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The Effects of a Newly Synthesized ATP-Sensitive Potassium Channel Opener, MJ-355, on Blood Pressure and Myocardial Ischemia-Reperfusion Injury in Rats

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ABSTRACT—ATP-sensitive potassium (K_{ATP}) channel openers, exerting a potent vasodilatory action, are useful in the treatment of cardiovascular disorders; e.g., hypertension and angina pectoris. This study was designed to evaluate the effect of MJ-355 (6-cyano-3,4-*trans*-3,4-dihydro-2,2-dimethyl-2*H*-3-hydroxy-4-[2-oxo-5*S*-(1-ethoxyethoxymethyl)-1-pyrrolidinyl]-1-benzopyran), a newly synthesized K_{ATP} channel opener, on hemodynamics in spontaneously hypertensive rats and on myocardial ischemia-reperfusion injury in a rat model of 45 min left coronary artery occlusion followed by 1-h reperfusion. Intravascular injection of MJ-355 (0.005, 0.05 and 0.1 mg/kg) produced a dose-related reduction in mean arterial blood pressure. The depressor effect started 10–15 min after the administration and persisted for more than 3 h and was not accompanied by a reflex tachycardia. In myocardial ischemia, pretreatment of MJ-355 (0.02 mg/kg) significantly reduced the total number of ventricular premature contractions and ventricular tachycardia, total duration of ventricular fibrillation and the mortality. Additionally, a significant reduction in infarct size was noted in all of the MJ-355-treated groups. The hemodynamic and cardioprotective effects of MJ-355 were virtually abolished by pretreating the rats with glibenclamide (4 mg/kg, i.v. bolus), a selective K_{ATP} channel blocker. In conclusion, MJ-355, through the activation of K_{ATP} channels, exhibited antihypertensive and cardioprotective effects. It is suggested that MJ-355 should be useful in the treatment of hypertension and/or acute myocardial infarction.

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

It has been shown that the ATP-sensitive potassium (K_{ATP}) current participates in the response of the myocardium to hypoxia (1). Recent studies demonstrated existence of K_{ATP} channels in both coronary smooth muscles and myocytes, and they modulate coronary blood flow at rest and during various pathophysiological conditions (2, 3). The opening of K_{ATP} channels leads to outward flow of K^+ and membrane potential hyperpolarization to stabilize the cell membrane. As a result, the shortening the duration of action potential occurs, thus reducing Ca^{2+} cycling during electrical systole. Potassium channel opening also speeds up the loss of contractile force of the ischemic region and drives the myocardial cell faster into a resting state. Similarly, the activation of K_{ATP} channels produces a hyperpolarization of the smooth muscle membrane that causes vasorelaxant and antihypertensive effects. In addition, when tissue is hypoxic, autocrine products such as adenosine and endothelium-dependent hyperpolarizing factors are released. Via the K_{ATP} channel activation, they resulted in hyperpolarization and vasorelaxation (4, 5). Thus, it is thought that K_{ATP} channels exert a protective property in myocardial ischemic diseases.

A series of K_{ATP} channel openers have been reported to possess potential benefit in myocardial ischemia (5, 6). Cromakalim containing a benzopyran ring system is the prototype of K_{ATP} channel openers (7). However, the potent vasodilatation of K_{ATP} opening produce significant side effects such as reflex tachycardia, edema, headache and flushing (8). Therefore, additional work is needed to find new K_{ATP} openers to improve their clinical potential for treating various diseases. A newly synthesized benzo-

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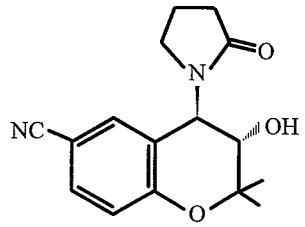
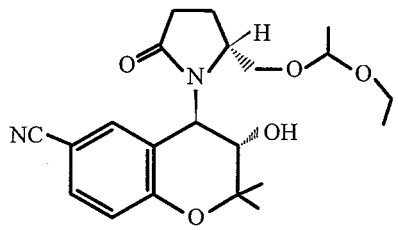


Cromakalim



MJ-355

Fig. 1. The chemical structures of cromakalim and MJ-355.

