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Ginsenoside Re prevents angiotensin II-induced gap-junction remodeling by activation of PPARγ in isolated beating rat atria

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ABSTRACT

Aims: Ginsenoside Re (G-Re), a major ginsenoside in ginseng, has many beneficial pharmacological effects on negative cardiac contractility, electromechanical alternans, antiarrhythmia, angiogenic regeneration and cardiac electrophysiological function. However, effects of G-Re on gap-junction remodeling are unclear. Therefore, this study aimed to investigate the effect of G-Re on angiotensin II (Ang II)-induced downregulation of connexin-40 (CX40) and -43 (CX43) in beating rat left atria.

Main methods: In this study, the isolated perfused beating rat atrial model was used and atrial gap-junction remodeling was induced by Ang II. In vivo hemodynamic experiments were analyzed with a biological recorder. Changes in protein expression were analyzed by western blot.

Key findings: G-Re attenuated Ang II-induced abnormal changes in heart rate, MAP, LVESP, LVEDP, + dp/dt max, − dp/dt min, P wave amplitude, P-R interval and P wave length. This indicated a dose-dependent preventive role against Ang II-induced hyper hemodynamics in rats. Atrial activities of p38 mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB) and activator protein 1 (AP-1) were significantly increased by Ang II, as was expression of atrial collagen I and matrix metalloproteinase 2 (MMP2). Atrial CX40 and CX43 expression was downregulated by Ang II. These Ang II-induced atrial effects were blocked by G-Re, as well as rosiglitazone, an agonist of peroxisome proliferator-activated receptor γ (PPARγ), in a dose-dependent manner. However, this inhibition was abolished by the PPARγ inhibitor GW9662.

Significance: G-Re may suppress Ang II-induced downregulation of CX40 and CX43, by activating PPARγ signaling, in isolated perfused beating rat atria.

1. Introduction

Ginseng, considered the “king” of herbs, is important in traditional Chinese medicine [1–3]. In vitro and in vivo results indicated that ginseng has potentially positive effects on heart disease through its various properties, including antioxidation, suppression of platelet adhesion, vasomotor regulation, improvement of lipid profiles and effects on various ion channels [4].

The pharmacological properties of ginseng are mainly attributed to ginsenosides, its major bioactive constituents [5]. One component of ginseng, ginsenoside Re (G-Re), belongs to the panaxatriol group. G-Re is a major ginsenoside and a primary ingredient in the leaves, berries, and roots of ginseng [6]. Peng et al. [7] reported that G-Re had many beneficial pharmacological effects on the cardiovascular system, such as negative effects on cardiac contractility and electromechanical alternans, anti-arrhythmia, angiogenic regeneration and cardiac electrophysiological function. Wang et al. [8] showed that G-Re had an antiarrhythmic effect. They concluded that G-Re exerted major antiarrhythmic actions in cat ventricular myocytes. The antiarrhythmic effects of G-Re may involve intracellular sites regulating Ca^{2+} homeostasis [8]. Jin et al. [9] showed that G-Re (47 nmol/L) decreased the contractile force and frequency of isolated atria in a dose-dependent manner. They suggested that the mechanism of decreased autorhythmicity by Ge-Re involved Ca^{2+} channel blockade.

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Table 1
Scheme of the in vivo experimental protocol.

Cont	Cont	Cont	Cont	Ang II	Ang II	Ang II	Ang II	Ang II	Ang II
Cont	Cont	G-Re	G-Re	G-Re	G-Re	G-Re	G-Re	G-Re	G-Re
Cont	Cont	G-Re	G-Re	G-Re+ Ang II	G-Re+ Ang II	G-Re+ Ang II	G-Re+ Ang II	G-Re+ Ang II	G-Re+ Ang II
Cont	GW9662	GW9662 + G-Re	GW9662 + G-Re	GW9662 +G-Re +Ang II	GW9662 +G-Re +Ang II	GW9662 +G-Re +Ang II	GW9662 + G-Re +Ang II	GW9662 +G-Re +Ang II	GW9662 +G-Re +Ang II

Each rectangle = 10 min.

