Autonomic Neuroscience: Basic and Clinical xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Short communication

# Altered cardiac gene expression of noradrenaline enzymes, transporter and β-adrenoceptors in rat model of rheumatoid arthritis

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ARTICLE INFO

*Keywords:*
Pristane
Rheumatoid arthritis
Heart ventricles
Noradrenaline
Gene expression
Rats

ABSTRACT

Baseline sympathetic activity was found to be elevated in rheumatoid arthritis (RA) patients and it is related to increased cardiovascular risk in these patients. Although many studies have highlighted the association between RA and increased cardiac sympathetic activity, the underlying mechanistic links remain unclear. The aim of the present study was to understand how diseases-triggered changes in gene expression may result in maladaptive physiological changes. Our results suggest that the equilibrium between noradrenaline synthesis, release and reuptake was disrupted in the ventricles of arthritic rats. In the acute phase of the arthritic process, decreased gene expression of MAO-A might lead to accumulation of noradrenaline in myocardial interstitial space, whereas increased gene expression of NET protected cardiomyocytes from the deleterious effects of enhanced noradrenaline. During the chronic phase, reduced expression of $\beta_1$-adrenoceptor and decreased efficiency of noradrenaline reuptake contribute to progressive damage of the myocardium and limits heart efficiency.

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting 0.5–1% of population (Symmons, 2002). Autonomic dysregulation is reported in RA, juvenile chronic arthritis and arthritic animals that support increased sympathetic activity (Koopman et al., 2011). Kavelaars et al. (1998) found that patients with juvenile chronic arthritis excrete more of the central noradrenaline metabolite MHPG in urine, which is suggestive for a higher noradrenergic turnover in the brain, as well as by the higher heart rate at rest in patients than healthy controls. Functional noradrenergic transmission consists of a balance between noradrenaline synthesis, secretion and reuptake. Noradrenergic activity is dependent on the synthesis of noradrenaline as determined by the rate limiting enzyme tyrosine hydroxylase (TH); noradrenaline released from cardiac sympathetic nerve terminals is removed from the neuroeffector junction by the neuronal noradrenaline transporter (NET) and metabolized to dihydroxyphenylglycol (DHPG) via monoamine oxidase-A (MAO-A) (Esler et al., 1990).

Many studies demonstrated that the sympathoneural system can exert quite different, even opposite, effects on immune responses and inflammation. In the early phases of RA, the sympathoneural system confers proinflammatory effects, and in the late, chronically phase of disease, the sympathoneural system has antiinflammatory effects (Härle et al., 2005).

Although many studies have highlighted the association between RA and increased cardiac sympathetic activity, the underlying mechanistic links remain unclear. Hence, it is important to examine regulation of more specific variables, such as the gene expression of noradrenaline enzymes, transporter and adrenoceptors in the acute and chronic phase of the arthritic process.

The aim of the present study was to understand how diseases-triggered changes in gene expression may result in maladaptive physiological changes.

## 2. Materials and methods

### 2.1. Animals

The congenic rat strain DA.1F (originating from Zentralinstitut für Versuchstierzucht, Hannover, Germany) was bred and maintained

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http://dx.doi.org/10.1016/j.autneu.2017.10.003
Received 10 August 2017; Received in revised form 4 October 2017; Accepted 4 October 2017

under specific pathogen free (SPF) conditions in the animal facility of the Institute of Biomedical research, Medical University of Vienna (MUV), Austria. Experiments were performed on male rats of 8–12 weeks of age. Rats were housed in groups of two to three in plastic cages with 12 h light/dark cycles. Standard rodent chow and water was provided ad libitum. All experiments were approved by the local ethical committee of the MUV. Arthritis was induced by an intradermal injection at the base of the tail with 150 µl of pristane oil (2,6,10,4-tetramethylpentadecane; Sigma-Aldrich). Twenty rats were treated with pristane oil and 10 additional rats served as controls. Arthritis development was monitored in all four limbs using a macroscopic scoring system as described (Tuncel et al., 2016). Briefly, one point was given for each swollen or red interphalangeal joint, one point was given for each swollen or red metacarpophalangeal joint, and one to five points were given for a swollen ankle (the maximum score per limb and rat was 15 and 60, respectively). Both cardiac ventricles from rats 0, 21 and 50 days after injection of pristane were dissected, snap-frozen in liquid nitrogen and stored at − 70 °C until analyzed.

### 2.2. Measurement concentration of norepinephrine and DHPG

Both cardiac ventricles from rats 0, 21 and 50 days after injection of pristane were dissected, snap-frozen in liquid nitrogen and stored at − 70 °C until analyzed. Concentration of norepinephrine and DHPG were determined by PLC and electrochemical detection as previously described (Jovanovic et al., 2014).

### 2.3. RNA isolation and real time RT-PCR

Total RNAs from heart ventricles was extracted using TRIzol® Reagent (Thermo Fischer Scientific, MA USA). The ventricles were homogenized in 1 ml TRIzol® Reagent per 100 mg of tissue using electrical homogenizer (IKA-WERKE, GmbH & Co, Germany). Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Bead (GE Healthcare Life Sciences, PA USA) and pd. $(N)_6$ primer according to manufacturer's protocol. PCR reaction were performed in the ABI Prism 7000 Sequence Detection System at 50 °C for 2 min., 95 °C for 10 min., followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. TaqMan PCR reaction were carried out using Assay-on-Demand Gene Expression Products (Thermo Fischer Scientific, MA USA) for TH (ID:Rn00562500_m1), for NET (ID:Rn00580267_m1), for $\beta_1$ (ID:Rn00824536_s1), for $\beta_2$ (ID:Rn00560650_s1), and for MAO-A (ID:Rn01430950_m1). A reference endogenous control was included in each analysis to correct the differences in the inter-assay amplification efficiency and all transcripts were normalized to cyclophilin A (ID:Rn00690933) expression.