pyran derivative, MJ-355 (6-cyano-3,4-*trans*-3,4-dihydro-2,2-dimethyl-2*H*-3-hydroxy-4-[2-oxo-5*S*-(1-ethoxyethoxymethyl)-l-pyrrolidinyl]-1-benzopyran) showed promise (Fig. 1). The present study was undertaken to evaluate the antihypertensive effect of MJ-355 in spontaneously hypertensive rats (SHR) and cardioprotective action on ischemia-reperfusion injury in Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

The study was conducted in strict accordance with the principles for the Care and Use of Laboratory Animals adopted and promulgated by The Japanease Pharmacological Society. Male SHR (16 weeks of age) and SD rats weighing 250–350 g were used. Rats were caged individually in clear plastic cages and kept in an environmentally controlled room maintained at 23℃, relative humidity of 55%, and a light-dark cycle of 12 h/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 the left femoral vein for the administration of drugs. Via a pressure transducer (Statham P23ID; Gould, Cleveland, OH, USA), blood pressure and heart rate were recorded continuously on a biotechnometer (RS 3400, Gould).

Hemodynamics

In the SHR, after they were given the drug, measurements of mean arterial blood pressure and heart rate were made in the MJ-355 (0.005, 0.05 and 0.1 mg/kg)- and cromakalim (0.025, 0.05 and 0.1 mg/kg)-treated groups at 15, 30, 60, 90, 120 and 180 min. In SD rats, mean arterial blood pressure and heart rate were measured in the control, cromakalim (0.005 mg/kg)-, MJ-355 (0.005 and 0.02 mg/kg)- and glibenclamide (4 mg/kg)-treated groups after drug treatment in the periods of ischemia and reperfusion. The product of heart rate and systolic blood pressure (rate-pressure produce: RPP), an index of myocardial oxygen consumption, was calculated in all of the experimental groups.

Experimental preparation for myocardial ischemia

In SD rats, tracheotomy was performed and an intubating cannula was connected to a rodent ventilator (New England, Medway, MA, USA). Tidal volume was set at 0.8–1.0 ml/100 g body weight and respiratory rate was set at 60–80 beats/min. The respiratory rate was adjusted to keep the arterial blood pH and blood gases in the physiological range (pH: 7.35–7.45; Pco_2: 30–35 mmHg; Po_2: 85–100 mmHg). 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) was used to display the ECG continuously throughout the experiment. All signals, including the ECG and hemodynamic data, were recorded on biotechnometer (RS 3400, Gould).

By way of 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 needle was passed around the left main coronary artery close to its origin. The thread was then made into a knot as an occluder. Another thick cotton thread was put through the former knot as a releaser loosening the knot by pulling the thread to produce reperfusion. The left coronary artery was occluded for 45 min, followed by 1 h of reperfusion.

Experimental protocol of ischemia-reperfusion

After performing all surgical procedures, the animals were randomized into one of seven experimental protocols: i) control group, rats received an equal volume of vehicle dimethyl sulfoxide (DMSO, 0.1%) 15 min before occlusion; ii) MJ-355 I-0.005 group, rats were injected with MJ-355 (0.005 mg/kg, i.v. bolus) 15 min before occlusion; iii) MJ-355 I-0.02 group, MJ-355 (0.02 mg/kg, i.v. bolus) was given 15 min before occlusion; iv) MJ-355 R-0.02 group, MJ-355 (0.02 mg/kg, i.v. bolus) was administered 5 min prior to reperfusion; v) cromakalim group, rats received cromakalim (0.005 mg/kg, i.v. bolus)

15 min before occlusion; vi) glibenclamide/MJ-355 group, rats were pretreated with glibenclamide (4 mg/kg, i.v. bolus), a selective K_{ATP} channel blocker (9), 20 min before MJ-355 (0.02 mg/kg) injection in the pre-ischemic period; vii) glibenclamide group, rats were pretreated with glibenclamide (4 mg/kg, i.v. bolus) 20 min prior to occlusion. Following drug treatments, the coronary artery was then 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 by releasing the snare after 45 min of ischemia. Blood pressure, heart rate and ECG were continuously monitored throughout the experimental period.

Arrhythmias

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

Determination of infarct size

After completing all measurements, staining of the risk area was accomplished with reocclusion of the coronary artery and injection of 0.4−0.5 ml methylene blue (3%) into the venous cannula to denote the area at risk. After 3 min, the heart was excised and the atria were removed. The entire ventricular area immersed in Krebs solution contained 95% oxygen (4℃) was sectioned into 2- to 3-mm slices from the apex to the base. The slices were incubated with nitroblue tetrazolium (0.1%) at 37℃ 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 using a computer-aided planimetry.