The cardiac gap junction is an intercellular communicating protein in cardiomyocytes that is indispensable for effective heart pumping functions [10]. Among various gap junctional proteins, connexin-43 (CX43), localized preferentially at the intercalated discs of individual cell ends, plays a major role in interventricular myocyte connections. In diseased myocardium, CX43 showed an altered redistribution, for example, decreased or non-anisotropic expression, in the infarct border zone or in failing or hypertrophied hearts [11]. Such altered CX43 expression, called "gap-junction remodeling," is believed to impair intercellular communication and augment susceptibility to ventricular tachyarrhythmias [12]. Accumulating recent evidence demonstrated that peroxisome proliferator-activated receptor-γ (PPARγ) is critical in cardiac fibrosis [13] and that G-Re can activate PPARγ [14]. However, the role of G-Re-activated PPARγ in atrial gap junction remodeling is unclear. Angiotensin II (Ang II) treatment can lead to myocyte hypertrophy and cardiac fibrosis (cardiac remodeling) by transforming growth factor (TGF)-β1 stimulation and subsequent activation of its downstream signaling molecules, NAD(P)H oxidase, protein kinase C, p38 MAPK and nuclear activator protein 1 (AP-1), in cardiomyocytes. Ultimately, this can lead to myocyte hypertrophy and cardiac fibrosis [15]. Thus, in our study, Ang II was used as a stimulus to investigate effects of G-Re on gap-junction remodeling in isolated perfused beating rat left atria, as well as the associated mechanisms.

2. Materials and methods

2.1. Preparation of perfused beating rat atria

Sprague–Dawley rats of both sexes, weighing 250–300 g, were used. The experimental procedures were approved by the Animal Care and Use Committee of Yanbian University and were in accordance with the animal welfare guidelines of the U.S. National Institutes of Health. The permit number was SCXK (Ji) 2011-006. The rats were decapitated and isolated perfused beating left atria were prepared as previously described [16]. Soon after preparing each perfused atrium, transmural electrical field stimulation with a luminal electrode was started at 1.5 Hz (0.3 ms, 30–40 V) and the atrium was perfused with HEPES buffer solution using a peristaltic pump (1.0 mL/min) that allowed atrial pacing. The HEPES buffer contained (in mmol/L) 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose and 10.0 HEPES (pH 7.4 with NaOH), as well as 0.1% bovine serum albumin.

2.2. Experimental protocols

2.2.1. In vivo infusion of Ang II and G-Re

To define effects of Ang II and G-Re on hemodynamics, Ang II and G-Re (Sichuan Weikeqi Biological Technology, Sichuan, China) were infused into rats anesthetized with pentobarbital sodium (3%, 3 mL/kg) (Table 1). After exposure with a longitudinal incision in the groin and neck, the femoral artery, femoral vein and carotid artery were dissected and cannulated with polyethylene tubes, each secured with a ligation. The cannula in the femoral vein was connected to a peristaltic pump (Minipuls 3, Villiers le Bel, France) for infusion of Ang II or G-Re and those in the carotid and femoral arteries were each connected to a pressure transducer. The cannula in the carotid artery was placed in the left ventricle to record the left ventricular end systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP). The cannula in the femoral artery recorded the mean arterial pressure (MAP). Blood pressure (BP), heart rate (HR) and electrocardiography (ECG) lead II were recorded using a biological recorder (RM6240C, Cheng Du, China). The rats were randomly divided into four groups: (1) Ang II (35.0 μg/100 g in 1.0 mL 0.9% NaCl) group; (2) G-Re + Ang II group. (This group was further divided into high dose (317.0 μg/100 g in 1.0 mL 0.9% NaCl), medium dose (31.7 μg/100 g in 1.0 mL 0.9% NaCl) and low dose (3.17 μg/100 g in 1.0 mL 0.9% NaCl) G-Re groups); (3) GW9662 (0.19 μg/100 g in 1.0 mL 0.9% NaCl) + G-Re + Ang II group; (4) Rosiglitazone (0.24 μg/100 g in 1.0 mL 0.9% NaCl) + Ang II group. Animals were stabilized for 30 min and then infused through the femoral vein with G-Re, GW9662, rosiglitazone or saline (1.0 mL 0.9% NaCl), as indicated. After 40 min, Ang II was infused through the femoral vein, followed by continuous observation for an hour. Immediately after perfusion, cardiac tissues were collected, frozen in liquid nitrogen and stored at − 80 °C for subsequent analysis by western blotting.

2.2.2. In vitro perfusion of Ang II and G-Re

All rats were randomly divided into 7 groups (n = 5 per group) as follows: control group, Ang II group, G-Re group, rosiglitazone group, Ang II + G-Re group, Ang II + rosiglitazone group and Ang II + G-Re + GW9662 group (Table 2).