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Effects of KR-31378, a novel ATP-sensitive potassium channel activator, on hypertrophy of H9c2 cells and on cardiac dysfunction in rats with congestive heart failure

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Abstract

The present study was performed to evaluate the effects of (2S, 3S, 4R)-*N*″-cyano-*N*-(6-amino-3, 4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2H-1-benzopyran-4yl)-*N*′-benzylguanidine (KR-31378), a novel mitochondrial ATP-sensitive potassium channel activator, on hypertrophy of H9c2 cells and on cardiac dysfunction in rats with congestive heart failure. In rat heart-derived H9c2 cells treated with hypertrophic agonists, such as angiotensin II, phenylephrine, isoproterenol, and urotensin II, cell size was significantly increased by 27–47%. The increases in cell size induced by the hypertrophic agonists were inhibited by treatment of KR-31378 in a concentration-dependent manner. This was confirmed by the results showing that KR-31378 inhibited the angiotensin II-induced increase in cell protein content. The effect of KR-31378 on the angiotensin II-induced increase in cell size was reversed by mitochondrial ATP-sensitive potassium channel blockers, 5-hydroxydecanoate or glibenclamide. In rats with congestive heart failure, induced by permanent coronary artery occlusion for 8 weeks, KR-31378 significantly reversed the cardiac dysfunction (increase in ratios of stroke volume or cardiac output to body weight) induced by myocardial infarction without reducing infarct size. In addition, KR-31378 significantly inhibited atrial hypertrophy (decrease in ratio of right atrium to body weight) and decreased the serum pro-atrial natriuretic peptide level, a biochemical marker of heart failure. These results suggest that KR-31378 suppresses hypertrophy induced by hypertrophic agonists in H9c2 cells and improves cardiac dysfunction in rats with congestive heart failure induced by myocardial infarction, and that the effects may be mediated by the activation of mitochondrial ATP-sensitive potassium channels.

Keywords: Cell hypertrophy; Congestive heart failure; KR-31378; Myocardial infarction; ATP-sensitive potassium channel

1. Introduction

Congestive heart failure is a common clinical syndrome characterized by abnormalities of cardiac function and morphology. Despite impressive advances in diagnosis and therapeutics, congestive heart failure remains the leading cause of death worldwide. Cardiac hypertrophy represents the most important factor in the development of congestive heart failure, and is currently the subject of intense investigative interest. Recently, some experimental evidence has suggested that ATP-sensitive potassium channel opening on the sarcolemma (Noma, 1983) and the inner membrane of the mitochondria (Inoue et al., 1991) of cardiomyocytes is involved in the signal transduction pathway leading to cardiac hypertrophy (Sanada et al., 2002; Xia et al., 2004). The opening of sarcolemmal ATP-sensitive potassium channels protects H9c2 cells against chronic mild hypoxia (Crawford et al., 2003) and impairment at the level of sarcolemmal ATP-sensitive potassium channels induces heart failure in humans (Bienengraeber et al., 2004). However, although protection was initially thought to occur via the ATP-sensitive potassium channels in the sarcolemma of myocytes, several studies have shown that these channels in the mitochondrial inner membrane may play an important role in cardioprotection (Garlid et al., 1996, 1997; Liu et al., 1998) and may prevent mitochondrial Ca^{2+} overload

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(Holmuhamedov et al., 1999). Thus far, a number of investigators have found that several ATP-sensitive potassium channel activators, such as nicorandil, pinacidil, and diazoxide, possess cardioprotective and anti-arrhythmic effects during acute ischemia/reperfusion (Das et al., 2001; Rousou et al., 2004).

