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Irisin relaxes mouse mesenteric arteries through endothelium-dependent and endothelium-independent mechanisms

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ABSTRACT

Irisin, a newly discovered myokine, has been shown to produce modest weight loss and improve glucose intolerance in mice. The purpose of this study was to investigate the effects of irisin on vascular activity and the mechanisms involved. Experiments were performed on mouse mesenteric arteries. We demonstrated that irisin induced relaxation in mesenteric arteries with or without endothelium in a concentration-dependent manner. It was further demonstrated that the irisin-induced vasorelaxation effects on endothelium-intact mesenteric arteries were reduced by pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME) or 1H-[1, 2, 4] oxadizolo [4, 3-a] quinoxalin-1-one (ODQ). However, pretreatment with indomethacin (INDO), a nonselective cyclooxygenase inhibitor did not modulate irisin-induced relaxation. In addition, the contraction due to extracellular Ca^{2+} influx and intracellular Ca^{2+} release was also inhibited by irisin. In summary, these results suggested that the endothelium-dependent relaxation of irisin is mediated by the nitric oxide (NO)-guanosine 3′, 5′-cyclic phosphate (cGMP)-dependent pathway but not the prostaglandin I_2 (PGI_2)-cyclic adenosine monophosphate (cAMP)-dependent mechanism. Endothelium-independent relaxation may be depend on inhibiting Ca^{2+} influx through blocking VDCCs and intracellular Ca^{2+} release through both IP3R and RyR channels.

1. Introduction

Vasoreactivity is of fundamental importance in a variety of cardiovascular diseases such as hypertension, headache, and stroke [1].

Irisin, a newly discovered myokine that is released upon proteolytic cleavage of the fibronectin type III domain containing protein 5 (FNDC5), is secreted by skeletal muscles and is increased with exercise [2]. Irisin promotes the browning of subcutaneous white adipocytes by stimulating expression of uncoupling protein 1 (UCP-1), and increasing energy expenditure through cross-talk between skeletal muscles and adipose tissues [2,3]. Irisin has been proposed as a bridge between exercise and metabolic homeostasis. Irisin levels are associated with obesity, type 2 diabetes mellitus, and insulin resistance [4,5,6]. Intriguingly, a lot of clinical studies have suggested that irisin may have potential therapeutic effects on cardiovascular diseases. These studies showed that circulating irisin levels are positively associated with endothelium-dependent vasodilation in newly diagnosed type 2 diabetic patients [7], and lower levels of irisin are independently associated with endothelial dysfunction in obesity [8]. Although different effects of irisin have been discovered, little is known about its potential vascular activity.

Therefore, in the present study, we assessed the endothelium-dependent and -independent effects of irisin on mouse mesenteric arteries, and investigated the mechanisms involved.

2. Materials and methods

2.1. Chemicals and reagents

Phenylephrine hydrochloride (PE), acetylcholine chloride (ACh), ethylene glycol-bis (β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), Nω-nitro-L-arginine methyl ester (L-NAME), 1H-[1,2,4]oxadizolo[4,3-a]quinoxalin-1-one (ODQ), indomethacin (INDO), caffeine, and nifedipine were purchased from Sigma Aldrich (St Louis, MO, USA). All other reagents were of analytical purity. Irisin was kindly supplied by Prof. Dongqi Tang. INDO and nifedipine

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were dissolved in ethanol. Other chemicals were dissolved in distilled water.

2.2. Animals

Male C57BL/6J mice (10–12 weeks old) purchased from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) were used for myography. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Shandong University of Laboratory Animals Care and Use Committee.

2.3. Dissection and mounting of mesentery arteries

Animals were anesthetized with intraperitoneal injections of pentobarbital sodium (Nembutal sodium solution, 50 mg/kg, Wuhan Entai Technology, Wuhan, China). The small intestine was exposed by a median incision of the abdomen. Second-order mesenteric artery branches were gently dissected and placed in a Petri dish containing cold (0–4 °C) Krebs–Henseleit solution (K–H solution). The solution contained (in mM): NaCl 118.0, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, and glucose 11.1, equilibrated with 95% O_2–5% CO_2 mixed gas to maintain a pH of 7.4. The second-order mesenteric vessels were cleaned of adhering fat and connective tissue and sectioned into 2 mm rings. To measure the isometric force, arterial rings were mounted in a Multi Myograph System (Danish Myo Technology, Aarhus, Denmark) as previous described [9]. Briefly, two stainless steel wires were passed through the vessel lumen and separately fixed to two jaws of the myograph chamber. The mounted rings were immersed in temperature-controlled (37 °C) chamber baths containing 5 ml kH solution. During the course of the experiment, the solution was continuously oxygenated with 95% O_2–5% CO_2 mixed gas.