Drugs

MJ-355, a 5′-substituted analog of cromakalim, is an intermediate of the rigid tetracyclic analogs of cromakalim and dissolved in 0.1% DMSO. MJ-355 was prepared from the readily available 6-cyano-2,2-dimethyl-2*H*-1-benzopyran and (*S*)-(+)-5-(hydroxymethyl)-2-pyrrolidinone in three steps. The synthesized MJ-355 (melting point: 130−132℃) was characterized by ^{1}HNMR (400 MHz, $CDCl_3$, 1.07 (t, 3H), 1.15 (d, 3H), 1.18 (s, 3H), 1.51 (s, 3H), 1.94−2.00 (m, 1H), 2.23−2.25 (m, 1H), 2.30−2.36 (m, 1H), 2.61−2.65 (m, 1H), 3.0 (br, 1H), 3.25−3.33 (m, 2H), 3.34−3.40 (m, 1H), 4.03 (d, 1H), 4.08−4.25 (m, 2H), 5.05 (br, 1H), 6.78 (dd, 1H), 7.33 (s, 1H), 7.34 (d, 1H)); mass spectrometry (EI, 70ev, m/z 388 (M^+), 370, 355, 281, 160, 84 (base)); and by IR (KBr, 3325, 2980, 2225, 1640, 1490 cm^{-1}). We purchased methylene blue and nitroblue tetrazolium from Sigma Chem. Co., St. Louis, MO, USA, which was prepared in distilled water, and glibenclamide and cromakalim, respectively, from RBI (Natick, MA, USA) and Biomol. Chem. Co. (Plymouth, PA, USA) and prepared in DMSO.

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 mortality in control, MJ-355-, cromakalim- and glibenclamide-treated groups were subjected to the chi-square test. The other parameters were compared by a analysis of variance (ANOVA). If this analysis indicated a significant difference among the 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.

RESULTS

Antihypertensive effects of MJ-355 in anesthetized SHR

The blood pressure and heart rate changes produced by MJ-355 are shown in Figs. 2 and 3. The action of MJ-355 began around 10−15 min after intravenous administration. MJ-355 at 0.005, 0.05 and 0.1 mg/kg produced a dose-dependent reduction in mean arterial blood pressure, which reached the maximum around 30 min after administration. The depressor effect of the high dose (0.1 mg/kg) persisted for more than 3 h. Pronounced bradycardia accompanied the hypotensive effect of MJ-355 at 0.005 and 0.05 mg/kg, which occurred during the first hour of administration, then gradually returned to baseline values 2 h after administration. Treating SHR with cromakalim (0.025, 0.05 and 0.1 mg/kg) also produced a depressor effect in a dose-dependent manner. Cromakalim at 0.05 and 0.1 mg/kg showed a similar hypotensive effect to that of MJ-355 at the same doses. However, the depressor effect of cromakalim elicited reflex tachycardia. After transient tachycardia, heart rate of rats treated with low dose of cromakalim then gradually decreased and recovered to baseline. The intermediate dose of cromakalim showed a bradycardic effect after 60−90 min administration. At high dose, reflex

tachycardia persisted for 2 h. In addition, as shown in Fig. 3B, the depressor and bradycardic actions of MJ-355 (0.02 mg/kg) were blocked by pretreatment with glibenclamide (4 mg/kg, i.v. bolus).

The hemodynamic changes of MJ-355 in myocardial ischemia-reperfusion

The hemodynamic data are summarized in Table 1. When the coronary artery was occluded, mean arterial blood pressure immediately dropped about 20 mmHg, and then it increased gradually. Rats in the control group showed no significantly hemodynamic changes during the ischemic and reperfusion periods. Pretreatment of 0.005 and 0.02 mg/kg MJ-355 and cromakalim produced a significant decrease in mean arterial blood pressure during the pre-occlusion period. MJ-355 at 0.005 mg/kg and cromakalim did not significantly change heart rate, whereas 0.02 mg/kg MJ-355 produced bradycardia 15 min after administration in pre-occlusion period. These three drug treatments did not significantly affect the hemodynamics during ischemia and reperfusion periods as compared with the control group. Similarly, pretreatment with glibenclamide did not significantly change mean arterial blood pressure, heart rate and RPP during pre-occlusion, ischemic and reperfusion periods as compared with the control group.