The compound (2S, 3S, 4R)-*N″*-cyano-*N*-(6-amino-3, 4-dihydro-2-dimethoxymethyl-3-hydroxy-2-methyl-2H-1-benzopyran-4yl)-*N′*-benzylguanidine, known as KR-31378, was synthesized at the Korea Research Institute of Chemical Technology (KRICT, Daejon, Korea) and is an attractive candidate for use as a mitochondrial ATP-sensitive potassium channel activator (Lee et al., 2001). KR-31378 has shown to significantly reduce myocardial infarction induced by ischemia/reperfusion in rats and dogs (Lee et al., 2001; Yoo et al., 2001). The anti-ischemic effects of KR-31378 have also been verified in focal ischemic brain damage in rats (Hong et al., 2002; Kim et al., 2004). In particular, KR-31378 has been reported to be a highly selective ATP-sensitive potassium channel activator without peripheral vasorelaxant and hypotensive activity, unlike other ATP-sensitive potassium channel activators (Lee et al., 2001). To date, although the acute effects of KR-31378, including its anti-ischemic action, have been well established, little is known concerning the long-term effects of this treatment against cardiac hypertrophy or congestive heart failure. Accordingly, we investigated whether KR-31378 directly blocks hypertrophy in H9c2 cells treated with hypertrophic agonists and improves cardiac dysfunction in rats with chronic heart failure induced by myocardial infarction, which has been extensively studied as a common cause of congestive heart failure in humans. In addition, we investigated whether the mitogen-activated protein kinase (MAPK)-dependent pathway in cellular hypertrophy induced by angiotensin II is involved in the anti-hypertrophic effects of KR-31378.

2. Materials and methods

2.1. Cell culture

H9c2 cells were purchased from the American Tissue Culture Collection (Manasas, VA, USA). Cells were subcultured weekly in 100-mm Corning dishes containing 10 ml Dulbecco's modified Eagle's medium (DMEM; Cambrex Bio Science, Walkersville, MD, USA) with 10% fetal bovine serum (FBS; Life Technologies, Rockville, MD, USA) and antibiotics (25 U/ml penicillin and 25 U/ml streptomycin). For the study of cellular hypertrophy, cells were seeded at a density of 3×10^4 cells per 35-mm well of six-well plates and cultured for 24 h in DMEM containing 10% FBS. The cells were washed with serum-free medium (DMEM without serum) and then treated with KR-31378 (3–30 μM), 5-hydroxydecanoate (100 μM), glibenclamide (50 μM), or a combination of these drugs under serum-free conditions for 24 h. Cells were treated with potential hypertrophic agonists, angiotensin II (100 nM), isoproterenol (1 μM), phenylephrine (10 μM), or urotensin II (100 nM), in quiescence medium (DMEM with 0.5% FBS) with or without a fresh supply of KR-31378, 5-hydroxydecanoate, glibenclamide, or a combination of these drugs. Cells were incubated for another 4 days at 37 °C in a humidified atmosphere containing 5% CO_2 to induce hypertrophic responses.

2.2. Cell area measurement

After washing with phosphate-buffered saline, adherent cells were fixed with 1% glutaraldehyde (Sigma, St. Louis, MO, USA) in phosphate-buffered saline for 30 min and stained with 0.1% Crystal violet (Sigma) for 10 min. Images were obtained using a digital camera attached to a Leica DMIL inverted microscope (Leica, Chatsworth, CA, USA) for analysis. Four random photographs were taken from each sample, and at least 70 individual cells were examined in each group. Cell size was analyzed using Image-Pro PLUS software (MediaCybernetics, Silver Spring, MD, USA). The data shown represent the image analysis from three independent experiments.

2.3. Protein content per cell measurement

Cells in 6-well plates were collected by scraping into 100 μl PRO-PREP™ protein extraction solution (Intron Biotechnology, Seoul, Korea). Protein concentrations were measured using Bradford protein assay kit (Bio Rad, Hercules, CA, USA). Cell protein content was determined by dividing the total amount of protein by the cell number, which was determined by a Coulter Counter (Beckman Coulter, Fullerton, CA, USA) after trypsinizing and fixing the cells.

2.4. Western blot analysis for MAP kinases

To investigate whether KR-31378 affects angiotensin II-induced MAPK activation, we examined the effects of three concentrations of KR-31378 (3, 10, and 30 μM) on angiotensin II-induced activation of c-Jun NH_2-terminal kinase (JNK1), p38 MAPK and extracellular signal-regulated protein kinase (ERK 1/2). After cell lysates from H9c2 cells treated with angiotensin II or isoproterenol for 30 min were prepared as described above, equal amounts of protein (30 μg) were loaded in 10% SDS-polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane (Hybond-C Extra, Amersham, Piscataway, NJ, USA). The membranes were blocked in 5% dry milk for 1 h, and then probed with primary rabbit anti-JNK1, p38 MAPK, or ERK 1/2 monoclonal antibody overnight. Following washing and probing with horse radish peroxidase-conjugated goat anti-rabbit immunoglobulin G antibody for 30 min, immunoreactive bands were detected by Western blotting kit (Pierce, Rockford, IL, USA). All antibodies were purchased from Cell Signaling (Beverly, MA, USA) and were used at a 1:1000 dilution.

