Changes of serum neurohormone after renal sympathetic denervation in dogs with pacing-induced heart failure

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Abstract: Background: Neurohormonal activation is a commonly cited array of phenomena in the body’s physiologic response to heart failure (HF). The aim of the present study was to determine the change law of serum neurohormones after renal sympathetic denervation (RSD) in dogs with pacing-induced HF. Methods: Twenty-eight beagles were randomly divided into control group, RSD group, HF group and HF + RSD group. The control group was implanted pacemakers without pacing; the RSD group underwent renal artery ablation without pacing; the HF group was implanted pacemakers with ventricular pacing at 240 bpm for 3 weeks; and HF + RSD group underwent renal artery ablation and with ventricular pacing at 240 bpm for 3 weeks. Blood samples were taken at baseline, and 3, 6, 9, 12, 15, 18, 21 days in all the dogs for neurohormones measurement. Results: After 3 weeks, the systolic femoral artery pressures in the HF and HF + RSD groups were reduced after pacing 3 weeks. There was an increase significantly in BNP, angiotensin II, aldosterone, endothelin-1 and decrease in renalse after 3 weeks when compared with baseline in HF group. RSD significantly suppressed the changes of plasma neurohormones concentration in experimental HF, but RSD had not obviously impact on the levels of plasma neurohormones during 3 weeks in RSD group. Conclusions: RSD attenuates the changes of levels of plasma neurohormones in the activated renin-angiotensin-aldosterone system (RAAS) but had not obviously effect in the normal physiology of RAAS.

Keywords: Renal sympathetic nerve, ablation, neurohormone, heart failure

Introduction

Chronic heart failure (HF) is a progressive syndrome that results in a poor quality of life for the patients. Despite advances in the control of cardiovascular diseases such as myocardial infarction, the incidence and prevalence of chronic HF continue to increase [1]. Neurohormonal activation is a commonly cited array of phenomena in the body’s physiologic response to HF. Under normal physiological conditions, neurohormonal activation compensates for the reduced cardiac output. The regulation of these systems is altered in chronic HF. Two main physiological systems have been shown to be overactive in chronic HF: the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nerve system [2, 3]. In our previous studies, we demonstrated that plasma angiotensin II (Ang II) and aldosterone concentrations were attenuated by renal sympathetic denervation (RSD), and these reductions may have prevented atrial substrate remodeling [4]. In another study, we found that the long-term RSD suppressed the increased levels of circulating Ang II and inhibited ventricular substrate remodeling during rapid ventricular pacing [5, 6]. The other studies have demonstrated that renal sympathetic nerve had impact on the levels of atrial natriuretic peptide and brain natriuretic peptide (BNP) [7, 8]. Whether RSD had impact on the other neurohormones and the change law of serum neurohormones after RSD in HF is unknown. Therefore, the purpose of the present study was to determine the change law of serum neurohormones after RSD in dogs with pacing-induced HF.
Methods

Animal handling was performed in accordance with the Wuhan Directive for Animal Research and the current Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996). The ethics committee at Wuhan University approved the study protocol.

Experimental models

Twenty-eight beagles of both genders and weighing 13.1 ± 2.3 kg were divided into four groups: 1) the control group (n = 8), 2) the RSD group (n = 7), 3) the HF group (n = 7) and 4) the HF + RSD group (n = 6). Intramuscular injection of 25 mg/kg ketamine sulfate was used before pentobarbital sodium premedication. All of the dogs were pre-medicated with sodium pentobarbital (30 mg/kg, IV), intubated, and ventilated with room air supplemented with oxygen from a respirator (MA01746, Harvard Apparatus Holliston, USA). Continuous ECG monitoring was performed.

The pacemakers (Shanghai Fudan University, China) in the control group were implanted in a

Figure 1. Changes of BNP during 3 weeks in the four groups. Results showed that there had a gradually increased during pacing and there was an increase significantly after 3 weeks when compared with baseline in HF and HF + RSD groups (ΔP < 0.01). When compared with that in the HF group, level of BNP was lower in the HF + RSD group after 3 weeks (#P < 0.01). There was no significant difference at baseline and after 3 weeks in the control and the RSD groups.

Figure 2. Changes of Ang II during 3 weeks in the four groups. There was an increase significantly after 3 weeks pacing when compared with baseline in HF group (ΔP < 0.01). There was no significant difference at baseline and after 3 weeks in the control, the RSD and the HF + RSD groups.
subcutaneous pocket and attached to a pacing lead (1646T, St. Jude Medical, Inc., USA) in the right ventricular apex under fluoroscopic visualization via the right external jugular vein. The dogs received antibiotics after surgery, and all of the animals recovered for 3 weeks without pacing. The dogs in the HF group received antibiotics and recovered for 3 days after pacemakers were implanted. Then, the dogs underwent ventricular rapid pacing at 240 beats per minute (bpm) for 3 weeks.

