Antihypertensive Actions of Methylripiaiochromene A from *Orthosiphon aristatus*, an Indonesian Traditional Medicinal Plant

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Methylripiaiochromene A (MRC) was isolated from the leaves of *Orthosiphon aristatus* (Lamiaceae) and subjected to the examination of several pharmacological actions related to antihypertensive activity. The following four findings were revealed from the present study: 1) MRC caused a continuous decrease in systolic blood pressure and a decrease in heart rate after subcutaneous administration in conscious male SHRSP; 2) MRC exhibited the concentration-dependent suppression of contractions induced by high K\(^+\), l-phenylephrine or prostaglandin E\(_{2\alpha}\) in endothelium-denuded rat thoracic aorta, 3) MRC showed a marked suppression of contractile force without a significant reduction in the beating rate in isolated bilateral guinea pig atri, and 4) MRC increased urinary volume and the excretion of Na\(^+\), K\(^+\) and Cl\(^-\) for 3 h after oral administration with a load of saline in fasted rats. These findings indicate that MRC possesses some actions related to a decrease in blood pressure, i.e. vasodilating action, a decrease in cardiac output and diuretic action. Furthermore, it is presumed that the traditional use of this plant in the therapy of hypertension may be partially supported by these actions with MRC.

Key words methylripiaiochromene A, Orthosiphon aristatus, antihypertension, vasodilation, cardiac output, diuretic

The leaves of *Orthosiphon aristatus* (Bl.) Mo. (Lamiaceae, "kumis kucing in Javanese), has been prescribed in traditional Indonesian medicine (jamu) for the treatment of hypertension and diabetes. Some investigators have reported the chemical constituents of this medicinal plant. However, pharmacological studies of these constituents have barely been carried out, although Schut and Zwaving have reported that two flavonoids, i.e. sinensetin and 3’-hydroxy-5,6,7,4’-tetra-methoxyflavone, exhibit diuretic activity. We recently performed chemical studies of the chloroform-soluble portion from the water decoction of the leaves, and isolated one new benzochromene (orthochromene A), two new isopimarane-type diterpenes (orthosiphonones A and B), together with four known compounds, methylripiaiochromene A (MRC), acetovanillochromene, and orthosiphons A and B. In this paper we studied several pharmacological actions related to the antihypertensive activity of MRC (Fig. 1), which was a major chemical component, with 2.3% yield from the water decoction of the leaves. It is generally established that blood pressure is closely related to peripheral resistance, cardiac output, and plasma and extracellular fluid volume. So, we examined the suppressive effects of MRC on contractions induced by three agonists in isolated rat thoracic aorta, and on the contractile force and the beating rates in isolated guinea pig atrial preparations. Furthermore, we studied the diuretic action closely related to plasma and extracellular fluid volume in rats.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 250—300 g (Japan SLC, Inc., Hamamatsu) and male Hartley guinea pigs weighing about 400 g (Japan SLC, Inc., Hamamatsu) were used for the present study. Stroke-prone spontaneously hypertensive rats (SHRSP) were originally provided from Dr. Okamoto at Kinki University and bred at our institute. The animals were housed under the following environmental conditions: temperature of 24±2 °C, humidity of 55±10% and a 12 h light/dark cycle, and they were given commercial food and tap water ad libitum.

Materials MRC was prepared from *Orthosiphon aristatus* purchased at Yogyakarta in Indonesia, and purified in our laboratory (purity >99%). This compound was suspended in 0.5% Tween 80 solution by means of an ultrasonic homogenizer (US-150, Nihonseiki). The following chemicals were used: prostaglandin E\(_{2\alpha}\) (PGF\(_{2\alpha}\)), l-phenylephrine hydrochloride, papaverine hydrochloride and dimethyl sulfoxide (Wako Pure Chemical Industries, Ltd., Osaka); dl-propanolol and hydrochlorothiazide (Sigma Chemical, St. Louis, U.S.A.); Tween 80 (Kanto Chemical Co., Inc., Tokyo); acetylcarnine chloride (ACH) (Ovisor\(^{1,2}\), Daiichi Pharmaceutical Co., Ltd., Tokyo). All other chemicals were of the highest grade available.