2.5. Induction of heart failure by myocardial infarction in rats

This study conformed to the *Guidelines for the Care and Use of Laboratory Animals*, published by the U.S. National

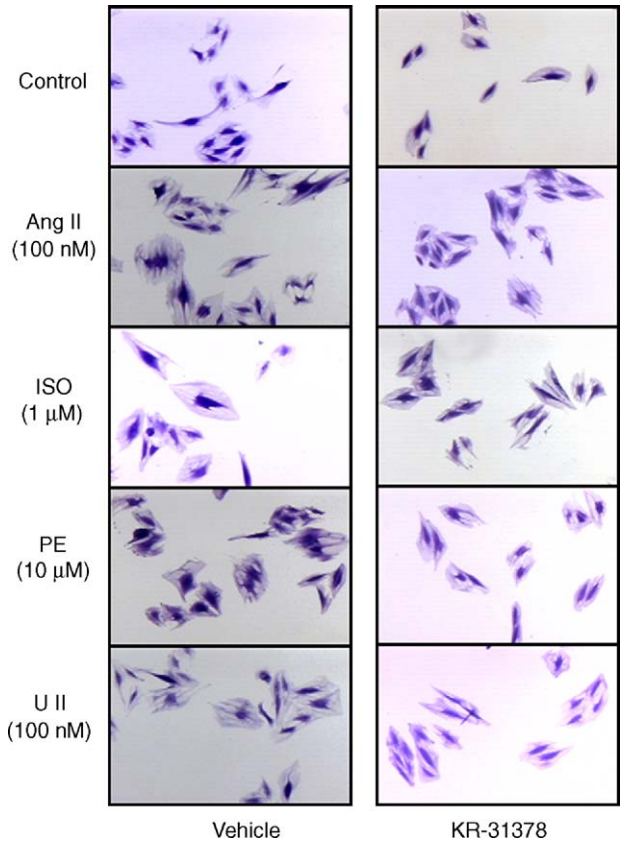

Fig. 1. Morphological changes of H9c2 myocytes indicating an increase in cell surface area after treatment with the hypertrophic agonists, angiotensin II (Ang II), isoproterenol (ISO), phenylephrine (PE) and urotensin II (U II), and the inhibitory effects of KR-31378.

Institutes of Health. Male Sprague–Dawley rats (body weight 300–360 g; Orient, Seoul, Korea) were anesthetized with ketamine (120 mg/kg, i.p.; Hanlim Pharm., Seoul, Korea), intubated and connected to a rodent ventilator (SAR 830/P ventilator, CWE, Ardmore, PA, USA) for artificial ventilation with ambient air (stroke volume, 10 ml/kg; 60 strokes/min) (Lee et al., 2001, 2005). Body temperature was maintained at 37 °C with a homeothermal blanket control unit. A left thoracotomy was performed and the heart was gently exposed. The heart was gently exteriorized by pressure on the chest, and a ligature (5-0 silk) was placed in position around the left anterior descending coronary artery; and the artery was either ligated or left untied. After surgery, all rats received metamizol (100 mg/kg, i.m.) for 2 days to prevent infection. The rats were randomly classified into five groups: sham-operated group, myocardial infarction followed by vehicle treatment, and myocardial infarction followed by KR-31378 treatment (3, 10, and 30 mg/kg). For KR-31378 treatment, an initial administration of the drug (3, 10, and 30 mg/kg, i.p.) was made 3 h after ligation. Twenty-four hours after surgery, all animals were treated with oral administration of either vehicle (0.5% carboxymethylcellulose) or KR-31378 (3, 10, and 30 mg/kg per day) for 8 weeks. To check for any increases in body weight, the animals were weighed every 7 days.

2.6. Measurement of cardiac functions in rats subjected to myocardial infarction

At 8 weeks after surgery, the rats were anesthetized with pentobarbital sodium (60 mg/kg i.p.; Hanlim Pharm). A 2F micronanometer-tipped catheter (SPR-838, Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle for measurement of left ventricular end-systolic pressure, left ventricular end-diastolic pressure, blood pressure, and heart rate. The rates of maximum positive and negative left ventricular pressure development ($+dP/dt_{max}$ and $-dP/dt_{max}$) were also determined. All data were stored and analyzed with a cardiac pressure–volume analysis program (PVAN 3.3, Millar Instruments) via a pressure–volume system (MPVS400, Millar Instruments). To determine stroke volume, cardiac output, and stroke work, a pressure–volume loop was generated by simultaneously recording the left ventricular pressure and the volume in the working heart.

