Biochemical Pharmacology 123 (2017) 40–51

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Glycine prevents pressure overload induced cardiac hypertrophy mediated by glycine receptor

Yan Lu [a,b,1], Xudong Zhu [a,1], Jinjie Li [a], Ru Fang [a], Zhuoyun Wang [a], Jing Zhang [a], Kexue Li [a], Xiaoyu Li [a], Hui Bai [a], Qing Yang [a], Jingjing Ben [a], Hanwen Zhang [a], Qi Chen [a,*]

[a] Atherosclerosis Research Center, Key Laboratory of Cardiovascular Disease and Molecular Intervention, Nanjing Medical University, Nanjing 210029, People's Republic of China
[b] Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, People's Republic of China

ARTICLE INFO

Article history:
Received 28 June 2016
Accepted 4 November 2016
Available online 9 November 2016

Chemical compounds studied in this article:
Glycine (Pubchem CID: 750)
Angiotensin II (Pubchem CID: 172198)

Keywords:
Cardiac hypertrophy
Glycine
Glycine receptor
ERK1/2 signaling

ABSTRACT

As a major amino acid, glycine has multiple functions in metabolism, growth, immunity, cytoprotection, and survival. The aim of this study was to determine the effects of glycine on pathologic cardiac hypertrophy and the mechanism underlying it. Pre-treatment with glycine significantly attenuated murine cardiac hypertrophy induced by transverse aortic constriction or by administration of angiotensin II (Ang II). This action was associated with a suppressive extracellular signal-regulated kinase 1/2 phosphorylation in myocardium. The cardioprotective effect of glycine disappeared when endogenous glycine receptor α2 was knocked down by mRNA interference in rats. Co-culture experiments revealed that glycine could also antagonize Ang II stimulated release of transforming growth factor β and endothelin-1 by cardiomyocytes, which prevented an over-production of collagens in rat fibroblasts. These results, for the first time, demonstrate that glycine may be a novel cardioprotector against pressure overload induced cardiac hypertrophy. Thus, glycine would be useful in the prevention of cardiac hypertrophy and heart failure.

1. Introduction

Despite great advances in the understanding and treatment of heart failure, the disease remains a leading cause of death worldwide [1,2]. Heart failure is structurally characterized by pathologic hypertrophy of the myocardium which temporarily preserves pump function and reduces ventricular wall stress. However, prolonged cardiac hypertrophy can cause arrhythmias, dilated cardiomyopathy and heart failure [3,4]. In contrast to physiological hypertrophy, pathological hypertrophy is characterized by accumulation of interstitial collagen and cell death, both of which contribute to increased risk for myocardial infarction, arrhythmia and sudden death. Therefore, it would be of great therapeutic interest to prevent pathological hypertrophy.

Glycine is a major amino acid in mammals and other animals. It plays an important role in metabolism, growth, development, immunity, cytoprotection, and survival [5,6]. Recent studies have shown a few beneficial effects of glycine on cardiomyocytes under ischemia-reperfusion (I/R) conditions. For example, 3 mM glycine increases the cell viability of isolated rat hearts after I/R [7]. Infusion of glycine into animal donor hearts is good for right ventricular function after transplantation [8]. Glycine can inhibit the LPS induced increase in cytosolic Ca^{2+} concentration and tumor necrosis factor-α (TNF-α) production in cardiomyocytes by activating a glycine receptor (glyR) [9]. The antioxidant N-2-mercaptopropionyl glycine has been reported to attenuate cardiac hypertrophy induced by TAC in mice [10]. However, whether glycine has an impact on cardiac hypertrophy is unknown.

In the current study, we demonstrate that glycine significantly attenuates murine left ventricular (LV) hypertrophy and cardiac fibrosis induced by either transverse aortic constriction (TAC) or angiotensin II (Ang II) administration. Mechanistically, we show that the cardioprotective effect of glycine may be via glyR α2 coupling to inhibition of extracellular signal-regulated kinase (ERK) phosphorylation and preventing production of transforming growth factor-β (TGF-β) and endothelin-1 (ET-1) by cardiomyocytes.

2. Materials and methods

2.1. Animals and treatments

All aspects of the animal care and experimental protocols were in accordance with the Guide for the Care and Use of Laboratory

* Corresponding author at: Atherosclerosis Research Center, Key Laboratory of Cardiovascular Disease and Molecular Intervention, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, People's Republic of China.
E-mail address: qichen@njmu.edu.cn (Q. Chen).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bcp.2016.11.008
0006-2952/

Animals (NIH publication, 8th edition, 2011) and approved by the Experimental Animal Care and Use Committee of Nanjing Medical University. Male 8-week-old C57BL/6J mice, male Sprague-Dawley rats each weighing 250 ± 30 g, and neonatal Sprague-Dawley rats (0–3 day old) were obtained from the Animal Center of Nanjing Medical University. All animal experiments performed in this study adhered to the protocols approved by the Institutional Animal Care and Use Committee of the Nanjing Medical University. Cardiac hypertrophy and heart failure were induced by TAC surgery or by Ang II administration by using an osmotic pump as described [11]. Glycine (Sigma-Aldrich, St Louis, USA) was suspended in saline for intraperitoneal injection. Mice were anaesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Rats were anaesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (8 mg/kg).

2.2. Measurement of plasma concentration of glycine

Glycine was injected into mice and the plasma was collected for further use at different time points. The plasma was blown dry by nitrogen and then dissolved in mobile phase. Agilent HPLC was coupled to a Micromass Quattro Ultima mass spectrometer with an ESI (Electrospray Ionisation) source, together with a Discovery C18 column (50 mm × 2.1 mm × 5 μm) for study. The mobile phase solvents were H_2O containing 0.1% TDFHA and ACN containing 0.1% TDFHA. Gradient composition started from 10% ACN and was maintained for 1 min. It was rapidly raised to 15% at 3 min, to 20% at 5 min, to 25% at 6 min, to 40% at 7 min, to 75% at 8.90 min, and finally to 98% at 9 min till the end of the analysis. Analysis was performed by using HPLC–ESI–MS/MS for a more specific and sensitive detection [12,13].