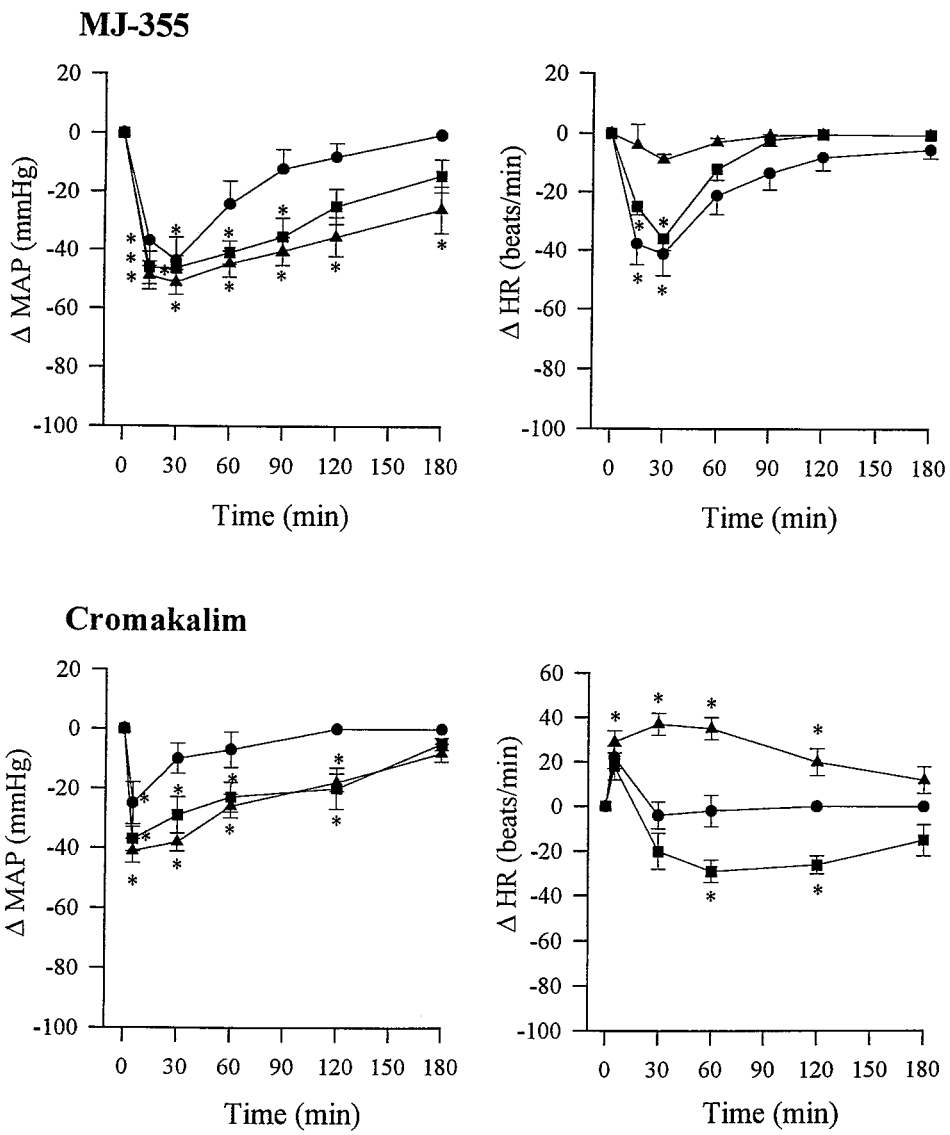


Fig. 2. Dose-response curves for changes in mean arterial blood pressure (MAP) and heart rate (HR) of SHR following intravenous administration of MJ-355 (●: 0.005 mg/kg, ■: 0.05 mg/kg, ▲: 0.1 mg/kg) and cromakalim (●: 0.025 mg/kg, ■: 0.05 mg/kg, ▲: 0.1 mg/kg). Data are expressed as the mean±S.E.M. of 8 observations. * indicates significant difference ($P<0.05$) as compared with the baseline values.