2.7. Measurement of heart weight and serum pro-atrial natriuretic peptide levels in rats subjected to myocardial infarction

After hemodynamic measurements, a blood sample was taken from the right carotid artery for determination of the pro-atrial natriuretic peptide level. Thereafter, the animals were sacrificed, and the heart was quickly removed and weighed. The left (including septum) and right ventricles were separated and weighed. The serum pro-atrial natriuretic peptide level was determined using a commercial immunoassay kit (Biomedica, Vienna, Austria) according to the recommendations of the manufacturer.

2.8. Statistical analysis

All values are expressed as the mean±S.E.M., except the values from the H9c2 cell study which are expressed as the mean±S.D. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls

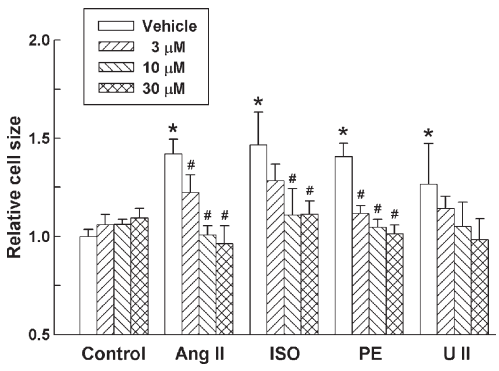

Fig. 2. Effects of KR-31378 on hypertrophy induced by angiotensin II (Ang II), isoproterenol (ISO), phenylephrine (PE) and urotensin II (U II) in H9c2 cells. Cell size was analyzed using Image-Pro PLUS software, and the values represent the relative area±S.D. ($n=3$). *$P<0.05$, significantly different from control group (no treatment). #$P<0.05$, significantly different from their respective vehicle group treated with hypertrophic agonist alone.

Table 1
Effects of KR-31378 on angiotensin II (Ang II)-induced hypertrophy of H9c2 cells

	Control	Vehicle (Ang II alone)	Ang II+KR-31378
Protein (μg/well)	53±3.5	65±4.9[a]	52±4.2[b]
Cells per well (× 10^4)	2.8±0.06	2.7±0.08	2.8±0.1
Protein per cell (ng)	1.89±0.12	2.41±0.16[a]	1.86±0.12[b]

Values are expressed as mean±S.D. (n=3).
[a] P<0.05, significantly different from control group (no treatment).
[b] P<0.05, significantly different from vehicle group (Ang II alone).

test for multiple comparisons (Sigma Stat, Jandel, San Rafael, CA, USA). In all comparisons, the difference was considered to be statistically significant at P<0.05.

3. Results

3.1. Effects of KR-31378 on cellular hypertrophy induced by hypertrophic agonists in rat-derived H9c2 cells

In control H9c2 cells treated with angiotensin II, phenylephrine, isoproterenol, or urotensin II, cell size was significantly increased by 1.42±0.08, 1.47±0.17, 1.41±0.07, or 1.27±0.21 times, respectively (Figs. 1 and 2). The increase in cell size induced by angiotensin II, phenylephrine, isoproterenol, or urotensin II was inhibited by pretreatment with KR-31378 in a concentration-dependent manner. In particular, cellular hypertrophy induced by angiotensin II or phenylephrine was significantly inhibited by 3 μM KR-31378 and completely blocked by a concentration of 10 μM. To confirm the image analysis results, the cell protein content of H9c2 cells was measured 4 days after induction of the hypertrophic response. As shown in Table 1, the protein content (2.41±0.16 ng) in angiotensin II-treated cell increased by 27.5% compared with that of the untreated controls (1.89±0.12 ng). In contrast, the protein content (1.86±0.12 ng) in 10 μM KR-31378-treated cell