2.3. TAC surgery

Cardiac hypertrophy was induced in mice and rats by constricting to transverse aorta. Briefly, a small midline skin cut was made just above the sternum of the animal. Muscles were gently separated until trachea was visible. Partial left side thoracotomy to the second rib was performed with blunt ended spring scissor and the sternum was retracted using a chest retractor. Blunt tip 45° angled forceps were used to gently separate the two lobes of thymus and to clean fat tissue from the aortic arch. A small piece of a 7-0 silk suture (presoaked in sterile saline) was placed between the innominate and left carotid arteries using a 90 degree curved forceps. Two loose knots were tied around the transverse aorta and a small piece of a 27 gauge blunt needle was placed parallel to the transverse aorta. The first knot was quickly tied against the needle, followed by the second one. The needle was promptly removed in order to yield a constriction of 0.4 mm in diameter.

2.4. Ang II pump implantation

Mini-osmotic pumps (model 2004, ALZET technical services, USA) filled with Ang II (1.5 mg/kg/day) or saline were dorsally implanted into mice for 21 days. Glycine (700 mg/kg/day) was peritoneally injected into mice a week before the surgery.

2.5. Cardiac imaging

Transthoracic 2-dimensional M-mode echocardiography was performed with Vevo 770 (VisualSonics, Toronto, Canada) equipped with a 30-MHz transducer. Percentage of fractional shortening, LV wall thickness, and LV mass and ejection fraction (EF) was calculated as described [14].

2.6. Antibodies and reagents

Antibody against glyR α2 and GAPDH were from Abcam Biotech (San Francisco, USA). Glycine, Ang II and antibody against α-actinin were obtained from Sigma. Antibodies against cRaf, p-cRaf, mitogen-activated protein kinase kinase1/2 (MEK1/2), p-MEK1/2, ERK1/2, p-ERK1/2, p38, p-p38, c-Jun N-terminal kinase (JNK) and p-JNK were obtained from Cell Signaling Technology (Boston, USA). Plasma concentrations of TGF-β and TNF-α were determined by using mouse TGF-β ELISA and rat TNF-α ELISA Kit (Excell, Shanghai, China). Plasma concentrations of ammonia and urea were determined by using blood ammonia assay kit and blood urea assay kit (Jiangcheng, Nanjing, China).

2.7. Isolation of neonatal rat cardiomyocytes and fibroblasts

Neonatal rat cardiomyocytes (NRCMs) were prepared by enzymatic digestion of hearts obtained from newborn (0–3 day old) Sprague–Dawley rat pups by using percoll gradient centrifugation. For *in vitro* experiments NRCMs and fibroblasts were pre-treated with glycine (5 mM) for 30 min followed by addition of Ang II (1 μM). Cells were harvested and used for the mRNA and protein studies.

3.3. Protective effect of glycine on Ang II induced cardiac hypertrophy

We also examined the effect of glycine on the Ang II induced cardiac hypertrophy in mice. Consistent with the results from the TAC model, we found that glycine could protect the mouse heart against Ang II induced hypertrophy by echocardiography measurements and heart appearance analysis (Fig. 3a–c). Histomorphometric analysis showed an inhibitive effect of glycine on cardiac fibrosis in Ang II administrated mice (Fig. 3d–g). Furthermore, pretreatment with glycine could inhibit Ang II induced phosphorylation of cRaf-MEK1/2-ERK1/2 in the mouse myocardium (Fig. 3h and i).

Antagonistic effect of glycine on Ang II induced cardiac hypertrophy was further investigated by using NRCMs *in vitro*. We found that treatment with Ang II (1 μM) resulted in a significant up-regulation of hypertrophic genes including ANP, BNP, and β-MHC in NRCMs. This action could be markedly blocked by pretreatment of NRCMs with glycine dose-dependently (Fig. 4a). Treatment with 5 mM glycine significantly decreased the NRCMs surface area in the presence of Ang II (Fig. 4b and c). Glycine pre-treated NRCMs also exhibited a suppressive phosphorylation of cRaf-MEK1/2-ERK1/2 in the presence of Ang II (Fig. 4d and e). As such, these data demonstrated that glycine could prevent Ang II induced murine cardiac hypertrophy *in vivo* and *in vitro*.

3.4. Cardiac protection of glycine is mediated via a glycine receptor

It is known that glyR plays an important role in mediation of cytoprotection of glycine [6,19,20]. GlyR is an ionotropic or ligand-gated receptor consisting with different sub-units including glyR α1, α2, α3, and α4. To investigate the role of glyR in glycine antagonizing cardiac hypertrophy, we first examined the expression of glyR in the heart. It is interesting that only glyR α2 was expressed in the cardiomyocyte but not in the cardiofibroblast in rats as measured by RT-PCR (Fig. 5a), immunofluorescence staining (Fig. 5b), and western blot (Fig. 5c). Moreover, we found that expression of glyR α2 was increased in adult rat cardiomyocytes compared with NRCMs (Fig. 5c).

When endogenous glyR α2 was knocked down (~70%) in cultured NRCMs by using a siRNA (Fig. 5d), the antagonistic effect of glycine on Ang II induced up-regulation of ANP, BNP and β-MHC disappeared (Fig. 5e). Consistently, inhibitive effects of glycine on cRaf-MEK1/2-ERK1/2 phosphorylation were also abolished in