Effects of MJ-355 on ischemia-induced arrhythmias

Ventricular arrhythmias occurred immediately after the ligation of the left coronary artery. Major arrhythmias occurred between 5 and 20 min post-occlusion, including VPC, VT and VF. In Fig. 4, MJ-355 (0.005 and 0.02 mg/kg) significantly reduced the total number of VPC, whereas cromakalim markedly increased the total number of VPC (Fig. 4A). MJ-355 at 0.02 mg/kg significantly reduced the total number of VT. Cromakalim and MJ-355 at low dose had no beneficial effect on VT (Fig. 4B). Treatment of MJ-355 and cromakalim significantly reduced the total duration of VF (Fig. 4C). Cromakalim and MJ-355 at 0.02 mg/kg also significantly reduced the mortality (Table 2). Although it did not showing statistical differences in the incidence of arrhythmias and mortality when compared with the control group, pretreatment with glibenclamide significantly increased the total number of VPC and VT during the ischemic period.

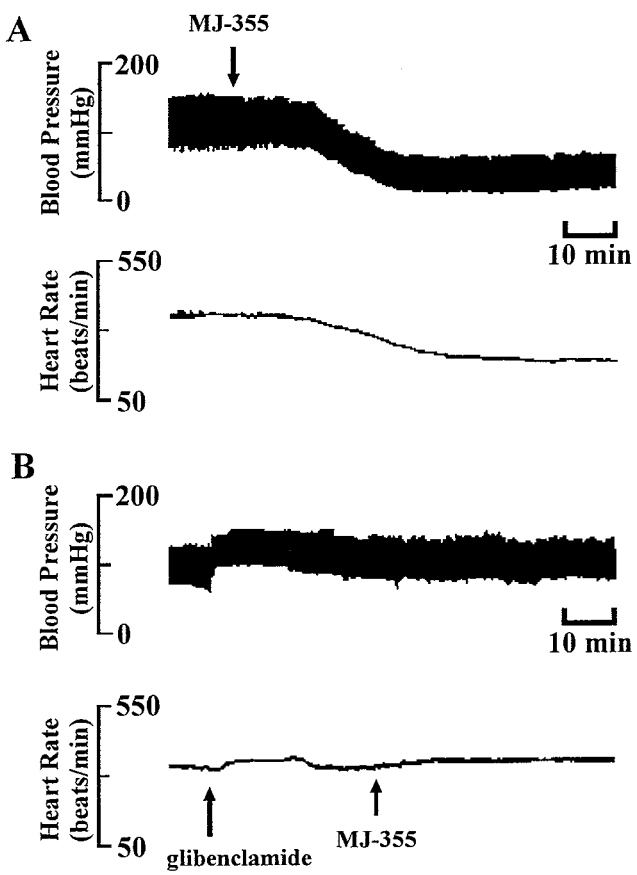


Fig. 3. The effects of MJ-355 on blood pressure and heart rate of SHR with or without pretreatment of glibenclamide. The traces represent the effect of MJ-355 (0.01 mg/kg, i.v. bolus) on blood pressure and heart rate in SHR (A) and the effect of pretreatment of glibenclamide (4 mg/kg, i.v. bolus) on changes in blood pressure and heart rate in response to MJ-355 (0.02 mg/kg, i.v. bolus) (B).

Infarct size

All experimental groups clearly demonstrated 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. 5). A significant reduction in infarct size, expressed as a percentage of the area at risk, was found in all of the MJ-355- and cromakalim-treated groups when compared with the control group (control: 38.7±3.3%; MJ-355 I-0.005: 20.2±2.1%, I-0.02: 16.4±2.3%, R-0.02: 20.7±2.7%; cromakalim: 20.3±2.6%; $P<0.05$ vs control). The infarct size was reduced to a similar extent among the drug-treated groups, except for the glibenclamide/MJ-355 group which was 40.6±7.9%. However, myocardial infarct size was significantly increased in the glibenclamide group (68.4±3.2%, $P<0.05$ vs control).

DISCUSSION

The present study was designed to evaluate the antihypertensive and cardioprotective effects of MJ-355, a newly synthesized benzopyran derivative, in rats. MJ-355 produced a dose-related reduction in mean arterial blood pressure with a delay of 10−15 min after administration. These results implied that MJ-355 should not be a direct vasodilator and the depressor effect of MJ-355 may be mediated through its active metabolite(s). Results from the in vitro study demonstrated that MJ-355 produced no vasodilatory effect in isolated rat aortic and mesenteric artery rings (data not shown). Interestingly, although MJ-355 produced a pronounced depressor effect, no reflex tachycardia was observed. In contrast, many other vasodilators elicit reflex tachycardia. The bradycardic effect of MJ-355 makes it a potential antihypertensive agent. However, the underlying mechanism of the bradycardia remains unknown. It may result from central suppression of the baroreflex or direct inhibition of cardiac tissue (e.g., SA node). Furthermore, pretreatment of glibenclamide abolished the hypotensive and bradycardic effects of MJ-355, suggesting that the hemodynamic changes were achieved via the opening of K_{ATP} channels.