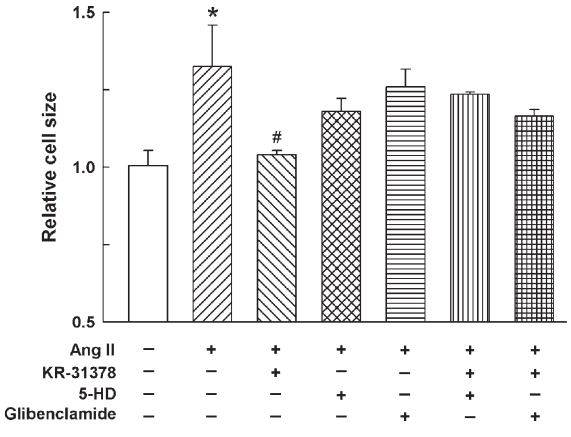

Fig. 3. Antagonistic activities of ATP-sensitive potassium channel blockers 5-hydroxydecanoate (5-HD) and glibenclamide on the effects of KR-31378 in H9c2 cells with hypertrophy induced by angiotensin II (Ang II). Values are expressed as mean±S.D. for all groups (n=3). *P<0.05, significantly different from control group (no treatment). #P<0.05, significantly different from their respective vehicle group treated with hypertrophic agonist alone.

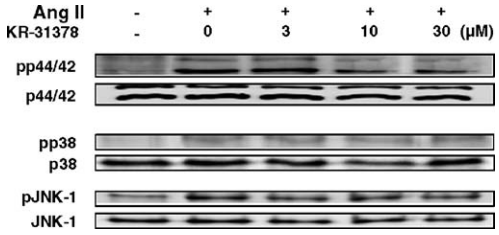

Fig. 4. Effects of KR-31378 on phosphorylated mitogen-activated protein kinases (MAPKs) induced by angiotensin II (Ang II). Equivalent amounts of protein were used for Western blot analysis with total extracellular signal-regulated protein kinase (ERK 1/2), c-Jun NH_2-terminal kinase (JNK1), and p38 MAPK antibodies. The data represent the results from a minimum of three separate experiments.

reverted almost to the untreated control level. As shown in Fig. 3, the mitochondrial ATP-sensitive potassium channel blockers, 5-hydroxydecanoate and glibenclamide (Jaburek et al., 1998), either partially or completely reversed the inhibitory effects of KR-31378 on cellular hypertrophy induced by angiotensin II.

3.2. Inhibitory effects of KR-31378 on angiotensin II-induced MAPK activation

As shown in Fig. 4, angiotensin II caused the rapid activation of JNK1, p38 MAPK, and ERK 1/2 after 30 min without affecting their total levels in H9c2 cells. KR-31378 inhibited the angiotensin II-induced activation of ERK 1/2 in a concentration-dependent manner. The observed inhibitory effects of KR-31378 on angiotensin II-induced pERK 1/2 activation were not attributable to the decrease in total ERK 1/2 protein levels. In contrast, KR-31378 did not block angiotensin II-induced p38 MAPK or JNK1 activation.

3.3. Effects of KR-31378 on myocardial infarction-induced cardiac hypertrophy in rats

Table 2 shows the time course for change of body weight at 0–8 weeks after ligation of the left anterior descending coronary artery in the rats. Body weight was not significantly different among all groups, although there was a trend for body weight to be slightly less in the group treated with 30 mg/kg KR-31378.

Table 2
Changes of body weight in rats with congestive heart failure induced by myocardial infarction

Week	Sham-operated (n=21)	Vehicle (n=21)	KR-31378		
			3 mg/kg (n=21)	10 mg/kg (n=18)	30 mg/kg (n=22)
0	331±7.7	334±4.6	341±3.7	336±4.3	331±5.0
1	366±8.7	346±4.9	352±2.9	346±4.9	341±4.7
2	395±10.1	372±5.3	379±4.3	370±5.6	363±5.5
3	410±13.1	398±5.7	403±5.7	393±6.2	381±6.2
4	425±15.3	417±6.5	424±6.5	418±7.4	399±7.2
5	440±16.3	436±7.3	442±7.3	435±8.3	414±7.8
6	453±17.1	451±7.9	456±8.0	452±9.1	429±8.7
7	464±17.7	464±7.5	472±8.5	468±10.1	442±9.1
8	479±7.7	483±8.7	488±8.9	485±11.2	459±9.9

Values are expressed as mean±S.E.M.

