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Angiotensin-(1–7) prevents atrial tachycardia induced-heat shock protein 27 expression

Xuewen Wang, PhD, Wenfeng Shangguan, PhD, Guangping Li, PhD*

Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular disease, Department of Cardiology, Tianjin Institute of Cardiology, Second Hospital of Tianjin Medical University, Tianjin, China

Abstract

Objective: We aimed to investigate the effects of angiotensin-(1–7) [Ang-(1–7)] on heat-shock protein 27 (HSP27) in a canine model of induced tachycardia.

Methods: Eighteen dogs were randomized into three equal treatment groups: sham, pacing and pacing + Ang-(1–7) group. The dogs in the last two groups were subjected to 2 weeks of rapid atrial pacing (500 bpm). The effects of Ang-(1–7) on HSP27 were assessed by real-time polymerase chain reaction and western blot.

Results: The expression levels of atrial HSP27 mRNA and protein were significantly ($P < 0.05$) higher for the pacing group than the sham group and significantly ($P < 0.05$) lower for the pacing + Ang-(1–7) group than the pacing group. There was no significant difference between the HSP27 expression levels in the right and left atria among all three groups.

Conclusions: Our findings suggest that the overexpression of HSP27 may possibly be occurring as an adaptive response that allows atrial tissues to cope with rapid atrial pacing, and an inhibiting effect of Ang-(1–7) on atrial remodeling may be one of the mechanisms responsible for the attenuation of HSP27 up-regulation induced by rapid pacing.
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Keywords: Angiotensin-(1–7); Atrial fibrillation; Heat shock protein 27

Introduction

Atrial fibrillation (AF) results in atrial remodeling with electrical, autonomic, contractile, as well as structural changes that shorten and increase atrial effective refractory period (AERP) dispersion; inhibit atrial conduction [1–2], enhance collagen production and hyperplasia of fibroblasts; and lead to myocyte separation. These changes impede intra-atrial conduction and cause localized blockade of conduction, which is conducive to the triggering and maintenance of AF [3–4]. Heat-shock proteins (HSPs) are a protein family that plays a protective role against various cellular stresses, including those that may induce AF under different circumstances such as surgery [5–6]. In particular, atrial expression levels of HSP27 levels have been shown to have an inverse correlation with the durations of persistent and paroxysmal AF and the extent of myolysis [5]. Further, Brundel et al. [7] have shown that HSP27 may be one of the most important HSPs protecting myocytes against AF-induced atrial remodeling by delaying or preventing AF progression. An animal study [8] has provided the primary evidence showing that treatment with HSP27 protects against atrial tachycardia-induced remodeling, thereby highlighting the therapeutic potential of HSP27 induction in clinical AF.

Other causative factors, and therefore potential therapeutic targets, involved in the generation of AF are the renin–angiotensin system (RAS), oxidative stress, and inflammation [9]. Ang-(1–7) is an active molecule of the RAS that can counter most activities of Ang II, such as vasoconstriction and hypertension; it is produced via the neutral endopeptidase pathway [10] or the angiotensin-converting enzyme 2 pathway [11]. By establishing an experimental model of chronic AF in dogs, we have previously [12–14] shown that Ang-(1–7) contributes to the inhibition of AF-induced structural and electrical remodeling of the atrium. However, the exact effect of Ang-(1–7) on HSP27 in preventing atrial remodeling still remains elusive. Therefore, in the present study, we aimed to examine the effects of Ang-(1–7) on the expression level of HSP27 released due to rapid atrial pacing in a canine model of AF.

* Corresponding author at: Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular disease, Department of Cardiology, Tianjin Institute ofCardiology, Second Hospital of Tianjin Medical University, Tianjin 300211, China.

E-mail address: tjcardiol@126.com (G. Li).

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Material and methods

Experimental model

Mongrel dogs (weight: 11–15 kg; n = 18) were randomized into three equal groups: sham, pacing (PAC), and pacing + Ang-(1–7) [PAC + Ang-(1–7)]. The animals were handled with humane care, and approval for the experimental protocol was provided by the Experimental Animal Administration Committee of Tianjin Medical University and Tianjin Municipal Commission for Experimental Animal Control.