### 2.4. Western blot analysis

Heart ventricles were homogenized in RIPA Lysis Buffer System (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, sc-24948). After centrifugation (12,000 rpm, 20 min at 4 °C), the supernatant was taken and protein concentrations were determined by the method of Lowry et al. (1951). 30 µg of the ventricles protein extract separated by 10% SDS-poly-acrilamide gel electrophoresis were transferred to a supported PVDF membrane (Immobilon-P membrane, Merck Millipore, Massachusetts, USA). The membranes were blocked in 5% non-fat dry milk in Tris-Buffered Saline-Tween 20 (TBST) for 1 h. All following washes (three times for 15 min.) and antibody incubation (overnight at 4 °C for primary antibody and 1 h at 4 °C for secondary antibody) were also performed in TBST at ambient temperature on a shaker. For measuring TH, NET, adrenergic $\beta_1$ receptor, adrenergic $\beta_2$ receptor and MAO-A protein levels, a polyclonal anti-TH primary antibody, rabbit (ab51191, dilution 1:1000, Abcam, Cambridge, UK), a polyclonal anti-NET primary antibody, rabbit (ab41559, dilution 1:1000, Abcam, Cambridge, UK), polyclonal anti-$\beta_1$ primary antibody, rabbit (ab3442, dilution 1:1000, Abcam, Cambridge, UK) and polyclonal anti-$\beta_2$ primary antibody, rabbit (ab182136, dilution 1:1000, Abcam, Cambridge, UK), and monoclonal anti-MAO-A, primary antibody, rabbit (ab126751, dilution 1:1000, Abcam, Cambridge, UK) were used respectively. Washed membrane was further incubated in the horseradish peroxidase conjugated secondary anti-rabbit antibody for luminol based detection (ab6721, dilution 1:5000, Abcam, Cambridge, UK). Secondary antibody was then visualized by Immobilon Western Chemiluminescent HPR Substrate (Merck Millipore, Massachusetts, USA).

### 2.5. Statistical analysis

The results are reported as means ± S.E.M. Significance of the differences in concentrations of norepinephrine, DHPH and gene expression levels of the examined catecholamine biosynthetic and degradation enzymes, transporter and β-adrenoceptors in the ventricles between naive and rats with pristane-induced arthritis were estimated by one-way ANOVA test. The Tukey post hoc test was used to evaluate the differences between the groups. Statistical significance was accepted at $p < 0.05$.

## 3. Results

No changes were observed in concentration of noradrenaline in the ventricles during acute and chronic phase of pristane-induced arthritis compared to naive control group (Fig. 1a). However, DHPG levels were markedly reduced in chronic phase in comparison with acute phase and naive ($p < 0.05$) (Fig. 1b).

Pristane-induced inflammation differently affected mRNA and protein levels of $\beta_1$- and $\beta_2$-adrenoceptors in heart ventricles. $\beta_1$-Adrenoceptors mRNA ($p < 0.01$) and protein levels ($p < 0.05$) were significantly up-regulated by inflammation during acute phase. However, mRNA ($p < 0.05$) and protein levels ($p < 0.05$) of $\beta_1$-adrenoceptors were markedly decreased in the chronic phase of arthritis compared to naive and compared to acute phase (mRNA levels $p < 0.001$; protein levels $p < 0.001$) (Fig. 1c). In contrast, gene expression of the $\beta_2$-adrenoceptors was unaffected by pristane-induced inflammation (Fig. 1d).

In the present study, no significant differences in the levels of TH mRNA and protein between naive and acute and chronic phase of pristane-induced arthritis were found (Fig. 2a). Analysis of date concerning NET indicates that pristane-induced arthritis led to increase in mRNA ($p < 0.01$) and protein level ($p < 0.05$) of NET during acute phase. On the other hand, the chronic phase of arthritis lead to reduction of elevated levels of NET mRNA ($p < 0.05$) and protein ($p < 0.05$) compared to naive and compared to acute phase (mRNA levels $p < 0.001$; protein levels $p < 0.01$) (Fig. 2b). During the acute phase of pristane-induced arthritis, mRNA ($p < 0.05$) and protein levels of MAO-A ($p < 0.01$) were significantly decreased compared to naive, whereas during the chronic phase of disease there was further reduced gene expression of MAO-A (mRNA levels $p < 0.05$) in the ventricles (Fig. 2c).

## 4. Discussion

The sympathetic tone in RA patients is increased and an increase in sympathetic activity would not suppress the proinflammatory process in the joint, but rather, it would increase the risk of cardiovascular disease in these patients (Härle et al., 2005). In the present study, we have shown marked changes in the levels of gene expression of noradrenaline biosynthetic and degrading enzymes, transporter and β-adrenoceptors in heart ventricles during the acute and chronic phase of experimental arthritis. Our data show that the amount of noradrenaline as well as TH mRNA and protein levels in the ventricles of arthritic animals in the acute phase were unchanged, whereas gene expression of MAO-A was decreased. These data suggest that decrease in the