Table 3
Effects of KR-31378 on cardiac morphometric parameters in rats with congestive heart failure induced by myocardial infarction

	Sham-operated (n=21)	Vehicle (n=21)	KR-31378		
			3 mg/kg (n=21)	10 mg/kg (n=18)	30 mg/kg (n=22)
HW, g	1.202±0.021	1.274±0.023	1.247±0.029	1.235±0.027	1.185±0.026
HW/BW, g/kg	2.511±0.033	2.638±0.035	2.572±0.044	2.551±0.042	2.585±0.033
Scar weight, g	–	0.179±0.01	0.177±0.008	0.168±0.005	0.166±0.01
Scar weight/BW, g/kg	–	0.373±0.023	0.376±0.018	0.356±0.012	0.370±0.025
LAW, g	0.036±0.001	0.049±0.002 [a]	0.047±0.003	0.054±0.003	0.046±0.002
LAW/BW, g/kg	0.076±0.002	0.102±0.003 [a]	0.097±0.005	0.110±0.006	0.099±0.004
RAW, g	0.058±0.003	0.068±0.003	0.060±0.003	0.058±0.003	0.059±0.003
RAW/BW, g/kg	0.120±0.005	0.141±0.004 [a]	0.123±0.006 [b]	0.120±0.004 [b]	0.127±0.005 [b]
LVW, g	0.893±0.015	0.940±0.018	0.918±0.021	0.905±0.018	0.873±0.019
LVW/BW, g/kg	1.867±0.026	1.947±0.029	1.894±0.033	1.872±0.033	1.907±0.027
RVW, g	0.214±0.005	0.216±0.004	0.222±0.005	0.218±0.006	0.207±0.005
RVW/BW, g/kg	0.447±0.009	0.448±0.008	0.458±0.008	0.450±0.009	0.452±0.009

Values are expressed as mean±S.E.M. BW, body weight; HW, heart weight; LAW, left atrium weight; LVW, left ventricular weight; RAW, right atrium weight; RVW, right ventricular weight.
[a] $P<0.05$, significantly different from sham-operated group.
[b] $P<0.05$, significantly different from vehicle group.

As shown in Table 3, the ratios of scar weight to body weight were similar in all experimental groups. The ratios of heart weight to body weight, left ventricle to body weight, and right ventricle to body weight were not significantly different among all groups. However, the ratios of left atrium to body weight and right atrium to body weight in the vehicle-treated group (0.102±0.003 and 0.141±0.004 g/kg, respectively, $P<0.05$) were significantly greater than those in the sham-operated group (0.076±0.002 and 0.120±0.005 g/kg, respectively). In particular, the increase in the ratio of right atrium to body weight induced by ligation of the left anterior descending coronary artery (vehicle group) was significantly inhibited by treatment with KR-31378 (0.123±0.006, 0.120±0.004, and 0.127±0.005 at 3, 10, and 30 mg/kg, respectively, $P<0.05$).

3.4. Effects of KR-31378 on myocardial infarction-induced cardiac dysfunction in rats

The hemodynamic data obtained 8 weeks after surgery are summarized in Table 4. The stroke volume, stroke work, and left ventricular contractility ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) in the vehicle group were significantly decreased compared with those in the sham-operated group. The decreases in these parameters were not significantly inhibited by treatment with KR-31378. However, the decrease of cardiac output in vehicle group (33.4±2.07 ml/min) as compared with that in the sham-operated animals (44.3±1.14 ml/min) was significantly improved by treatment with 30 mg/kg KR-31378 (40.7±2.30 ml/min, $P<0.05$). Furthermore, as shown in Fig. 5, the indexes of stroke volume and cardiac output were significantly reduced in the vehicle group (0.179±0.011 and 69.3±4.32 ml/min/kg, respectively, $P<0.05$) as compared with those of the sham-operated group (0.233±0.008 and 93.3±3.35 ml/min/kg, respectively), which were recovered by treatment with KR-31378 in a dose-dependent manner (0.229±0.015 and 90.5±6.37 ml/min/kg at 30 mg/kg, respectively, $P<0.05$). No significant differences were observed in heart rate, left ventricular end-systolic pressure and left ventricular end-diastolic pressure.