The dogs in the RSD group and the HF + RSD group underwent double renal artery ablation prior to ventricular rapid pacing. Briefly, a quadrupole radiofrequency ablation catheter was introduced into each renal artery via femoral artery [5]. We applied radiofrequency ablations lasting up to 90 sec each and of 6 watts within each renal artery. After ablation, pacemakers were implanted. Antibiotics were administered for 3 days and the dogs underwent ventricular rapid pacing at 240 bpm for 3 weeks in the HF + RSD group and dogs in the RSD group recovered for 3 weeks without pacing.

**ELISA assay**

Blood samples were taken at baseline, and 3, 6, 9, 12, 15, 18, 21 days in all the dogs. Four milliliters of venous blood was collected in EDTA vacutainers and centrifuged at 2310 g, for 10 min at 4°C (Beckman Coulter, Avanti J-E). The serum was separated and kept in microtubes and stored at -80°C until assay. The levels of BNP, Ang II, aldosterone, renalase, prostaglandin E2 (PGE2), endothelin-1 (ET-1) and erythropoietin (EPO) were examined by ELISA (Canis Elisa Kit, Jiangcheng Bioengineering Institute, China). Serum creatinine was also examined by ELISA.

**Statistical analysis**

The values are shown as the mean ± SD. Two-sample independent Student’s t tests were used to compare the means of 2 groups. ANOVA with Neuman-Keuls tests were used to compare the means of continuous variables among multiple groups, and in the case of a significant difference this was further analysis was undertaken with a Tukey-Kramer test. All the statistical tests were 2-sided, and a probability value < 0.05 was required for statistical significance.

**Results**

The similar results of changes of blood pressures and heart rate were reported in our previous study [6]. Briefly, after 3 weeks, the systolic femoral artery pressures in the HF and HF + RSD groups were reduced after pacing 3 weeks. The heart rates and level of serum creatinine were not altered in the four groups prior to or after the study period. There had a decrease trend in the RSD group, but there was not significant difference at baseline and after 3 weeks. The heart rate had a decreased trend in the RSD, HF and HF + RSD groups, but there was not significant difference at baseline and after 3 weeks.
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Changes of BNP

Figure 1 shows the results of plasma BNP concentration in the four group dogs. There had a gradually increased during pacing and there was an increase significantly after 3 weeks pacing when compared with baseline in HF and HF + RSD groups (HF: 108 ± 17 pg/mL vs 253 ± 31 pg/mL, P < 0.01; and HF + RSD: 114 ± 16 pg/mL vs 185 ± 26 pg/mL, P < 0.01). When compared with that in the HF group, level of BNP was lower in the HF + RSD group after 3 weeks (185 ± 26 vs 253 ± 31 pg/mL, P < 0.01). There was no significant difference at baseline and after 3 weeks in the control and the RSD groups.

Changes of Ang II and aldosterone

Figures 2 and 3 show the results of plasma Ang II and aldosterone concentration in the four group dogs. There was an increase significantly after 3 weeks pacing when compared with baseline in HF group (Ang II: 149 ± 19 vs 241 ± 26 pg/mL, P < 0.01; Aldosterone: 223 ± 26 vs 384 ± 39 pg/mL, P < 0.01). The plasma Ang II and aldosterone concentration had an increase trend in the HF + RSD group (Ang II: 147 ± 21 vs 169 ± 24 pg/mL, P = 0.06; Aldosterone: 225 ± 22 vs 252 ± 35 pg/mL, P = 0.08) and a decrease trend in the RSD group (Ang II: 151 ± 19 vs 132 ± 17 pg/mL, P = 0.06; Aldosterone: 225 ± 26 vs 201 ± 22 pg/mL, P = 0.06), but there was not significant difference at baseline and after 3 weeks. There was no significant difference at baseline and after 3 weeks in the control and the RSD groups.

Changes of ET-1

As shown in Figure 4, the ET-1 concentration in the HF group was increased remarkably after 12 days (79 ± 9.1 vs 108 ± 11 pg/mL, P < 0.01), and the elevation was maintained thereafter. After 3 weeks of pacing, the concentrations of ET-1 increased significantly compared with baseline (79 ± 9.1 vs 115 ± 13 pg/mL, P < 0.01). There was no significant difference at baseline and after 3 weeks in the control, the RSD and the HF + RSD groups.

Changes of renalase

As shown in Figure 5, the plasma renalase concentration decreased after 6 days and the decreased level was maintained thereafter in the HF group. Compared with baseline, the levels of plasma renalase were lower after 3 weeks in the HF and the HF + RSD groups (HF: 246 ± 27 ng/mL vs 174 ± 18 ng/mL, P < 0.01; and HF + RSD: 254 ± 32 ng/mL vs 217 ± 21 ng/mL, P < 0.01). When compared with that in the HF group, level of renalase was higher in the HF + RSD group after 3 weeks (217 ± 21 vs 174 ± 13 pg/mL, P < 0.01). There was no significant difference at baseline and after 3 weeks in the control and the RSD groups.

