprotection and that K_{ATP} openers inhibit the inefficient ATP hydrolysis that occurs in the mitochondria under ischemic conditions (Grover, 1997). Based on the hemodynamic data (Table 1) during myocardial ischemia–reperfusion, (−)-MJ-451 did not significantly produce rate-pressure product alterations, indicating that the cardioprotective effects of (−)-MJ-451 may not be mediated via a reduction of cardiac oxygen consumption. Therefore, more effort should be devoted to investigating the correlation between K_{ATP} openers and ATP preservation during myocardial ischemia.

K_{ATP} channel openers have received increasing interest for their potential antiarrhythmic action. It has been shown that K_{ATP} channel openers produce both antiarrhythmic (Takahashi et al., 1991; Carlsson et al., 1992) and proarrhythmic activities (Wolleben et al., 1989; Chi et al., 1990). Interestingly, agents that block K_{ATP} channels also show antiarrhythmic (Pasnani and Ferrier, 1992; Billman et al., 1993) and proarrhythmic activities (Pasnani and Ferrier, 1992; D'Alonzo et al., 1992). The mechanisms of K_{ATP} channel modulators for mediating antiarrhythmic or proarrhythmic actions are unclear. It has been suggested that the antiarrhythmic effect of the K_{ATP} channel openers can ameliorate triggered activity (Carlsson et al., 1992; D'Alonzo et al., 1994), whereas they tend to exacerbate re-entry arrhythmias (Wolleben et al., 1989; Chi et al., 1990). On the contrary, blockers of K_{ATP} channels may alleviate re-entrant arrhythmias (Pasnani and Ferrier, 1992; D'Alonzo et al., 1992), whereas they may cause deterioration of triggered arrhythmias (Spinelli et al., 1991; D'Alonzo et al., 1994). The variability of findings is a result of the experimental conditions of and the proarrhyth-

mic effect of K_{ATP} channel openers may be dose-dependent. We also found that a high dose of (−)-MJ-451 (50 μg/kg) evoked adverse rhythms such as ventricular fibrillation and increased mortality (90%) (data not shown).

Recently, an increasing number of studies with preconditioning of the myocardium have demonstrated that opening of K_{ATP} channels during ischemia may contribute to an endogenous protective mechanism (Grover, 1997). Therapy with K_{ATP} channel openers may offer a ''chemical preconditioning'' that gives the heart an improved ability to withstand transient oxygen deprivation and consequently suffer less tissue damage during acute myocardial infarction (Lawson, 1996). This increases the interest in exploring the therapeutic potential of K_{ATP} channel openers. In conclusion, (−)-MJ-451, via opening K_{ATP} channels, produces a potent antihypertensive effect without reflex tachycardia. Also, it suppresses ventricular arrhythmias induced by myocardial ischemia and reduces infarct size after reperfusion. (−)-MJ-451 produced its cardioprotective effect without affecting hemodynamic variables. Therefore, (−)-MJ-451 is suggested to be a cardioselective K_{ATP} opener and a potential therapeutic agent in the treatment of hypertension or acute myocardical infarction.

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