2.4. Vessel viability testing

The arterial rings were given a resting tension of 3 mN and then equilibrated for 60 min prior to the experiment. After equilibration, the viability of the vessel and integrity of the endothelium was assessed by its stable and reproducible responses to the addition of 1 μM PE and 1 μM ACh. Vessel segments with >50% relaxation to ACh were considered endothelium intact. In order to produce endothelium-denuded arteries, the interior of the vessel was rubbed with a stainless steel wire to remove the endothelium. The absence of ACh-induced relaxation was taken as evidence that the vessel segments were successfully denuded of endothelium.

2.5. Measurements of vascular reactivity in isolated mesenteric arteries

In order to determine the vasodilative effect of irisin on endothelium-intact and endothelium-denuded mesenteric arteries, 1 μM PE was employed to induce a steady contraction in the arteries, and then the concentration-dependent responses of irisin (0.1–100 μM) were examined. The time-matched vehicle control (double distilled water) group was also constructed. To evaluate the role of endothelium-derived vasodilative factors, endothelium-intact arteries were incubated with each of the following inhibitors: 100 μM L-NAME (nitric oxide synthase inhibitor), 1 μM INDO (a non-selective inhibitor of cyclooxygenase), and 3 μM ODQ (soluble guanylate cyclase inhibitor) for 30 min before the addition of 1 μM PE and irisin.

To test the effect of irisin on regulating Ca^{2+} influx, experiments were performed under nominally Ca^{2+}-free conditions. Endothelium-denuded mesenteric arteries were washed with a Ca^{2+}-free KH-solution before 1 μM PE was applied. Then cumulative concentrations of $CaCl_2$ (0.01–3 mM) were added in the absence or presence of a 10 min pre-incubation of 1 μM and 100 μM irisin to obtain a concentration–response curve. To verify the contribution of voltage-dependent Ca^{2+} channels, concentration–response curves to $CaCl_2$ in endothelium-denuded mesenteric arteries were also assessed by comparing the 100 μM irisin-induced vasorelaxant response in the presence or absence of 1 μM nifedipine (a voltage-dependent Ca^{2+} channel blocker). The maximal contraction obtained with the control concentration–response curve for $CaCl_2$ was taken as 100%, and all values were calculated as a percentage of the maximal response. To investigate the effect of irisin on intracellular Ca^{2+} release from sarcoplasmic reticulum (SR)-induced contraction, the first transient contraction was induced by 1 μM PE or 20 mM caffeine after exposing the endothelium-denuded arteries to a Ca^{2+}-free solution with 50 μM EGTA for 15 min. Then, the arteries were washed three times and incubated with normal KH-solution for at least 30 min to refill the intracellular Ca^{2+} stores. Soon afterwards, the normal KH-solution was rapidly replaced with a Ca^{2+}-free solution and incubated for another 15 min. The second contraction was induced by 1 μM PE or 20 mM caffeine in a Ca^{2+}-free KH-solution in the absence or presence of a 10 min pre-incubation of irisin. The ratio of the second contraction to the first contraction was calculated.

2.6. Statistical analysis

Data are expressed as means ± S.D.. The number (n) of arterial rings is from different mice. Relaxation in each arterial ring is expressed as the percentage of the contraction induced by 1 μM PE. Statistical comparisons were made between two groups with the *t*-test and between multiple groups by ANOVA. All statistical analyses were performed using SPSS v.18.0 statistical analysis software (SPSS Inc., Chicago USA). P values < 0.05 were considered statistically significant.

3. Results

3.1. Endothelium-dependent and -independent relaxation induced by irisin

To study the effect of irisin on endothelium-dependent and –independent relaxation of mesenteric arteries, the concentration-dependent responses of irisin (0.1–100 μM) were examined. Compared with the control (distilled water), irisin elicited significantly concentration-dependent relaxations in mesenteric arteries pre-contracted by 1 μM PE (Fig. 1A). The relaxing effect of irisin was significantly reduced but not abolished when the endothelium was removed from the mesenteric artery (Fig. 1B).

3.2. Irisin-evoked relaxation in arteries was dependent on endothelium-derived vasodilative factors

To investigate mechanisms underlying the vasorelaxant effects of irisin on endothelium-dependent pathways, endothelium-intact arteries were pre-incubated with 100 μM L-NAME, 1 μM INDO, or 3 μM ODQ before being contracted with 1 μM PE. Pre-incubation with L-NAME (Fig. 2A) or ODQ (Fig. 2B) partially attenuated the irisin-induced relaxation of endothelium-intact arteries pre-contracted by 1 μM PE. By contrast, pre-incubation with the nonselective cyclooxygenase inhibitor INDO did not modulate the irisin-induced relaxation (Fig. 2C).

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