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Ferulic acid relaxed rat aortic, small mesenteric and coronary arteries by blocking voltage-gated calcium channel and calcium desensitization via dephosphorylation of ERK1/2 and MYPT1

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ABSTRACT

Ferulic acid, a natural ingredient presents in several Chinese Materia Medica such as Radix *Angelicae Sinensis*, has been identified as an important multifunctional and physiologically active small molecule. However, its pharmacological activity in different blood vessel types and underlying mechanisms are unclear. The present study was to investigate the vascular reactivity and the possible action mechanism of FA on aorta, small mesenteric arteries and coronary arteries isolated from Wistar rats. We found FA dose-dependently relieved the contraction of aorta, small mesenteric arteries and coronary arteries induced by different contractors, U46619, phenylephrine (Phe) and KCl. The relaxant effect of FA was not affected by L-NAME (eNOS inhibitor), ODQ (soluble guanylate cyclase inhibitor), and mechanical removal of endothelium in thoracic aortas. The contraction caused by 60 mM KCl (60 K) was concentration-dependently hindered by FA pretreatment in all three types of arteries. In Ca^{2+}-free 60 K solution, FA weakened Ca^{2+}-related contraction in a concentration dependent manner. And FA relaxed both fluoride and phorbol ester which were PKC, ERK and Rho-kinase activators induced contraction in aortic rings with or without Ca^{2+} in krebs solution. Western blotting experiments in A7r5 cells revealed that FA inhibited calcium sensitization via dephosphorylation of ERK1/2 and MYPT1. Furthermore, the relaxation effect of FA was attenuated by verapamil (calcium channel blocker), ERK inhibitor, and fasudil (ROCK inhibitor). These results provide evidence that FA exhibits endothelium-independent vascular relaxant effect in different types of arteries. The molecular mechanism of vasorelaxation activity of FA probably involved calcium channel inhibition and calcium desensitization.

1. Introduction

Ferulic Acid (FA), (E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid ($C_{10}H_{10}O_4$, Fig. 1), is a natural polyphenol and exists not only in multiple Chinese Materia Medica, for example Radix *Angelicae Sinensis* and Rhizoma *Chuanxiong*, but also fruits, cereals and vegetables (Chen et al., 2009; Zhao et al., 2014). Previous studies have shown that FA exhibits multiple pharmacological effects including antioxidant, anti-inflammatory, angiogenesis and anticancer properties (Fukuda et al., 2015; Mancuso and Santangelo, 2014). FA is also known as a aldose reductase inhibitor (Yawadio, 2007) and improves cardiovascular and kidney structure and function in hypertensive rats (Alam et al., 2013). So, FA has been proposed as a potential treatment agent for various disorders such as cardiovascular diseases, hypertension, neurodegenerative diseases, cancer and diabetes mellitus (El-Bassossy et al., 2016; Muthusamy et al., 2016; Shen et al., 2016; Song et al., 2016). Several commercial available drugs named Piperazine Ferulate Tablets, Sodium Ferulate Tablets and Sodium Ferulate Injection have been approved by China State Food and Drug Administration.

In the present study we mainly focus on the vascular activity of FA. Because of the poor solubility of FA in water, previous studies mainly focused on the water soluble derivatives such as ferulic acid ethyl ester,

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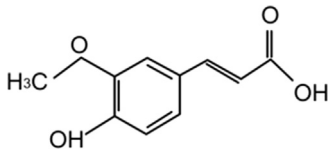

Fig. 1. The chemical structure of Ferulic Acid.

ferulate nitrate and sodium ferulate over the past years (Chen et al., 2009; Wang et al., 2012). In the aspect of vascular relaxation effect of FA, the role of endothelium is full of paradox. Furthermore, pharmacological effects of FA and its underlying mechanism during vascular relaxation procedure in vascular smooth muscle cells are also need to be further elucidated. Calcium influx through calcium channel and calcium sensitization plays crucial roles in vascular contraction. So, we hypothesized that FA inhibited vascular contraction via blocking calcium channel and suppressing calcium sensitization.

Thus, the present study investigated the vessel reactivity of FA in different vascular beds including aortic arteries, small mesenteric arteries and coronary arteries. And whether its underlying mechanisms were related to calcium channel and calcium sensitization signaling pathway were also studied both in various arteries, vascular endothelial cells and smooth muscle cells.

2. Materials and methods

2.1. Chemicals and antibodies

Acetylcholine (Ach), 9, 11-dideoxy-9a,11a-methanoepoxy Prostaglandin F2a (U46619), L-NAME, ferulic acid (FA), nifedipine, ODQ, and phenylephrine (Phe), were bought from Sigma. ERK inhibitor (ERKi), verapamil and fasudil were supplied by Cayman. Ferulic acid were dissolved in dimethyl sulfoxide (DMSO), and other drugs were prepared in distilled water. Antibodies were obtained from Cell Signaling Technology.