Effects of MRC on Systolic Blood Pressure and Heart Rate in Conscious SHRSP Male 13 week-old SHRSP were used in this study. MRC was subcutaneously administered at doses of 50 mg/kg and 100 mg/kg to 8 animals in each group, and 0.5% Tween 80 solution as a vehicle control was given to 9 animals in the same manner. Systolic blood pressure and heart rate were measured at 1, 3.5, 6, 8.5, 24 and 48 h after the administration by a tail-cuff method (BP monitor, UR-5000, Ueda Avancer Corp., Tokyo) with pre-heating at 37 °C for 12 min.

Fig. 1. Chemical Structure of Methylripiaiochromene A from *Orthosiphon aristatus* © 1999 Pharmaceutical Society of Japan
Effects of MRC on Contractile Responses Induced by High K\(^+\), l-Phenylenephrine or PGF\(_{2\alpha}\) in Isolated Rat Thoracic Aorta  Male Wistar rats were sacrificed and their thoracic aorta were isolated. Each aorta spiral strip (approx. 2.5 mm x 20 mm) was prepared following the removal of connective tissue, and suspended in a 30 ml organ bath filled with Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.55 mM CaCl\(_2\), 2 mM KH\(_2\)PO\(_4\), 1.18 mM MgSO\(_4\), 7 mM H\(_2\)O, 1.18 mM KH\(_2\)PO\(_4\), 11.1 mM NaHCO\(_3\), 1.11 mM glucose) maintained at 37 °C. The solution was continuously aerated with a gas mixture of 95% O\(_2\) and 5% CO\(_2\). A resting tension of 0.5 g was applied to each preparation and the contractile force of its spontaneous movement was measured under an isometric condition by means of a force displacement transducer (TB-652T, Nihon Kohden) connected to an amplifier (EF-601G, Nihon Kohden), and the beating rate was determined by a heart rate counter (AT-601G, Nihon Kohden) coupled to the contractile responses. These atrial movements were described on a thermal pen recorder. After the spontaneous contractile force and the beating rate became constant, MRC was cumulatively applied. The contractile force obtained before the treatment with MRC was taken as 100%.

Effects of MRC on Urinary Volume and Electrolyte Excretion in Rats Five groups of 5 Wistar rats were employed. Food and water were not given to the rats for 18 h and 3 h before the administration of test materials, respectively. MRC suspended in 0.5% Tween 80 was administered orally at three dose levels of 25, 50 and 100 mg/kg in a volume of 5 ml/kg, immediately followed by the oral administration of 20 ml/kg of saline to hydrate the animals. The rats were individually placed in metabolic cages, and their urine was collected for the ensuing 3 h, followed by the measurement of urine volume. The urine was subjected to the determination of Na\(^+\), K\(^+\) and Cl\(^-\) concentrations using an automatic electrolyte analyzer (Dri-chem 800V, Fuji Medical System Co., Ltd., Tokyo). The urinary excretion of each electrolyte was calculated from the volume and concentration of the urine, and represented by a value per kg of body weight. The intake of food and water was restricted during the collection of urine. Hydrochlorothiazide (HCT) (25 mg/kg) as a positive compound and 0.5% Tween 80 solution as a vehicle were also tested in the same manner.

Statistics Data are expressed as the means±S.E. Comparison of mean values between the two groups was performed by the unpaired Student’s t-test. Statistical analysis among more than three groups was evaluated using one-way analysis of variance (ANOVA) followed by Dunnnett’s post-hoc test. All statistical analyses were carried out using Stat View™ (Abacus Concepts, Inc., Berkeley, CA). A probability (p) value of less than 0.05 was considered to be significantly different.

