Assessment of the Anti-anginal Effect of Tetramethylpyrazine Using Vasopressin-Induced Angina Model Rats

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Intravenous tetramethylpyrazine has been widely used in China as a complementary and/or alternative medicine to treat patients with ischemic heart disease. We assessed the anti-anginal effect of tetramethylpyrazine (10 mg/kg, intravenously (i.v.), n=6) in comparison with that of its vehicle, saline (1 mL/kg, i.v., n=6), using vasopressin-induced angina model rats. First, Donryu rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally (i.p.)), and the surface lead I electrocardiogram was continuously monitored. Administration of vasopressin (0.5 IU/kg, i.v.) to the rats depressed the S-wave level of the electrocardiogram, indicating the onset of subendocardial ischemia. However, pretreatment with tetramethylpyrazine suppressed the vasopressin-induced depression of the S-wave level, which was not observed following pretreatment with its vehicle alone (saline), suggesting that tetramethylpyrazine ameliorated the vasopressin-induced subendocardial ischemia in vivo. These results may provide experimental evidence for the empirically known clinical efficacy of tetramethylpyrazine against ischemic heart disease, and could provide clues to better understanding its in vivo mechanism of action.

Key words: tetramethylpyrazine; vasospastic angina; ST-segment depression

While β-blockers, Ca2+ antagonists and/or nitrates have been used for the treatment of patients with angina pectoris according to therapeutic guidances of western medicine,1) tetramethylpyrazine (TMP), an extract of the herb Ligusticum wallichii FRANCH. (chuanxiong), has been widely applied for the treatment of ischemic heart disease as a complementary and/or alternative medicine in China.2) Its molecular and cellular levels of mechanisms of actions have been reported to include Ca2+ antagonism,3) activation of Ca2+-activated potassium current,4) stimulation of cyclic adenosine monophosphate production5) and enhancement of endothelium-dependent nitric oxide synthesis.6) Moreover, pretreatment of tetramethylpyrazine was shown to ameliorate a vasoconstrictor terlipressin-induced decrease of cardiac index in portal hypertensive rats.7) However, in vivo experimental evidence for empirically known clinical efficacy of tetramethylpyrazine is still limited. In the present study, we assessed the effects of tetramethylpyrazine on the vasopressin-induced angina model of rats, which has been shown to be useful and reliable in evaluating the anti-spastic effect of a drug in vivo.8)

MATERIALS AND METHODS

Experiments were performed by using 12 male Donryu rats, weighing approximately 200–300 g (Japan SLC, Inc., Shizuoka, Japan). Animals were kept at 23±1°C under a 12-h light–dark cycle, where food and water were available. All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 15-53-251) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animal of Toho University.

Cardiovascular Variables The rats were divided into two groups: tetramethylpyrazine group (n=6) and vehicle group (n=6). The animals were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally (i.p.)). The right femoral vein and artery were cannulated for drug administration and for measuring blood pressure, respectively. Additional pentobarbital sodium (10–20 mg/kg, intravenously (i.v.)) was injected through the femoral vein to maintain an adequate depth of anesthesia, which was judged by jaw muscle tone. Body temperature was maintained at 37°C with a heating pad. The surface lead I electrocardiogram was obtained from the limb electrodes. The blood pressure and electrocardiogram were continuously monitored with a polygraph system (RM-6000; Nihon Kohden Co., Tokyo, Japan), which were analyzed with a real time full automatic data analysis system (WinVAS3 ver 1.1R24v; Physio-Tech Co., Ltd., Tokyo, Japan).

Experimental Protocol The protocol of the whole experiment was shown in Fig. 1. After the assessment of basal control values (C), vehicle saline (1 mL/kg, i.v.) was infused over 1 min. Six minutes after the start of the vehicle administration, vasopressin (VP) (0.5 IU/kg, i.v.) was infused over 1 min to induce ischemic attack. More than 30 min after the start of initial vasopressin administration, tetramethylpyrazine (10 mg/kg, i.v.) or vehicle (1 mL/kg, i.v.) was infused over 1 min. Six minutes after the start of tetramethylpyrazine or vehicle administration, vasopressin (0.5 IU/kg, i.v.) was infused over 1 min. The dose of tetramethylpyrazine in this study was determined by previous experimental studies2–7) as well as clinical experiences as a traditional Chinese medicine.

Drugs Tetramethylpyrazine was obtained from Medisan Pharmaceuticals Co., Ltd. (Harbin, China). Vasopressin was purchased from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Tet-
ramethylypyrazine was dissolved in saline at a concentration of 10 mg/mL. Vasopressin was diluted with saline to a concentration of 0.5 IU/mL. The other drugs used were pentobarbital sodium (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan).

**Statistical Analysis** The depression of the S-wave level was measured in three consecutive recordings of lead I electrocardiogram, which was used as a marker to estimate the severity of the subendocardial ischemia. The effects of tetramethylpyrazine (TMP) and vehicle on the vasopressin (VP)-induced changes in the heart rate from that just before vasopressin administration (ΔHR), mean blood pressure from that just before vasopressin administration (ΔMBP) and S-wave level from that just before vasopressin administration (ΔS) were calculated in order to precisely analyze the impact of vasopressin on each cardiovascular variable. Data are presented as the mean ± standard error of the mean (S.E.M.). The statistical significances within a parameter were evaluated by repeated-measures ANOVA followed by Contrasts as a post-hoc test for mean values comparison. A p value < 0.05 was considered to be statistically significant.