2.2. Animals

Male Wistar rats weighting 200–250 g were supplied by the Laboratory Animal Service Center, Longhua Hospital, Shanghai University of Traditional Chinese Medicine. Animals were housed in a room with constant temperature (23 ± 2 °C) and humidity (55 ± 5%), exposed to a 12 h light and dark cycle, and free access to food and water. All experiments described below were in accordance with the Animal Experimentation Ethics Committee of Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine.

2.3. Artery preparation

Wistar rats were killed by carbon dioxide suffocation. After scarification, aortas, small mesenteric arteries and heart were quickly isolated and immersed in oxygenated (95% O_2/5% CO_2) chilled Krebs solution with the following composition (mM):119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_2, and 11 D-glucose. Fat and connective tissues were removed carefully. Then arties were cut into ring segments in length of 4 mm for aortas, and 2 mm for main mesenteric and coronary arteries. In some rings, the endothelium was mechanically removed by gently rubbing the internal surface of the ring using stainless steel wire.

2.4. Measurement of isometric vascular tone

Isometric tension of aortic rings were recorded in 20-ml organ bath (Danish Myo Technology, Aarhus, Denmark), while main mesenteric and coronary arteries were measured in wire myograph. The organ chambers were filled with Krebs solution bubbled with 95% O_2 and 5% CO_2 at 37 °C (pH 7.4). Each ring was stretched to different resting tensions: 25 mN for aortas, 5 mN for small mesenteric arteries and 2 mN for coronary arteries. Before each experiment, the rings were equilibrated for 60–90 min and stimulated with 60 mM KCl at least 3 times to obtain a reproducible maximal contractile response. The integrity of endothelium was assessed by the ability of acetylcholine (10 μM) to induce more than 80% relaxation of rings pre-contracted with Phe (1 μM) or U46619 (30 nM). In endothelium-denuded rings, the relaxation to Ach was less than 10%. Ca^{2+}-free Krebs solution was prepared by the omission of $CaCl_2$ and the addition of 0.5 mM EGTA.

2.5. Experimental procedure

2.5.1. Effect of FA on contraction induced by Phe, KCl and U46619

1 μM Phe, 60 mM KCl, or 30 nM U46619 was applied to contract different kind of arteries and cumulative concentration response of FA (0.03, 0.1, 0.3, 1, 3 mM) were examined. Phe was not added to the study of coronary arteries as it could not cause any contraction. The experiments were repeated in addition of the solvent, DMSO at 1:1000 v/v (volume of DMSO per volume of final solution volume), to the contracted arteries did not result in any relaxation, thus verifying that the relaxations observed are most likely due to the action of FA (Fig. s1).

2.5.2. Role of endothelium in FA-induced relaxation

To elucidate the role of endothelium in FA-mediated relaxation, aortic rings were 30 min pre-incubation with nitric oxide synthase (NOS) inhibitor L-NAME (1 mM), NO-sensitive guanylyl cyclase inhibitor ODQ (3 μM) or mechanical removal of endothelium. Then concentration-response to FA (0.03–3 mM) was studied in aortic rings pre-contracted by 1 μM Phe.

2.5.3. Effect of FA on high K^+ (60 mM KCl)-induced contraction

Aortic rings, small mesenteric and coronary arteries were pretreated with 0.1 mM or 1 mM FA, or DMSO at 1:1000 v/v for solvent control for 30 min, followed by 60 mM KCl. The plateaued contraction force values were recorded. Contractions were expressed as the percentage of the maximal contraction induced by KCl (60 mM) in control group.

2.5.4. Effect of FA on extracellular Ca^{2+}-induced contraction

Aortic rings were challenged with high K^+ (60 mM KCl) containing Ca^{2+}-free Krebs solution to obtain the plateaued contraction, and then the cumulative concentration-response curves of $CaCl_2$ (0.1–10 mM) were obtained after 30 min incubation with 1 mM FA or DMSO at 1:1000 v/v for solvent control. And The L-type Ca^{2+} channel blocker nifedipine (100 nM) was used as positive control. The contractile responses to $CaCl_2$ were expressed as the percentage of the maximal contraction induced by KCl (60 mM) in standard Krebs solution.

2.5.5. Effect of FA on fluoride or phorbol ester induced vascular contraction

Aortic rings were contracted with sodium fluoride (NaF, 6 mM) or Phorbol 12-myristate 13-acetate (PMA, 10 μM) in Krebs solution with or without Ca^{2+}, and then the cumulative concentration-response curves of FA (0.03–3 mM) were record. The relaxation effects of FA were expressed as the percentage of the evoked tone.

2.5.6. Effect of calcium channel blocker, ERK inhibitor, and ROCK inhibitor on FA-induced vascular relaxation

Aortic rings pre-incubation with calcium channel blocker (Verapamil, 1 μM), ERK inhibitor (ERKi, 1 μM), and ROCK inhibitor (Fasudil, 10 μM) for 30 min, then concentration-response to FA (0.03–3 mM) was studied in aortic rings pre-contracted by 1 μM Phe.