RESULTS

Effects of MRC on Systolic Blood Pressure and Heart Rate in Conscious SHRSP At the beginning of the experiment, the systolic blood pressures of SHRSP divided into three groups receiving 50 and 100 mg/kg of MRC and the vehicle were 231±3, 239±3 and 236±3 mmHg, respectively. MRC caused continuous decreases in a dose-dependent manner. A decrease of 15 to 30 mmHg in the mean blood pressure was observed at 3.5 to 24 h after the subcutaneous administration at 100 mg/kg (p<0.05 or p<0.01), whereas no changes were observed in the control group (Fig. 2, A). MRC at 50 mg/kg caused a significant fall only at 8 h (p<0.05). The initial values of heart rate of the MRC groups and the control group were 334±4 (at 50 mg/kg), 340±7 (at 100 mg/kg) and 345±4 beats/min, respectively. MRC exerted significant (p<0.01) reductions of 75 and 45 beats/min only at 6 and 8.5 h, respectively, after dosing at 100 mg/kg (Fig. 2, B). The reduced changes in heart rate were recovered at 24 h,
 Unlike the blood pressure. A slight decrease in heart rate was observed only at 6 h after dosing with 50 mg/kg (p<0.01). The vehicle did not affect the heart rate during an examination of 48 h.

**Effects of MRC on Contractile Responses to High K⁺, l-Phenylephrine or PGF₂α in Isolated Rat Thoracic Aorta**

MRC was administered at doses of 50 mg/kg (A) and 100 mg/kg (B) to 8 animals in each group. The vehicle (0.5% Tween 80) was similarly given to 9 animals (C). Each point is expressed as the mean±S.E. of changes from the initial values. *p<0.05, **p<0.01, significantly different from the corresponding value in the vehicle control group on the respective time (Dunnet's multiple comparison).

Fig. 2. Time Courses of the Changes in Systolic Blood Pressure (A) and Heart Rate (B) after Subcutaneous Administration of Methylripari-ochromene A (MRC) in Conscious SHRSP.

Fig. 3. Relaxation by Methylripari-ochromene A on High K⁺, l-Phenylephrine or PGF₂α-Induced Precontractions in Rat Thoracic Aorta

The test compound was cumulatively applied to the preparations after contractions induced by 60 mm K⁺ (C), 3×10⁻³ m l-phenylephrine (D) or 10⁻³ m PGF₂α (E). The relaxation induced by 10⁻⁴ m papaverine was taken as 100%. Each point indicates the mean±S.E. of 4 experiments.

Unlike the blood pressure. A slight decrease in heart rate was observed only at 6 h after dosing with 50 mg/kg (p<0.01). The vehicle did not affect the heart rate during an examination of 48 h.

**Effects of MRC on Contractile Responses to High K⁺, l-Phenylephrine or PGF₂α in Isolated Rat Thoracic Aorta**

MRC at 1.1×10⁻⁴ M caused potent dilating action on high K⁺-induced contractions in the endothelium-denuded thoracic aorta strips, whereas changes were hardly observed on 10⁻⁵ M PGF₂α-induced contractions by a similar treatment with MRC. Figure 3 shows the concentration-response curves of suppressive actions on contractile responses to 60 mm KCl, 3×10⁻³ M l-phenylephrine or 10⁻³ M PGF₂α after the cumulative applications of 3.8×10⁻³ M to 1.1×10⁻⁴ M MRC. The IC₅₀ values of MRC on suppressive actions to the contractions induced by high K⁺, l-phenylephrine and PGF₂α were 0.83(±0.08)×10⁻⁴, 1.39(±0.17)×10⁻⁴ and 4.67(±0.25)×10⁻⁴ M, respectively.

Fig. 4. Effects of Methylripari-ochromene A(MRC) on the Contractile Responses to 72.2 mm K⁺ and Ca²⁺ (0.3–30 mm) in Rat Thoracic Aorta Strips Exposed to Ca²⁺-Free Medium.