**RESULTS**

**Effects on the Heart Rate** Basal control value (C) was 415 ± 25 bpm in the tetramethylpyrazine group (n = 6), and 337 ± 11 bpm in the vehicle group (n = 6). The time courses of changes in the heart rate from that just before the vasopressin administration (ΔHR) are summarized in Fig. 2. There was no significant difference in the control value of ΔHR. In the initial session, administration of the vehicle hardly affected the heart rate in the tetramethylpyrazine group, but it decreased the heart rate at 1 min after the start of infusion in the vehicle group compared with that just before the vasopressin administration. Additional administration of vasopressin decreased the heart rate for 1–5 min in both groups. In the second session, administration of tetramethylpyrazine increased the heart rate at 1 min after the start of infusion in the tetramethylpyrazine
group compared with that just before the vasopressin administration, whereas administration of the vehicle hardly affected the heart rate in the vehicle group. Additional administration of vasopressin decreased the heart rate in both groups.

**Effects on the Blood Pressure** Basal control value (C) was 92±3 mmHg in the tetramethylpyrazine group (n=6) and 112±9 mmHg in the vehicle group (n=6). The time courses of changes in the mean blood pressure from that just before the vasopressin administration (ΔMBP) are summarized in Fig. 2. Typical tracings showing the effects of tetramethylpyrazine on the vasopressin-induced changes in the blood pressure are depicted in Fig. 3. There was no significant difference in the control value of ΔMBP. In the initial session, administration of vehicle hardly affected the mean blood pressure in both groups. Additional administration of vasopressin increased the mean blood pressure for 1–5 min after the start of infusion in both groups. In the second session, administration of tetramethylpyrazine increased the mean blood pressure at 2 min after the start of infusion in the tetramethylpyrazine group compared with that just before the vasopressin administration, whereas administration of vehicle hardly affected the mean blood pressure in the vehicle group. Additional administration of vasopressin increased the mean blood pressure for 1–5 min after the start of infusion in both groups.

**Anti-anginal Effects** The time courses of changes in the S-wave level from that just before the vasopressin administration (ΔS) are summarized in Fig. 2. Typical tracings of the effects of tetramethylpyrazine on the vasopressin-induced changes in electrocardiogram are depicted in Fig. 3. Basal control value (C) was $-0.007\pm0.010$ mV in the tetramethylpyrazine group (n=6), and $-0.005\pm0.009$ mV in the vehicle group (n=6). There was no significant difference in the control value of ΔS. In the initial session, administration of the vehicle hardly affected the ΔS-wave level in both groups. Additional administration of vasopressin depressed the ΔS-wave level for 1–5 min after the start of infusion in both groups. Additional administration of vasopressin depressed the ΔS-wave level in the tetramethylpyrazine group, whereas it depressed the ΔS-wave level for 1–4 min after the start of infusion in the vehicle group.

**DISCUSSION**

In the present study, we compared in vivo anti-anginal effect of tetramethylpyrazine with that of its vehicle by using a well-established vasospastic angina model of rats. Intra-coronary administration of vasopressin has been shown to constrict small coronary arteries, causing subendocardial ischemia and Ca$^{2+}$ channel blocker is known to possess a potent coronary vasodilator effect, which has been widely used for patients with vasospastic angina. Tetramethylpyrazine significantly inhibited the vasopressin-induced subendocardial ischemia, which was not observed by the vehicle, indicating that tetramethylpyrazine may possess an anti-ischemic effect. Similar results were reported previously. Intravenous administration of 2–15 mg/kg tetramethylpyrazine was reported to increase the coronary blood flow from 37 to 74 mL/min but decrease the mean coronary vascular resistance from 1770 to 700 dyn·s·cm$^{-3}$ in a dose-related manner in anesthetized dogs. Moreover, pretreatment of tetramethylpyrazine (80 mg/kg, i.v.) suppressed the endothelin-1-induced coronary vasoconstriction in anesthetized dogs. Several potential mechanisms that may explain the coronary vasodilator effects of tetramethylpyrazine have been proposed. Tetramethylpyrazine has been shown to stimulate Ca$^{2+}$-activated potassium current, cyclic adenosine monophosphate production, and the endothelial nitric oxide synthase in addition to Ca$^{2+}$ channel blockade. While these previous knowledge regarding the coronary vasodilator action of tetramethylpyrazine may partly explain its currently observed anti-anginal effect, its precise mechanisms need to be elucidated.

Vasopressin in doses of 0.12, 0.4, 1.2 and 4 mU/kg/min has been reported to increase the left ventricular end-diastolic pressure in a dose-related manner in the pentobarbital sodium-anesthetized dogs. Since the elevation of the preload can increase the ventricular wall stress, resulting in aggravation of...
subendocardial ischemia, the effect of a drug on the preload to the left ventricle may have some potential to affect its anti-anginal effects. It has been shown that tetramethylpyrazine could significantly decrease the left ventricular end-diastolic pressure in isoproterenol-induced heart failure in rats. Thus, the pretreatment of tetramethylpyrazine might have ameliorated the vasopressin-induced increase of the left ventricular end-diastolic pressure, which could also contribute to the anti-anginal effect.

The currently observed results may provide an in vivo experimental evidence for empirically known clinical efficacy of tetramethylpyrazine against ischemic heart disease, and suggest that tetramethylpyrazine could be applied for patients with vasospastic angina.

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Conflict of Interest The authors declare no conflict of interest.

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