MJ-355 possesses a cardioprotective efficacy against ischemia-reperfusion injury in the rat model. This cardioprotection of MJ-355 is unlikely mediated through its hemodynamic effects because during the ischemia and reperfusion period, MJ-355 did not significantly alter the mean arterial blood pressure, heart rate and RPP compared to the control group. Besides, the rat heart lacks functional collaterals (10), which precludes blood supply to the ischemic myocardium during the 45-min ligation in this model. Therefore, the antiarrhythmic effects of MJ-355 is most likely achieved directly at ventricular myo-

Table 1. Hemodynamic effects of MJ-355, cromakalim and glibenclamide during ischemic and reperfusion periods

	Baseline	After drug (20 min)	Ischemia (min)					Reperfusion (min)			
			0	5	10	20	30	0	20	40	60
MBP (mmHg)											
Control	86.0±3.5	86.0±2.0	54.0±4.0	59.0±2.5	63.5±3.5	64.0±4.0	65.5±4.0	67.5±2.5	67.5±4.0	65.5±2.5	65.0±4.0
Cromakalim	85.5±5.0	63.0±7.0*	48.0±5.0	56.0±6.0	66.0±4.0	67.5±4.0	66.0±6.0	71.0±3.5	72.0±3.5	71.5±4.0	72.0±3.0
MJ-355 (0.005 mg/kg)	86.5±4.0	76.0±2.0*	60.0±3.0	57.5±3.5	59.5±3.5	62.5±4.0	66.5±3.5	72.0±5.0	76.0±5.0	75.5±5.5	76.5±5.5
MJ-355 (0.02 mg/kg)	85.0±3.0	75.0±2.0*	55.0±3.0	56.0±6.0	67.5±4.0	70.0±3.0	68.5±6.0	74.5±5.5	74.0±5.5	73.0±6.0	72.0±6.5
Glibenclamide	88.0±5.6	88.5±4.7	61.0±6.0	78.5±6.5	78.0±5.5	78.5±6.0	77.5±5.4	74.6±5.0	75.0±4.6	77.2±5.2	75.5±6.5
HR (beats/min)											
Control	382±9	382±9	360±8	367±7	367±11	367±9	363±7	324±6	351±8	352±9	364±8
Cromakalim	386±8	372±11	334±27	330±28	332±27	333±27	332±23	327±24	325±28	326±27	325±24
MJ-355 (0.005 mg/kg)	384±13	372±7	378±11	381±11	384±14	387±11	385±13	389±13	401±14	411±13	411±15
MJ-355 (0.02 mg/kg)	382±10	357±7*	376±10	384±8	383±8	385±8	383±7	350±9	358±8	366±8	378±9
Glibenclamide	365±8	370±7	354±8.0	357±8	365±9	370±12	378±10	360±11	365±9	380±12	395±9
RPP/1000 (mmHg × beats/min)											
Control	45±3	45±3	30±2	34±2	35±2	35±2	35±2	38±2	38±3	38±3	37±3
Cromakalim	45±4	42±4	24±3	34±4	35±5	35±5	34±4	35±4	32±6	30±7	24±7
MJ-355 (0.005 mg/kg)	46±3	41±1	33±2	40±2	42±2	43±2	43±3	44±4	44±3	45±2	45±2
MJ-355 (0.02 mg/kg)	46±2	39±3	29±1	34±1	39±2	41±1	42±1	43±3	43±3	42±3	43±4
Glibenclamide	43±5	44±4	27±3	35±4	37±3	38±3	42±4	40±2	41±3	43±3	45±4

Cromakalim: 0.005 mg/kg; Glibenclamide; 4 mg/kg; MBP: mean arterial blood pressure; HR: heart rate; RPP: rate-systolic blood pressure product. Values are expressed as means±S.E.M.
*$P<0.05$ vs the control group.