We then induced sustained AF in the animals with the same method used in our previous studies [12]. In brief, we administered sodium pentobarbital (intravenous; 30 $mg \cdot kg^{-1}$) to induce anesthesia. We introduced a sterilized unipolar, screw-in, J-pacing lead (St. Jude Medical, St. Paul, USA) into the right atrium via the right jugular vein and secured its distal end; atrial capture was confirmed with the external cardiac stimulator (TOP2001, Hongtong Co., Shanghai, China). The other tip of the pacing lead was attached to a programmed pacemaker (prepared at Shanghai Fudan University, China) placed within a subcutaneous pouch created in the cervical region. The lead was allowed to stabilize over an interval of 24 h. The pacemaker was set such that the atrium was stimulated at 500 bpm (cycle length, 120 ms). Surface electrocardiography was performed 24 h after implantation of the pacemaker and on alternate days thereafter to ensure maintenance of atrial capture at 1:1. Throughout the 2-week period of atrial pacing, the PAC + Ang-(1–7) group received Ang-(1–7) (dose: 6 $\mu g \cdot kg^{-1} \cdot h^{-1}$) through a catheter advanced into the left jugular vein by means of the ALZET® osmotic pump (DURECT, Cupertino, CA, USA). The systolic blood pressure was determined at baseline and at the end of the 2-week period. A multichannel physiological device (TOP2001, Hongtong Co., Shanghai, China) was used to record the electrocardiograph and blood pressure.

Real-time PCR analysis

The animals were killed and cardiectomy was performed. The right and left atria were excised, individually flash-frozen in liquid nitrogen, and stored separately at −80 °C for further analyses. Total RNA extraction from 100 mg of the excised atrial tissue was then performed using an RNA extraction kit (Takara Bio, Shiga, Japan). The integrity of the extracted RNA was verified by agarose gel electrophoresis. The amount of total RNA extracted was measured at a wavelength of 260 nm, by using a spectrophotometer (UV-240, Shimadzu Co., Kyoto, Japan). Then, the extracted RNA was maintained at −80 °C for subsequent analysis. RNA was reverse transcribed into single-strand cDNA using a kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The synthesized cDNA was used as a template for PCR amplification. For the amplification of HSP27 genes, specific oligonucleotide primer pairs were prepared using relevant GeneBank sequences. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the loading control, and the Gene Runner software package was used to prepare the required primers:

HSP27, 5′-CACGAAGAG AGGCAGGATGA-3′/5′-GAGGTGAGGATGTGGGCTAA-3′; and GAPDH, 5′-GGCGTGAACCATG AGAAGTAT-3′/5′-GTGGAAG-CAGGGATGATGTT-3′. The primers were obtained from BGI-Tech Ltd. (Tianjin, China). The PCR steps included initial denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 40 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 45 s. A 5-μL quantity of the obtained product was then subjected to 1% agarose gel electrophoresis and the target gene sequence was verified by using a DNA sequencer (ABI PRISM® 377, Applied Biosystems, Foster City, USA).

Western blot analysis

Samples of the left and right atrial tissue were collected from all animals and analyzed by western blot. Protein extraction was performed and the extract was subjected to 6% SDS-denaturing polyacrylamide gel electrophoresis for protein separation. Then, the extracted proteins were transferred to a polyvinylidene fluoride membrane (Merck Millipore, Billerica, MA, USA) and incubated overnight with the primary antibody at 4 °C. After washing the membranes, they were incubated with the secondary antibody conjugated with horseradish peroxidase. The proteins were then identified by enhanced chemiluminescence, and the levels of HSP27 protein were determined as a ratio of GAPDH levels. The antibodies against GAPDH and HSP27 were procured from Abcam Inc. (Cambridge, UK).

Statistical analysis

Grouped data were expressed as mean ± standard deviation. Intergroup comparisons were made using analysis of variance (ANOVA). The relationship between the protein expression levels of HSP27 was assessed with linear correlation analysis. A two-tailed P value of <0.05 was considered significant.

Results

Hemodynamic parameters

No statistically significant intergroup differences were detected in terms of the canine ventricular rate or systolic pressure before and after pacing. Table 1 shows the measurements of these values in detail.

Expression of HSP27 gene

The mRNA expression levels of HSP27 were significantly higher in the PAC group than in the sham group ($P < 0.05$, Fig. 1), whereas those in the PAC + Ang-(1–7) group were significantly lower than those in the PAC group ($P < 0.05$, Fig. 1). Further, none of the three groups showed any significant difference in the expression levels of the HSP27 gene between the right and left atrium ($P > 0.05$, Fig. 1).

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