3.5. Effects of KR-31378 on serum pro-atrial natriuretic peptide in rats with heart failure

As shown in Fig. 6, the serum pro-atrial natriuretic peptide level in the vehicle-treated group (1216±36.8 fmol/ml) was

Table 4
Effects of KR-31378 on hemodynamic parameters in rats with congestive heart failure induced by myocardial infarction

	Sham-operated (n=21)	Vehicle (n=21)	KR-31378		
			3 mg/kg (n=21)	10 mg/kg (n=18)	30 mg/kg (n=22)
HR, b/m	401±4.8	385±7.6	395±6.5	395±8.3	394±5.7
LVSP, mm Hg	136±2.5	133±2.6	131±3.0	134±3.4	128±3.0
LVEDP, mm Hg	6.73±0.47	7.79±0.38	6.71±0.32	7.37±0.39	7.34±0.35
SV, ml	0.111±0.002	0.086±0.005 [a]	0.094±0.006	0.095±0.004	0.103±0.006
CO, ml/min	44.3±1.14	33.4±2.07 [a]	36.8±1.97	37.0±1.18	40.7±2.30 [b]
SW, mm Hg ml	11.1±0.52	8.5±0.63 [a]	9.1±0.72	8.9±0.53	9.9±0.55
LV + dP/dt_{max}, mm Hg/s	10,123±233	8093±270 [a]	7937±273	7859±181	8269±312
LV − dP/dt_{max}, mm Hg/s	−10,308±243	−7676±273 [a]	−7550±245	−7487±189	−7725±280

Values are expressed as mean±S.E.M. HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; SV, stroke volume; CO, cardiac output; SW, stroke work; LV + dP/dt_{max}, positive left ventricular contractility; LV − dP/dt_{max}, negative left ventricular contractility.
[a] $P<0.05$, significantly different from sham-operated group.
[b] $P<0.05$, significantly different from vehicle group.

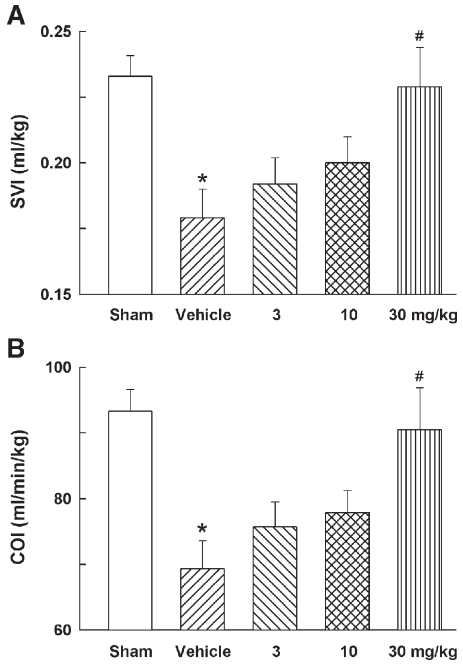

Fig. 5. Effects of KR-31378 on (A) stroke volume index (SVI) and (B) cardiac output index (COI) in rats with congestive heart failure induced by myocardial infarction. Values are expressed as mean±S.E.M. (n=18–22). *P<0.05, significantly different from the sham-operated group. $^{\#}P$<0.05, significantly different from the vehicle group.

significantly increased as compared with that in the sham-operated group (910.9±44.2 fmol/ml, P<0.05), and it was significantly attenuated with treatment of 30 mg/kg KR-31378 (1078.4±34.2 fmol/ml, P<0.05).

4. Discussion

This study demonstrates that KR-31378, a novel ATP-sensitive potassium channel activator, has a potent inhibitory effect against hypertrophy of H9c2 cells and cardiac dysfunction in rats with congestive heart failure induced by myocardial infarction.

In heart-derived H9c2 cell study, hypertrophic agonists, such as angiotensin II, phenylephrine, isoproterenol, and urotensin II, significantly increased cell size by 27–47%, which is in line with previously reported results (Huang et al., 2004). KR-31378 significantly inhibited the increase in cell size induced by all the types of hypertrophic agonists tested in the present study in a concentration-dependent manner (Fig. 2). This result was confirmed again by showing that KR-31378 reversed the angiotensin II-induced increase in cell protein content to the control level (Table 1). These results suggest that KR-31378 has potent anti-hypertrophic effects in H9c2 cells. The results of the present study also suggest that hypertrophic growth involves an intricate web of interconnected signaling pathways and that all these stimuli share common signaling cascades that could be attenuated by KR-31378. To verify whether the anti-hypertrophic effects of KR-31378 are mediated by ATP-sensitive potassium channel opening, we further investigated the potential effects of KR-31378, 5-hydroxydecanoate, glibenclamide, or a combination of these drugs on angiotensin II-induced hypertrophy of H9c2 cells. Both 5-hydroxydecanoate and glibenclamide either partially or completely blocked the effects of KR-31378 against angiotensin II-induced cellular hypertrophy (Fig. 3). These results suggest that the anti-hypertrophic effects of KR-31378 on H9c2 cells might be mediated by the opening of mitochondrial ATP-sensitive potassium channel, although it still remains controversial that 5-hydroxydecanoate selectively blocks mitochondrial ATP-sensitive potassium channel but not sarcolemmal ATP-sensitive potassium channels in cardiomyocytes and that mitochondrial ATP-sensitive potassium channel is likely to be mediators of cardioprotection (Hanley and Daut, 2005).

