ORIGINAL ARTICLE

Vasorelaxation Effect of Gastrodin on Isolated Thoracic Aorta Rings of Rats

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ABSTRACT  Objective: To study the effect of gastrodin on isolated thoracic aorta rings of rats and to investigate the potential mechanism. Methods: A perfusion model of isolated thoracic aorta rings of rats was applied. The effect of cumulative gastrodin (5, 50, 100, 150, 200, and 250 μmol/L) on endothelium-intact/aorta rings was investigated. The same procedure was applied to observe the effect of gastrodin on endothelium-intact/denuded aorta rings pre-contracted with 10 mmol/L phenylephrine hydrochloride (PE). The aorta rings incubated by 200 mmol/L gastrodin in the Ca²⁺-free (K-H) solution was contracted by using PE. The effect of 200 mmol/L gastrodin on endothelium-denuded aorta rings pre-contracted with 60 mmol/L KCl was also observed. Results: Compared with the denuded gastrodin group, the intact gastrodin group could significantly relax the PE-contracted aorta rings (P<0.01). In Ca²⁺-free (K-H) solution KHS, the PE-induced contraction rate of aorta rings pre-incubated by gastrodin was 6.5% ± 0.7%, which was significantly less than the control group (11.8% ± 0.9%, P<0.01). However, after 3 mmol/L CaCl₂ was added, the Ca²⁺-induced contraction in the gastrodin group (51.7% ± 2.4%) was similar to that in the control group (49.8% ± 2.8%). The contractile rate of rings in the KCl-contracted gastrodin group (96.3% ± 0.6%) was not significantly different from that in the control group (96.8% ± 1.2%). Conclusions: Gastrodin has the effect of vasorelaxation on isolated thoracic aorta rings of rats. The mechanism of the vasorelaxation of gastrodin may mainly work through the inhibition of inositol 1,4,5-trisphosphate receptor on the sarcoplasmic reticulum of the arterial smooth muscle, which leads to the reduction of the Ca²⁺ released from the sarcoplasmic reticulum.

KEYWORDS gastrodin, thoracic aorta ring, vasorelaxation, Chinese medicine

Gastrodia elata BI is a well-known Chinese medical herb that has been widely used in Asia for thousands of years. β-Gastrodin is the most effective and bioactive constituent extracted from the Gastrodia elata BI, demonstrating considerable effect on increasing cerebral blood flow and improving insufficient blood supply in basilar arteries. It can also protect the brain and heart from ischemia-reperfusion injury. Gastrodin injection has been widely used in clinical treatment of vertigo caused by carotid artery insufficiency. The clinical performance of gastrodin is enabled through improving arterial blood supply. However, the specific mechanism of gastrodin remains unclear. To further study the effect of gastrodin on vessels and its possible mechanism, a perfusion model of isolated thoracic aorta rings of rat was applied in the research experiment, providing further theoretical and experimental implication for clinical treatment on cardiovascular diseases.

METHODS

Drug, Reagents and Instruments

Gastrodin (batch 110807-200205, ≥98% purity) was provided by the National Institution of Food and Drug Control, China; phenylephrine hydrochloride (PE, batch No. H31021175) was from Shanghai Harvest Pharmaceutical Co., Ltd. China; acetylcholine (Ach, batch 12-331-86) was from Carbomer, USA; and ethylenebietraacetic acid (EGTA, batch No. 3594B23) was from Amresco, USA. The constituents of the Kreb-Henseleit (K-H) solution (KHS mmol/L) were NaCl 118, KCl 4.7, NaHCO₃ 25, KH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 2.5, and glucose 11.1, pH 7.4. The Ca²⁺-free KHS had no CaCl₂ with an addition of 0.05 mmol/L EGTA. Power lab 4/35-data acquisition system (manufactured by AD Instruments, Australia) was used to transduce the biological signals and the software system Lab Chart.
AD Instruments, Australia) was used to record and analyze the experimental data.