Changes of PGE₂ and EPO

We next examined the plasma PGE₂ and EPO concentration during 3 weeks. The results showed that the plasma PGE₂ and EPO concentration had no significant difference between the baseline and after 3 weeks in the four
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Figure 5. Changes of renalase during 3 weeks in the four groups. Level of renalase decreased after 6 days and the decreased level was maintained thereafter. Compared with baseline, the levels of plasma renalase were lower after 3 weeks in the HF and the HF + RSD groups (ΔP < 0.01). Level of renalase was higher in the HF + RSD group than in the HF group after 3 weeks (ΔP < 0.01). There was no significant difference at baseline and after 3 weeks in the control and the RSD groups.

Discussion

In the present study, we demonstrated that rapid-paced canine HF may lead to changes of plasma neurohormones concentration. We also found that catheter-based RSD significantly suppressed the changes of plasma neurohormones concentration in experimental HF, but RSD had not obviously impact on the levels of plasma neurohormones during 3 weeks in RSD group dogs. The present findings suggest that RSD can attenuate the changes of levels of plasma neurohormones in the activated RAAS and sympathetic nerve system but had not obviously effect in the normal physiology of RAAS and sympathetic nerve system.

Neurohormonal activation in HF

It is clear that chronic HF is associated with important neurohormonal over activation and alterations in the autonomic control of the cardiac function [9-11]. Two main physiological systems including the sympathetic nerve system and the RAAS are altered in chronic HF [12, 13]. In response to the ventricular dysfunction of HF, the sympathetic nerve system and RAAS activity persisted beyond that needed for adaptation, with chronic release of Ang II, aldosterone, and other neurohormones. The neurohormones ultimately became “maladaptive”. Active Ang II has several known biologic effects, including vasoconstriction, profibrotic and pro-inflammatory effects that contribute to untoward cardiac remodeling [14]. Neurohormonal antagonists were acting more directly on the myocardium. They were preventing the progression of ventricular remodeling and, in some cases, promoting reverse remodeling, thus improving myocardial function and favorably influencing the natural history of HF [15-17]. In the present study, we also found that the levels of BNP, Ang II, aldosterone, ET-1 increased and renalase decreased in experimental HF. Our results are in accordance with the previous investigations where it was shown changes of neurohormonal factors during HF [18-21]. Furthermore, we also found that the levels of BNP, Ang II and aldosterone had a gradually increased during pacing, while the level of ET-1 increased sharply and the level of renalase decreased sharply after several days. The cause of this finding is unclear. These results indicate that the hormones were regulated by different pathway.

Neurohormones and renal sympathetic nerve

The kidneys are richly innervated by efferent sympathetic nerve fibres and the renal sympathetic drive is markedly increased in HF. Even mild and low-frequency stimulation of efferent sympathetic nerves enhances sodium reab-
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sorption and stimulates renin release by the juxtaglomerular cells [22]. As renin is secreted into the circulation, it promotes increased levels of Ang I, ultimately increasing circulating Ang II. These hormone-dependent mechanisms of regulation play essential roles in normal physiology, but when chronically activated, these initially adaptive ion channel regulatory mechanisms may lead to dysfunction [23]. In the present study, we found that RSD could suppress increased the levels of BNP, Ang II, aldosterone, ET-1 and decreased renalase in HF dogs, but had not significant impact on these neurohormonal factors in normal physiological dogs. The previous studies had showed that RSD not only decrease the levels of BNP, Ang II and aldosterone in atrial fibrillation or obstructive sleep apnea animal model, but increase the level of renalase in HF model or adult rats with spontaneous hypertension [4, 7, 21, 24, 25]. All these animal models had the activation of RAAS and sympathetic nerve system. The detrimental effects of chronic sympathetic nerve system activation must also be considered in the context of activation of multiple neurohormonal systems, predominantly RAAS. Denervation of afferent renal nerves may lower global sympathetic tone, by interruption of afferent renal signals that stimulate the sympathetic nerve system [26]. Taken together, RSD may attenuate the overactivation effects of the sympathetic nerve system and serve a cardioprotective in the failing heart [6]. Our findings implied that RSD had potential effect in the cardiovascular disease with chronic activation of RAAS and sympathetic nerve system.

Study limitations

The present study has several limitations. First, we did not further to investigate the time course changes of neurohormones in myocardial tissues in the HF and the effect of neurohormones on the progression of HF. Thus, it is unknown whether the ventricular substrate remodeling begins shortly after the initiation of pacing or only after neurohormonal factors has taken place. Second, we performed the pacing and RSD duration for 3 weeks. Whether long-term RSD had similar impact on the plasma neurohormones in HF or normal physiological canines should be further investigated. Third, we did not evaluate the potential effects of the neurohormones after RSD. For example, our results showed that RSD inhibited the changes of plas-

ma renalase and ET-1 in HF model. Whether the effect had a cardioprotective or a deleterious function in the HF should be further investigated.

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Disclosure of conflict of interest

None.

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