Steroids 112 (2016) 47–53

Contents lists available at ScienceDirect

Steroids

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

# Chronic stress and excessive glucocorticoid exposure both lead to altered Neuregulin-1/ErbB signaling in rat myocardium

Ruili Dang [a,1], Yujin Guo [a,1], Ling Zhang [a,1], Lei Chen [b], Ranyao Yang [a], Pei Jiang [a,*]

[a] Institute of Clinical Pharmacy & Pharmacology, Jining First People's Hospital, Jining Medical University, Jining 272000, China
[b] Department of Pharmacy, Second Xiangya Hospital, Central South University, Changsha 410010, China

ARTICLE INFO

*Article history:*
Received 6 January 2016
Received in revised form 21 March 2016
Accepted 22 April 2016
Available online 29 April 2016

*Keywords:*
Chronic unpredictable mild stress
Dexamethasone
Neuregulin-1
Depression
Heart disease

ABSTRACT

Exposure to chronic stress or excess glucocorticoids is associated with the development of depression and heart disease, but the underlying mechanisms remain equivocal. While recent evidence has indicated that Neuregulin-1 (NRG1) and its ErbB receptors play an essential role in cardiac function, much is still unknown concerning the biological link between NRG1/ErbB pathway and the stress-induced comorbidity of depression and cardiac dysfunction. Therefore, we examined the protein expression of NRG1 and ErbB receptors in the myocardium of rats following chronic unpredictable mild stress (CUMS) or rats treated with two different doses (0.2 and 2 mg/kg/day, respectively) of dexamethasone (Dex). The stressed rats showed elevated expression of NRG1 and phosphorylated ErbB4 (pErbB4) in the myocardium, whereas ErbB2 and pErbB2 were inhibited. The lower dose of Dex enhanced myocardial NRG1/ErbB signaling, but as the dose is increased, while ErbB4 remained activated, the expression of ErbB2 and pErbB2 became compromised. Both CUMS and 2 mg/kg of Dex suppressed the downstream Akt and ERK phosphorylation. Although the lower dose of Dex increased myocardial antiapoptotic Bcl-xl expression, a significant decrease of Bcl-xl expression was found in rats treated with the higher dose. Meanwhile, both CUMS and two different doses of Dex induced proapoptotic Bax level. Combined, our data firstly showed (mal)adaptive responses of NRG1/ErbB system in the stressed heart, indicating the potential involvement of NRG1/ErbB pathway in the stress-induced cardiac dysfunction.



## 1. Introduction

Depressive patients are considerably more likely to suffer cardiovascular disease (CVD), and in patients with CVD, depression is a predictor of poor outcome (specially increased morbidity and mortality) [1]. The comorbidity of depression and CVD is associated with hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis or glucocorticoid excess in response to physiologic stress over time [2]. Chronic unpredictable mild stress (CUMS) is accepted as a valid animal model of depression and exposure to chronic stress or glucocorticoids can not only induce the rats to a depression-like state [3], but also cause myocardial injury and electrical remodeling [4,5]. However, the molecular signaling mechanisms by which stress or glucocorticoids induces the deteriorating effects on heart are unknown. The risk-multiplying connection between the two common medical conditions seems to be intricate and mediated by several factors, and the molecular mechanisms underlying the pathological interrelationship remain elusive.

Neuregulin-1 (NRG1), along with the erythroblastic leukemia viral oncogene homolog (ErbB) 2, 3, and 4 receptor tyrosine kinases, plays a critical role in multiple aspects of cardiac biology, and accumulating evidence has demonstrated the significance of NRG1/ErbB signaling in mediating adaptations of the heart to physiological and pathological stimuli [6]. NRG1 exerts its effect in a paracrine manner via the ErbB family. NRG1 binds to ErbB3 or ErbB4 (but not ErbB2) and leads to a conformational change in ErbB3 or ErbB4, which then dimerizes preferentially with ErbB2. Heterodimers with ErbB2 are implicated as a more potent signaling complex than homodimers [7]. In the adult heart, NRG1 receptors ErbB2 and ErbB4, but not ErbB3, are found on cardiomyocytes. The formation of dimers results in tyrosine phosphorylation and activates the corresponding downstream Akt and extracellular-regulated kinase (ERK) signaling pathway, which regulate a variety of cell-specific functions, including proliferation, differentiation and cell migration [8].

Since NRG1/ErbB pathway is responsive to myocardial stress and indispensable for cardiac structural maintenance and

* Corresponding author.
*E-mail address:* jpoet89@126.com (P. Jiang).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.steroids.2016.04.011
0039-128X/

functional integrity [9], there exists a possibility that this system might also play a role in the stress-induced cardiac dysfunction. However, to our knowledge, there are no published studies examining the NRG1/ErbB system in the heart of the animal model of depression. Thus, the main objective of the study was to investigate the effect of CUMS and dexamethasone (Dex) administration on NRG1/ErbB signaling in the heart.

## 2. Materials and methods

### 2.1. Animals

Male, Sprague-Dawley rats (210–240 g), supplied by the Experimental Animal Center of the Second Xiangya Hospital, were housed under standard conditions of temperature (23 ± 2 °C) and light (12:12 h light/dark cycle), with free access to food and water, except prior to sucrose preference test (SPT) or when they were submitted to CUMS (see below). All animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee in our university.