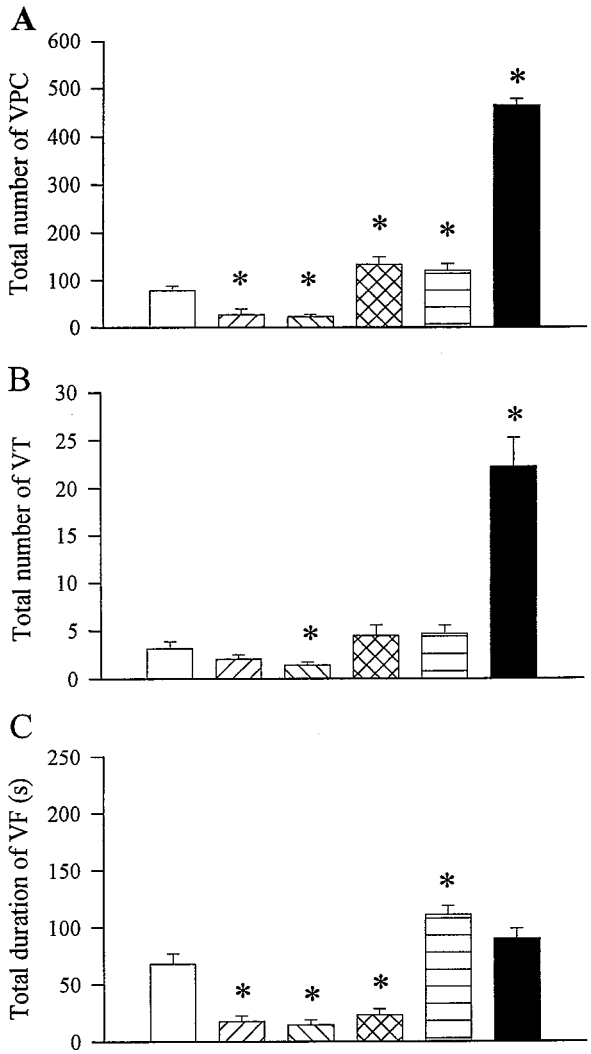


Fig. 4

Fig. 4. Effects of MJ-355, cromakalim and glibenclamide on ventricular arrhythmias in anesthetized SD rats. □: control, ▨: MJ-355 was given at 0.005 mg/kg prior to ischemia, ▧: MJ-355 was given at 0.02 mg/kg prior to ischemia, ▩: cromakalim 0.005 mg/kg was given before occlusion, ▤: glibenclamide (4 mg/kg) was pretreated 20 min before MJ-355 (0.02 mg/kg) injection in pre-ischemic period, ■: glibenclamide (4 mg/kg) was given prior to ischemia. The total number of ventricular premature contractions (VPC) (A) and ventricular tachycardia (VT) (B) and the total duration of ventricular fibrillation (VF) (C) are shown. Values are expressed as the mean±S.E.M., *$P<0.05$ vs the control group.

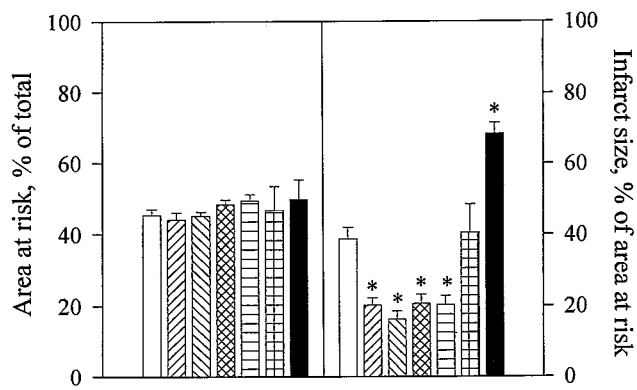


Fig. 5. Effects of MJ-355, cromakalim and glibenclamide on myocardial infarct size, expressed as a percentage of risk region, in SD rats undergoing 45 min of left coronary artery occlusion followed by 1 h of reperfusion. □: control, ▨: MJ-355 was given at 0.005 mg/kg prior to ischemia, ▧: MJ-355 was given at 0.02 mg/kg prior to ischemia, ▩: MJ-355 was given at 0.02 mg/kg prior to reperfusion, ▤: cromakalim (0.005 mg/kg) was given before occlusion, ▦: glibenclamide (4 mg/kg) was pretreated 20 min before MJ-355 (0.02 mg/kg) injection in pre-ischemic period, ■: glibenclamide (4 mg/kg) was given prior to ischemia. Values are expressed as the mean±S.E.M., *$P<0.05$ vs the control group.

