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# The secretion patterns and roles of cardiac and circulating arginine vasopressin during the development of heart failure

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ABSTRACT

**Objective**The aim of this study is to investigate local cardiac and circulating AVP secretion during heart failure and to determine whether AVP mediates ventricular remodeling.
**Methods**We assessed cardiac function and AVP levels of post-myocardial infarction (MI) heart-failure rats 3 weeks (n = 10), 4 weeks (n = 10), 6 weeks (n = 10), 9 weeks (n = 15) after the proximal left anterior descending coronary artery (LAD) ligation. Ten sham-operated rats were used as the control group. In vitro, cardiac microvascular endothelial cells (CMECs) were initiated from isolated Wistar rat hearts and subjected to Ang II to induce AVP expression and secretion. Besides, the effects of AVP stimulation on CMECs and cardiac fibroblasts (CFs) were studied using methylthiazol tetrazolium assay, Western blotting and real-time PCR.
**Results**With cardiac dysfunction, plasma and local cardiac AVP, aldosterone levels increased over time, peaking at 9 weeks post-MI. AVP levels were negatively correlated with serum $Na^+$ and LVEF but positively correlated with LVEDD and myocardial hydroxyproline. In CMECs treated with Ang II, AVP mRNA and protein expression increased. In addition, AVP promoted CFs proliferation and up-regulated the expression of endothelin-1 and connective tissue growth factor.
**Conclusion**CMECs are the cellular sources of elevated local heart AVP stimulated with Ang II/AT1. An intrinsic cardiac AVP system exists. Local cardiac and circulating AVP secretion were enhanced by deteriorating cardiac function. AVP may promote ventricular remodeling. Thus, AVP could be an important mediator of myocardial fibrosis in late-stage heart failure.



## 1. Introduction

Arginine vasopressin (AVP) is a neuroendocrine peptide that is primarily synthesised by the neurosecretory cells of the supraoptic and paraventricular hypothalamus nuclei and released via the posterior pituitary in response to hyperosmolarity, hypotension, or hypovolemia. AVP is attracting attention for its biological properties such as the regulation of body fluid osmolality, blood volume, and vascular tone (Baylis, 1987; Thibonnier, 2003). Studies have reported that AVP is the key factor in the development of chronic water retention and the main cause of hyponatremia (Adrogue and Madias, 2000). AVP receptor antagonists are under development (Finley et al., 2008): for example, conivaptan, the combined V1a/V2-receptor antagonist, recently received U.S. Food and Drug Administration approval for the treatment of hyponatremia in heart failure patients.

Changes in plasma nerve-endocrine-cytokines provide insights into the development of heart failure and guide the treatment and prognosis of heart failure. B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) are generally considered biomarkers for diagnosing heart failure; however, BNP and NT-pro-BNP have several limitations, particularly grey areas in diagnosis that are greatly affected by renal function, gender, age, obesity, body mass index, heart rate, genetic polymorphism and blood volume (Korenstein et al., 2007). Patients and rats with heart failure present elevated plasma AVP concentration and aggravated water retention (Francis et al., 2001; Goldsmith et al., 1983; Nakamura et al., 2006; Szatalowicz et al., 1981). AVP increases the cardiac preload and therefore promotes the progression of heart failure. In advanced heart failure, changes in neurohumoral factors and the inappropriate administration of diuretics result in renal haemodynamic abnormalities with refractory water retention and progressive renal impairment (Damman et al., 2014), which affect the diagnosis rate of BNP and NT-pro-BNP. AVP is considered the major neurohormone that mediates fluid retention in advanced heart failure. Hence, AVP is a promising biomarker for the diagnosis of advanced heart failure and disease severity evaluation at certain stages. The purpose of the present study is to investigate the plasma

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AVP concentration at various time points in the progression of heart failure.

AVP is present in plasma of homozygous Bratttelboro, centrally AVP-deficient and hypophysectomised rats (Balment et al., 1986; Kim et al., 1997), which led us to conclude that peripheral organs produce AVP. Earlier studies have shown the distribution of AVP in the aortas of rabbits and rats (Loesch et al., 1991; Simon and Kasson, 1995). Later, Hupf et al. (1999) observed that the presence of AVP was in the isolated perfused rat hearts and the release of AVP increased with pressure overload. Previous researches have suggested that cardiovascular tissue is responsible for the AVP autocrine. However, the patterns and regulative mechanisms of local cardiac AVP secretion remain unclear. In the current study, we focused on AVP expression in rat myocardial tissue with heart failure and explored the cellular origin of AVP.

Moreover, AVP plays an important role in cardiovascular homeostasis. Whereas the vascular effects of AVP are well characterised, AVP's direct cardiac actions are less clear. AVP has been observed to enhance the synthesis of protein, creating cell hypertrophy in cardiomyocytes and smooth vascular muscle cells (Geisterfer and Owens, 1989; Tahara et al., 1998). Nevertheless, the role of AVP in cardiovascular remodelling, especially myocardial fibrosis, has yet to be illustrated. Connective tissue growth factor (CTGF) mediates the development of myofibroblasts by enhancing transforming growth factor (TGF) β1's ability to induce fibroblasts to differentiate into myofibroblasts, a critical process in fibrosis (Zeisberg et al., 2000).