### 2.2. Chronic stress paradigm

Nonstressed rats were housed four per cage and remained undisturbed, whereas the stressed rats were caged individually and received the CUMS regimen for a period of 42 consecutive days. Following the previous protocol [10], the CUMS procedure consisted of once daily exposure to different stressors including soiled cages for 24 h, cage tilt (45°) for 24 h, immobilization for 2 h, tail clamping for 2 min, day-night reversal (12 h/12 h), food deprivation for 24 h and exposure to an empty water bottle for 1 h immediately following 24 h of water deprivation.

### 2.3. Dexamethasone administration

As the major contribution of glucocorticoid excess to the comorbidity of depression and CVD, the effect of dexamethasone (Dex) on NRG1/ErbB signaling was also assessed. The rats were housed separately and randomly allocated to one of the three groups: (1) vehicle (control), (2) 0.2 mg/kg Dex and (3) 2 mg/kg Dex. The animals in different groups received daily single intraperitoneal injection of vehicle, 0.2 mg/kg or 2 mg/kg Dex between 8:00 am and 8:40 am for 10 days. Dex-acetate was suspended in saline containing 0.2% Tween 80. The lower dose was commonly used to study the stress-caused effects of the glucocorticoid [11] and the choice of the higher dose was based on the previous findings to induce the rats to a depressive-like state [10,12].

### 2.4. Sucrose preference test

SPT is widely used for the measurement of stress-induced anhedonia state, a key depressive-like behavior in rats [13]. Prior to SPT, all the rats were housed individually and habituated to 48 h of forced 1% sucrose solution consumption in two bottles on each side. Then after 14 h water deprivation, we placed two pre-weighed bottles, one containing 1% sucrose solution and another containing tap water to each rat. The side (left and right) of the two bottles was randomly placed in order to avoid spatial bias. The bottles were weighed again after 1 h and the weight difference was considered to be the rat intake from each bottle. The preference for sucrose was measured as a percentage of the consumed 1% sucrose solution relative to the total amount of liquid intake.

### 2.5. Open field test (OFT)

OFT is a measure to evaluate the general locomotion and exploratory behavior. As previously described [14], the test was conducted in a room illuminated by a 60 W fluorescent bulb the day after SPT. Each rat was placed at the centre of an apparatus with a square arena (90 cm × 90 cm × 40 cm). The floor of the arena was equally divided into 25 squares. After 30 s adaptation, the number of crossings (squares of crossing with all paws) and rearings (standing on the hind-paws) was counted for 5 min. Each test session was videotaped and scored by an experienced observer blind to the experiment design.

### 2.6. Western blot analysis

For western blotting analysis, total protein was prepared from myocardium (dissected from cardiac apex), and the concentration was analyzed using Bradford method. Samples were loaded on pre-cast 12% SDS-PAGE gels with approximately 50 μg protein in each lane. Proteins in the gels were transferred to a PVDF membrane and blocked for 1 h in 5% non-fat dry milk in TBS-T (25 mM Tris, pH 7.5, 150 mM NaCL, 0.05% Tween-20). The following antibodies and concentrations were used over night at 4 °C; NRG1 (Santa Cruz, sc-28916; 1:400), ErbB4 (Santa Cruz, sc-283; 1:500), ErbB2 (Cell Signaling, 2165; 1:1500), p-ErbB4 (Tyr1056) (Santa Cruz, sc-33040; 1:300), p-ErbB2 (Tyr1248) (Cell Signaling, 2247; 1:1500), p-Akt (Ser473) (Cell Signaling, 4060; 1:3000), p-ERK (Thr202/Tyr204) (Cell Signaling, 4695; 1:2000) and β-actin (Proteintech, 66009-1-Ig; 1:4000). It was then probed with HRP-conjugated secondary antibody for 40 min. After washing, membranes were dipped in ECL and immunoblots were analyzed by using the Bio-profil Biolight PC software. The signals were normalized to β-actin as an internal standard.

### 2.7. Real-time PCR analysis

Total RNA was extracted from the myocardium using Trizol reagent (Invitrogen, USA) following the manufacturer's instructions. RNA concentration was determined for quantity and integrity using the spectrophotometry (Jingke, China). cDNA was produced using RevertAid First Strand cDNA Synthesis Kit (Thermo, USA). Quantitative PCR was performed on Bio-rad Cx96 Detection System (Bio-rad, USA) using SYBR green PCR kit (Applied Biosystems, USA) and gene-specific primers (Bax: forward, 5′-CCAGGACGCATCCACCAAGAAGC-3′, reverse, 5′-TGCCACA CGGAAGAAGACCTCTCG-3′; Bcl-xl: forward, 5′-CAGCTTCATATAACCCCAGG GAC-3′). Each cDNA was tested in triplicate. Relative quantitation for PCR product was normalized to β-actin as an internal standard.

### 2.8. Statistical analysis

Results from the experiment were reported as means ± SD and analyzed using SPSS version 13.0 software. Student's *t*-test was used to compare means of nonstressed and stressed groups. Differences in the variant doses of Dex treated groups were determined by using one-way ANOVA with Dunnett's *t*-test for *post hoc* comparisons. The prior level of significance was established at $p < 0.05$.

## 3. Results

### 3.1. Effects of CUMS and Dex on body weight gain and behaviors

Animals exposed to 6 weeks of CUMS showed significantly less weight gain when compared with nonstressed animals [nonstress: 144.6 ± 11.2 g; CUMS: 107.5 ± 16.9 g; $p < 0.01$]. As previously