Each point represents the mean±S.E. The effects of MRC were tested at concentrations of 1.1×10⁻⁵ M (○, n=5), 3.8×10⁻⁵ M (▲, n=5) and 1.1×10⁻⁴ M (△, n=3). The final concentration of Tween 80 as a vehicle was 0.0002% (□, n=12). The contractile response to 72.2 mm K⁺ before the exchange to Ca²⁺-free medium was taken as 100%.

**Effects of MRC on Contractile Responses to High K⁺ and Ca²⁺ in Rat Thoracic Aorta Exposed to Ca²⁺-Free Medium**

After treatment with previous exposure to Ca²⁺-free medium, MRC at 1.1×10⁻⁵ M, 3.8×10⁻⁵ M and 1.1×10⁻⁴ M restrained Ca²⁺-induced contractions of the rat thoracic aorta stimulated by excess K⁺ in a concentration-dependent manner (Fig. 4). Maximal contractions by 30 mm Ca²⁺, used as the highest concentration, were decreased to 73.8%, 47.0% and 21.0% after pretreatment with 1.1×10⁻⁵, 3.8×10⁻⁵ and 1.1×10⁻⁴ M MRC, respectively.

**Effects of MRC on Spontaneous Contraction of Isolated Guinea Pig Atria**

MRC decreased the contractile force of spontaneously moving atria after cumulative applications of 3.8×10⁻⁵ M and 1.1×10⁻⁴ M. The expressed changes were 18.8±2.6% (p<0.05) and 54.7±2.8% (p<0.01) by the low and high concentrations, respectively (Fig. 5). On the other hand, the beating rate was not significantly affected at either concentration, while slight reductions were observed in a part of preparations treated with the high con-
concentration. Propranolol at $10^{-5} \text{M}$ as a positive compound exerted significant suppressive action on both the contractile force and beating rate.

**Effects of MRC on Urinary Volume and Electrolyte Excretion in Rats** Urinary volume was increased about 3 times in 3 h after the oral administration of 100 mg/kg MRC ($p<0.01$), while it was not affected at 25 mg/kg (Table 1). At the middle dosage (50 mg/kg), an elevated tendency of the urinary volume was observed to some extent without statistical significance. On the other hand, the concentrations of $\text{Na}^+$, $\text{K}^+$ and $\text{Cl}^-$ in urine were not changed. Excretions of the three electrolytes determined by calculation were necessarily increased at 100 mg/kg ($p<0.05$ or $p<0.01$). HCT (25 mg/kg) as a positive control showed marked increases in the parameters of urinary volume, electrolyte excretion and $\text{Na}^+$ and $\text{Cl}^-$ concentration.

**Effects of Two Benzochromes on High $\text{K}^+$-Induced Contractile Responses in Isolated Rat Thoracic Aorta** Acetovanillochromene and orthochromene A caused potent dilating action on high $\text{K}^+$-induced contractions in the endothelium-denuded thoracic aorta strips. $IC_{50}$ values with these compounds were $1.01 \times 10^{-4} \text{M}$ and $1.32 \times 10^{-4} \text{M}$, respectively.

**DISCUSSION**

Javanese traditional medicines, called “jamu”, have brought benefits in the therapy of many kinds of disease.\(^{12}\) We have investigated the pharmacological property of a major chemical constituent (MRC, 2.3% yield from the water decoction) in the leaves of *Orthosiphon aristatus* ("kumis kucing" in Javanese), which is a popular jamu raw material used mainly to treat hypertension and diabetes.\(^{9}\) The present study certainly demonstrated that MRC caused continuous decreases in systolic blood pressure and heart rate after subcutaneous administration in conscious SHRSP. It is well known that blood pressure is reduced by a dilation of blood vessels and/or a decrease in cardiac output.\(^{10}\) so we investigated several cardiovascular actions expected to contribute to a decrease in blood pressure.