**Experimental Animals**

Wistar rats (260–300 g) were provided by the Animal Research Centre of Hubei Province [Hubei Province Animal Use Certificate No. SCXK (E) 2008-0005], with an equal number of males and females. All procedures done on the animals were according to the Regulations for the Administration of Affairs Concerning Experimental Animals of People's Republic of China, including standard laboratory conditions, housing, food, temperature, and execution method.

**Preparation of Isolated Thoracic Aorta Rings**

Rats were anesthetized with phenobarbitone and their thoracic aortas were removed immediately. Thoracic aortas were stripped from perivascular connective tissue and cut into rings 2–3 mm in length in the cold (under 4 °C) KHS saturated with gas mixture (95% O₂ and 5% CO₂). The rings were placed into the chamber organ bath of power lab filled with 10 mL KHS (37 °C) that was continuously infused with gas mixture (95% O₂ and 5% CO₂). After stabilization for 15 min, a constant resting tension of 2 g was applied for at least 90 min, during which period the KHS was replaced every 15 min. PE (10⁻⁶ mol/L) was used to contract the rings. Then, the rings were rinsed three times after reaching the maximum contraction amplitude. This process was conducted twice to induce the maximum contraction activity. Successful removal of the endothelium was demonstrated by Ach testing. Rings were stimulated with 10⁻⁶ mol/L PE. After reaching the maximum contraction, rings were relaxed by 10⁻⁵ mol/L Ach and the relaxation rate was then determined. The removal of endothelium was considered successful if the relaxation rate was lower than 20%. The rings were rinsed three times and equilibrated for 30 min before the next step. The relaxation rate was calculated as tension before gastrodin treatment – tension after gastrodin treatment/tension before gastrodin treatment × 100%. The contraction rate was calculated as tension after contraction – tension before contraction/tension before contraction × 100%.

**Effect of Gastrodin on Endothelium-intact Aorta Rings**

When the endothelium-intact thoracic aorta rings were prepared, increasing concentration of gastrodin was added accumulatively (5, 50, 100, 150, 200, and 250 μmol/L) to act directly on the endothelium-intact aorta rings in the gastrodin group. Isometric physiological solution was added in the control group. Changes in tension of aortic rings were recorded and the relaxation rate was calculated.

**Effect of Cumulative Gastrodin on Aorta Rings Pre-contracted with PE**

The endothelium-intact/denuded aorta rings were pre-contracted with 10⁻⁶ mol/L PE. Upon stabilization, the same procedure as above was performed. A dose-response curve was drawn and the values were shown as relaxation rate.

**Effect of Incubation of Gastrodin on Endothelium-Denuded Aorta Rings Contracted with PE in Ca²⁺-free and Ca²⁺-Recovered KHS**

After 120 min equilibration in KHS, the endothelium-denuded aorta rings were rinsed twice with Ca²⁺-free KHS (15 min for every time). Upon stabilization for 30 min, gastrodin (200 μmol/L) and isometric physiological solution were added to incubate the aorta rings for 30 min in the gastrodin group and the control group. Then, 10⁻⁶ mol/L PE was added into the medium to observe the changes in tension of the rings, and the variations in tension were recorded and compared. When the rings reached steady status, the 3 mmol/L CaCl₂ was added and the variation in tension was recorded and compared between the gastrodin group and the control group. The values were shown as contraction rate.

**Effect of Gastrodin on Endothelium-Denuded Aorta Rings Pre-contracted with KCl**

Upon stabilization, 200 μmol/L gastrodin was added in KCl-contracted endothelium-denuded group and isometric physiological solution was added in the control group. Variation of contraction was observed and recorded.

**Statistical Analysis**

All data were statistically analyzed as means ± standard deviation (x ± s) with Prism 5.0. The significant difference between two means (P<0.05) was established by Student’s two-tailed t-test for paired and unpaired data as appropriate.