Table 2. Effects of pretreatment with MJ-355, cromakalim and glibenclamide on the onset and incidence of ventricular arrhythmias and mortality following the left coronary artery occlusion in SD rats

Group	N	VPC		VT		VF		Mortality (%)
		onset (min)	incidence (%)	onset (min)	incidence (%)	onset (min)	incidence (%)	
Control	20	5.4±0.3	100	5.6±0.4	100	6.8±0.7	60	55
Cromakalim								
0.005 mg/kg	7	8.2±0.9*	100	8.3±0.8*	100	8.9±1.3	43	14*
MJ-355								
0.005 mg/kg	18	6.3±0.3	100	7.1±0.4	94	9.1±1.1	44	22
0.02 mg/kg	27	6.9±0.3	96	8.4±0.5*	74	9.6±0.8	26	11*
Glibenclamide								
4.0 mg/kg	7	4.2±0.4	100	4.3±0.8	100	6.3±0.8	57	43

N: number of rats, VPC: ventricular premature contractions, VT: ventricular tachycardia, VF: ventricular fibrillation. Values are expressed as the mean±S.E.M., *$P<0.05$ vs the control group.

cytes by suppressing the arrhythmogenic activity. This may be mediated through its metabolite(s). Recently, in a preliminary study, we found that an active metabolite of MJ-355, MJ-451 (6-cyano-3*S*,4*R*-dihydro-2,2-dimethyl-2*H*-3-hydroxy-4-[2-oxo-5*S*-(1-hydroxymethyl)-1-pyrrolidinyl]-1-benzopyran), shortened the duration of action potential in ventricular cells of guinea pig and possessed cardioprotective effects in rats of myocardial ischemia. In guinea pig isolated trachea, MJ-451 showed a marked relaxant activity, which also can be blocked by glibenclamide (11).

There is general agreement that K_{ATP} channel openers afford cardioprotection in myocardial ischemia. In the in vitro perfused rat heart subjected to transient global ischemia, administration of K_{ATP} channel openers before ischemia significantly improved the post-ischemic recovery of contractile function, increased time to ischemic contracture, enhanced reflow and reduced necrosis (12). In vivo studies showed that, when given prior to ischemia, the K_{ATP} channel openers bimakalim, pinacidil, cromakalim and aprikalim reduced infarct size (13–15). Also, our in vivo experiments confirmed that K_{ATP} channel openers indeed afforded protective effect against myocardial ischemia-reperfusion injury in anesthetized rats. Meanwhile, the protective effect of the K_{ATP} channel opener MJ-355 can be achieved by giving it after ischemia. The evidence that a K_{ATP} channel opener has a cardioprotective effect given at the reperfusion period is sparse. Mizumura et al. reported the similar result that the K_{ATP} channel opener bimakalim reduced infarct size in an anesthetized open-chest dog model when administered just before reperfusion (16).

Recently, K_{ATP} channel openers have received an increasing interest for their potential antiarrhythmic action. Since the identification of the K_{ATP} channels by Noma in cardiac cells (1), they play a role of a natural blocker. In the normally oxygenated heart, K_{ATP} channels are mainly in a closed state because of the high content of intracellular ATP. However, during myocardial ischemia, rapid activation of these channels generates a large repolarizing K^+ current to hyperpolarize the myocardium, leading to a decrease in voltage-dependent calcium current and myocardial contractility. Energy is conserved for the active extrusion of intracellular Ca^{2+} and Na^+ and mantains cell integrity and viability. On the other hand, numerous studies showed that K_{ATP} channel openers produced both antiarrhythmic (17, 18) and proarrhythmic activities (19–21). Interestingly, agents that block K_{ATP} channels also showed antiarrhythmic (22–24) and proarrhythmic activities (24, 25). The mechanisms of K_{ATP} channel modulators 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 (17, 26, 27), whereas they tend to exacerbate re-entry arrhythmias (19–21). On the contrary, blockers of K_{ATP} channels may alleviate reentrant arrhythmias (24, 25), whereas they may deteriorate triggered arrhythmias (27, 28). Results vary depending upon the condition of experiments. Furthermore, such a proarrhythmic effect of K_{ATP} channel openers may be dose-dependent. We also found that high dose of MJ-355 (0.05 mg/kg) evoked adverse rhythms such as VF and increased the mortality (88%) (data not shown).

In conclusion, MJ-355 is a potent and long-lasting antihypertensive agent. It not only suppressed ventricular arrhythmias induced by myocardial ischemia, but also reduced infarct size after reperfusion. Therefore, MJ-355 could be a potential therapeutic agent in the treatment of hypertension and acute myocardical infarction.

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