MRC exerted suppressive actions to contractions induced by three agonists in rat thoracic aorta. MRC exhibited high suppressive potency against three agonist-induced contractions in order of high $\text{K}^+$-$\alpha$-phenylephrine > PGF$_{2\alpha}$. It has been reported that a vasodilator exhibiting this order with these three agonists is likely to be a Ca$^{2+}$ channel blocker.\(^{13}\) Next, the properties of vasodilating action with MRC were examined under a Ca$^{2+}$-free condition, and it was found that MRC markedly suppressed Ca$^{2+}$-induced contractions in $K^+$-stimulated thoracic aorta exposed to a Ca$^{2+}$-free medium.

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**Table 1. Effects of Methyltripauchromene A (MRC) and Hydrochlorothiazide (HCT) on Urinary Volume (UrI. Vol), Electrolyte Concentration (Conc.) and Electrolyte Excretion (Exc.) in Rats**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Uri. Vol (ml/kg/3 h)</th>
<th>Na$^+$ Conc. (meq/l)</th>
<th>Na$^+$ Exc. (meq/kg/3 h)</th>
<th>K$^+$ Conc. (meq/l)</th>
<th>K$^+$ Exc. (meq/kg/3 h)</th>
<th>Cl$^-$ Conc. (meq/l)</th>
<th>Cl$^-$ Exc. (meq/kg/3 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>9.6±1.1</td>
<td>59.6±10.1</td>
<td>0.57±0.11</td>
<td>41.6±4.3</td>
<td>0.40±0.06</td>
<td>84.8±9.1</td>
<td>0.79±0.08</td>
</tr>
<tr>
<td>MRC</td>
<td>25</td>
<td>8.7±1.0</td>
<td>52.0±2.6</td>
<td>0.45±0.06</td>
<td>39.1±5.4</td>
<td>0.32±0.04</td>
<td>83.6±7.0</td>
<td>0.72±0.08</td>
</tr>
<tr>
<td>MRC</td>
<td>50</td>
<td>13.9±0.8</td>
<td>49.4±2.6</td>
<td>0.69±0.05</td>
<td>42.0±2.3</td>
<td>0.59±0.06</td>
<td>76.6±3.0</td>
<td>1.07±0.08</td>
</tr>
<tr>
<td>MRC</td>
<td>100</td>
<td>28.7±4.0**</td>
<td>58.0±4.5</td>
<td>1.66±0.27**</td>
<td>31.6±7.5</td>
<td>0.79±0.07**</td>
<td>67.4±5.0</td>
<td>1.89±0.25**</td>
</tr>
<tr>
<td>HCT</td>
<td>25</td>
<td>28.0±2.7**</td>
<td>117.8±5.5**</td>
<td>3.28±0.24**</td>
<td>28.2±2.5**</td>
<td>0.81±0.09**</td>
<td>132.4±5.0**</td>
<td>3.72±0.24**</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±S.E. ($n=5$). *$p<0.05$, **$p<0.01$, significantly different from the vehicle control (Dunnett’s multiple comparison).
Although these findings are not direct evidence, it is supposed that a decrease in a slow Ca	extsuperscript{2+} inward current may account for the observed suppressed contractions.

It is generally accepted that a decrease in the blood pressure can be caused by a decrease in cardiac output, to say nothing of a reduction in total peripheral resistance. Cardiac output is regulated both by the contractile force of the cardiomyocyte and by the heart rate. In the present study, MRC markedly suppressed the contractile force of isolated guinea pig atrial preparation without a significant change in the beating rate. This result suggests that MRC may cause a decrease in cardiac output.

Thus, MRC restrained the contractions of the cardiac muscle the same as vascular smooth muscle. These cardiac effects are not contradictory to the speculation that MRC may have a Ca	extsuperscript{2+} antagonistic action. The L-type Ca	extsuperscript{2+} channel has been extensively studied by numerous pharmacologists. Channel blockers of this type can suppress contractions of cardiac and smooth muscle cells as a result of their ability to reduce the intra-cellular influx of calcium ions through voltage-dependent channels. Many Ca	extsuperscript{2+} channel blockers, such as 1,4-dihydropyridines, diltiazem and verapamil, have been used to treat the cardiovascular diseases of hypertension and anginal pectoris with extreme benefit.