The exact signaling mechanisms leading to cardiac hypertrophy are not fully understood. Therefore, we examined the activation of the different MAPK cascades by angiotensin II and the potential action of KR-31378. In agreement with the study of Li et al. (2005), angiotensin II caused the rapid activation of JNK1, p38 MAPK, and ERK 1/2 after 30 min without affecting their total levels in H9c2 cells. In particular, KR-31378 inhibited the activation of ERK 1/2 induced by angiotensin II in a concentration-dependent manner. Although it has been difficult to determine whether the action of KR-31378 on ERK phosphorylation is a direct effect or a consequence of reduced intracellular Ca^{2+} overload, KR-31378 showed inhibitory effects on ERK phosphorylation. These findings suggest that the ERK 1/2 signaling pathway plays a pivotal role in the anti-hypertrophic effects of KR-31378 in H9c2 cells with angiotensin II-induced hypertrophy.

In rat models with chronic heart failure induced by permanent coronary artery occlusion for 8 weeks, the ratio of scar weight to body weight was similar in all experimental groups, indicating that all groups had the same potential for ischemic damage (infarct size) as a result of left anterior descending coronary artery occlusion. In the present study, the stroke volume and cardiac output indexes were significantly reduced in the rats with chronic heart failure induced by myocardial infarction (vehicle group) as compared with those of the sham-operated group, and that was recovered by treatment with KR-31378 in a dose-dependent manner. These results suggest that KR-31378 may improve cardiac function by increasing the stroke volume and cardiac output indexes,

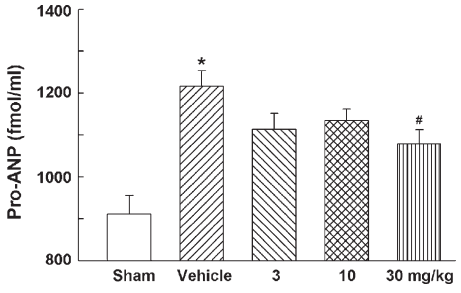

Fig. 6. Effects of KR-31378 on serum pro-atrial natriuretic peptide levels in rats with congestive heart failure induced by myocardial infarction. Values are expressed as mean±S.E.M. (n=18–22). *P<0.05, significantly different from the sham-operated group. $^{\#}P$<0.05, significantly different from the vehicle group.

without reducing infarct size (Table 4). Although the ratio of ventricle to body weight was not significantly different among all groups, the ratio of atrium to body weight in the vehicle-treated group was significantly increased as compared with that in the sham-operated group (Table 3), which is in good agreement with previous studies (We et al., 2002; Martinez and Villalobos-Molina, 2003). In the present study, KR-31378 significantly inhibited the increase in ratio of atrium to body weight (Table 3) and the increase in pro-atrial natriuretic peptide levels induced by heart failure in rats (Fig. 6). An increase in the plasma concentration of atrial natriuretic peptide in congestive heart failure has been reported to result from an increase in atrial natriuretic peptide secretion from the atria and ventricles caused by chronic stretching (Matsubara et al., 1990; Comini et al., 1995). Recently, it has been noted that an increase in atrial natriuretic peptide secretion in patients with atrial fibrillation is associated with atrial volume (Wozakowska-Kaplon and Opolski, 2005). These results suggest that inhibition of right atrial hypertrophy by KR-31378 may be attributable to an inhibition of increase in the size of its constituent cells induced by atrial fibrillation (Fang et al., 2003; Hagens et al., 2005).

Taken together, the results from the present study suggest that KR-31378 inhibits hypertrophy induced by the hypertrophic agonists in H9c2 cells and improves cardiac dysfunction in rats with congestive heart failure induced by myocardial infarction. Its effects might be mediated through the activation of mitochondrial ATP-sensitive potassium channels. KR-31378 could be potentially useful in the prevention and treatment of congestive heart failure.

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