Multiple neuroendocrine systems are involved in the development of heart failure, including the renin-angiotensin-aldosterone system (RAAS), the AVP system, the natriuretic peptide system, and the endothelial system. AVP shares several properties with RAAS and the endothelial system such as the regulation of hydromineral balance and vasoconstriction. Nonetheless, the exact relations among these systems have not been clarified. This study explores these neurohormonal systems activated in heart failure, with a focus on the role of AVP.

## 2. Materials and methods

### 2.1. Heart failure rat model in vivo

Normal male Wistar rats (N = 75) weighing 200 g to 250 g were obtained from the Experimental Animal Centre of Sun Yat-sen University (Guangzhou, China). All procedures were approved by the Experimental Animal Ethics Committee of Sun Yat-sen University. Experimental myocardial infarction-induced heart failure was produced by ligating the left anterior descending coronary artery, as previously described (Klocke et al., 2007). Ten sham-operated rats were used as the control group. Echocardiography was performed 3 weeks post-surgery. The rats with a left ventricular ejection fraction (LVEF) no higher than 45% and a weak cardiac impulse in the left ventricular anterior wall were randomly divided into five experimental heart failure groups: (1) the sham-operated group, which was used as a control group (sham, n = 10); (2) the 3 weeks post-infarction group (3w-HF, n = 10); (3) the 4 weeks post-infarction group (4w-HF, n = 10); (4) the 6 weeks post-infarction group (6w-HF, n = 10); and (5) the 9 weeks post-infarction group (9w-HF, n = 15). Twenty rats with LVEF >45 % or death were excluded.

### 2.2. Cell culture in vitro

Wistar rats aged 5–7 days were obtained from the Experimental Animal Center at Sun Yat-sen University. Primary rat cardiac microvascular endothelial cells (CMECs) were isolated as previously described (Nishida et al., 1993). The CMECs were grown in Dulbecco's modified Eagle's medium (DMEM, HyClone, USA) containing 10% foetal bovine serum (FBS, Gibco, USA), 10% newborn calf serum (NBS, Gibco, USA) and 100 μg/mL ECGS (Sigma, USA). The CMECs were characterised by typical cobblestone morphology and positive staining for CD31 (sc-1506, Santa Cruz Biotechnology, USA) and factor VIII (sc-33584, Santa Cruz Biotechnology, USA), which are surface markers for microvascular endothelial cells. The medium was changed every 2 days, and cells from passages 2–4 were used in all of the experiments. After starvation for 24 hours, the CMECs were exposed to Ang II, losartan, AVP, SR49059, or vehicle for 24 hours.

Primary cardiac fibroblasts (CFs) were isolated as previously described (Gao et al., 2009), and grown in supplemented DMEM media containing 10% FBS. The CFs were treated with AVP, SR49059, or vehicle.

### 2.3. Echocardiography measurements

Echocardiography was performed 3 weeks post-surgery and 1 day before the sacrifice to evaluate the changes in cardiac morphology and blood flow. The echocardiography was performed by an experienced operator using ESAOTE ultrasound Doppler equipment. M-mode tracings of the long-axis view of the left ventricle were captured, and the following indexes were collected: the left ventricular systolic diameter (LVESD), the left ventricular diastolic diameter (LVEDD), the left ventricular ejection fraction (LVEF), and the left ventricular fractional shortening (LVFS). The echocardiograph operator was blinded to the group allocation at all times. All of the echocardiograms were recorded for off-line analysis. The enumerated data were presented as the average of three cardiac cycles.

### 2.4. Tissue preparation and immunohistochemistry

The heart was arrested in diastole with an intraventricular injection of KCl (10%). The atria and the right ventricular free wall were excised; the ventricles were rinsed with isotonic saline and then dissected and weighed. The weights of the ventricles were normalised to the body weight and used as an index of ventricular hypertrophy. To estimate collagen production, the hydroxyproline level in the left ventricle was determined using the hydroxyproline assay according to the manufacturer's instructions (BioVision, USA).

Left ventricle tissue specimens were cross-sectioned at the level of the papillary muscle, fixed and dehydrated in 10% formaldehyde, and embedded in paraffin for immunohistochemistry of AVP (1:5000; Millipore, USA).

### 2.5. Enzyme-linked immunosorbent assay

Blood from the abdominal aortas was collected in sodium citrate anticoagulant tubes before sacrifice. The blood was centrifuged at 3000 r/min at 4 °C, and the supernatant was collected and kept at –80 °C. The myocardial tissue samples were grounded thoroughly with a glass homogeniser in phosphate-buffered saline solution (0.01 M, pH 7.4) and centrifuged at 3000 r/min for 20 minutes. The supernatant was collected for the detection of AVP and aldosterone. The cell culture medium of the CMECs was collected to determine AVP levels. The AVP and aldosterone levels were determined using a commercially obtained enzyme-linked immunosorbent assay (ELISA) kit (ADI-900-017,Enzo; 10004377, Cayman) according to the manufacturer's instructions.

### 2.6. Quantitative real-time PCR

Total RNA was extracted using Trizol (Sigma, USA) according to the manufacturer's instructions and quantified using NanoDrops spectrophotometer. Then 1 μg of the isolated total RNA was reverse transcribed using an Omniscript RT Kit (Qiagen, Australia) according to the manufacturer's protocol. The single-strand cDNA was