The effects of Ca	extsuperscript{2+} channel blockers on heart rate have been described in many reports. It is known that diltiazem and verapamil decrease the heart rate due to a suppression of atrioventricular conduction, whereas some 1,4-dihydropyridines, such as nifedipine and nicardipine, cause no action or a slight elevation in heart rate. In this study, MRC did not exert significant changes in the beating rate of guinea pig atrial preparations at the concentration which induced a marked negative inotropic action, although decreases in the heart rate were caused after an administration of MRC in conscious SHRSP. In this, there is an accountable disagreement between the reduced heart rate in SHRSP and no significant change in the beating rate of isolated guinea pig atria. We speculate that this disagreement may be caused by differences in animal species or in the experimental system in vivo and in vitro. The former possibility was confirmed using the isolated SHRSP atrial preparations. The result was similar to that obtained in the guinea pig preparation, namely, MRC induced a remarkable suppression of contractile force without a significant change in the beating rate. Thus, as the disagreement cannot be explained from the viewpoint of differences in animal species, then the point of differences in in vivo and in vitro experimental systems is brought to a focus.

The beating rate decreased to 165 beats/min after isolation of guinea pig atria, although the heart rate in conscious SHRSP was 340 beats/min. There was the difference between these basal rates in two experiments. In general, as the basal levels of the parameters before the treatment are higher, lowering of these reactions by test compounds are observed more significantly, for example, concerning blood pressure or body temperature. A higher dose is often necessary to obtain the decreased results in lower level preparations. This speculation may be supported from the present results that the beating rates were distinctly reduced in two guinea pig atrial preparations showing slightly high values before application of the higher concentration with MRC.

Diuretic actions were not related to an acute change in blood pressure, as shown by the above-mentioned results obtained by subcutaneous administration in conscious SHRSP. However, in the case of chronic treatment with traditional medicines, diuretic action is able to contribute to antihypertensive activity. From this viewpoint, we also investigated the diuretic action of MRC in rats, and it was found that MRC increased urinary volume and electrolyte excretions. MRC did not affect the concentrations of electrolytes in urine, while HCT (often used in the therapy of hypertension) induced remarkable elevations of Na\textsuperscript{+} and Cl\textsuperscript{−} concentrations in addition to an increase in urinary volume. The mechanism of HCT was proposed to be a suppression of Na\textsuperscript{+} reabsorption at the diluting segment of the distal tubule, which produced the increases in urinary volume and Na\textsuperscript{+} and Cl\textsuperscript{−} excretions. It has been reported that the early antihypertensive effects of thiazides is due to a decrease in cardiac output caused by a reduction of blood volume following diuretic effects, while its lasting effects are maintained by a decrease in peripheral vascular resistance. A mechanism of diuretic action with MRC has not been elucidated so far, but it is presumed that it is not similar to that with HCT, since there were no changes in urinary Na\textsuperscript{+} concentration by MRC.

MRC is one of three benzochromenes isolated from the leaves of Orthosiphon aristatus. The others, acetovanillomchromene and orthochromene A, were obtained in smaller yield, 0.11% and 0.087%, from the water decoction. IC\textsubscript{50} values with these compounds on the suppressive action to K\textsuperscript{+}-induced contractions in rat thoracic aorta were 1.01 × 10\textsuperscript{-6} M and 1.32 × 10\textsuperscript{-4} M, respectively, which were quite similar to that with MRC. Therefore, these compounds are expected to participate in the vasodilatation of this medicinal plant, together with MRC.

Finally, although experimental data in the present work cannot provide direct evidence concerning the mechanism of a decrease in blood pressure with MRC, it is suggested that the traditional treatment of hypertension with “kumis kucing” may be partially supported by a vasodilating action, a decrease in cardiac output and a diuretic action with the major chemical constituent, MRC.

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