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Original research article

The effects of vasoactive peptide urocortin 2 on hemodynamics in spontaneous hypertensive rat and the role of L-type calcium channel and CRFR2

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ABSTRACT

Background: Urocortin (UCN) is a newly identified vascular-active peptide that has been shown to reverse cardiovascular remodeling and improve left ventricular (LV) function. The effects and mechanism of urocortin 2 (UCN2) *in vivo* on the electrical remodeling of left ventricle and the hemodynamics of hypertensive objectives have not been investigated.
Methods: UCN2 (1 μg/kg/d, 3.5 μg/kg/d or 7 μg/kg/d) was intravenously injected for 2 weeks and its effects on hemodynamics in spontaneously hypertensive rats (SHRs) observed. The whole-cell patch clamp technique was used to explore the effects of UCN2 on the electrical remodeling of left ventricular cardiomyocytes. The flow cytometry method was used to determine the content of fluorescence calcium in myocardium.
Results: UCN2 improved the systolic and diastolic function of SHRs as demonstrated by decreased left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), increased $+dp/dt_{max}$ and $-dp/dt_{max}$ and decreased cAMP level. UCN2 inhibited the opening of L-type calcium channel and decreased the calcium channel current of cardiomyocytes. In addition, UCN2 also decreased the contents of fluorescence calcium in SHR myocardium. However, astressin2-B (AST-2B), the antagonist of corticotropin-releasing factor receptor 2 (CRFR2), could reverse the inhibitory effects of UCN2 on calcium channel.
Conclusion: UCN2 can modulate electrical remodeling of the myocardium and hemodynamics in an experimental model of SHR *via* inhibition of L-type calcium channel and CRFR2 in cardiomyocytes.

Introduction

Hypertension is one of the major health problems around the world. It is found to be the key trigger of many events leading to various pathophysiological alterations and potentially to a fatal outcome [1,2]. In the cardiovascular system, hypertension can enhance the heart overload, which in turn leads to cardiac remodeling and ultimately to heart failure. The development of left ventricular (LV) remodeling constitutes a key component of hypertension and a variety of cardiovascular diseases. Vascular and left ventricular hypertrophy confers a definite risk of increased cardiovascular morbidity and mortality that is independent of the arterial blood pressure [3]. On the other hand, because electrical remodeling has emerged as an important pathophysiologic mechanism underlying both hypertrophic and failure phenotypes of a variety of cardiovascular diseases, identification of regulators that can tackle electrical remodeling is essential for the control of hypertension-related cardiac pathology.

Many recent reports have shown that a newly isolated vascular-active peptide urocortin (UCN) can reverse cardiovascular remodeling and improve LV functions and hence prevent a series of complications, such as myocardial infarction and atherosclerosis [4]. UCN is a 40-amino-acid peptide related to the hypothalamic hormone corticotrophin releasing factor (CRF) family, the central mediator of the hypothalamic-pituitary-adrenal axis and stress response in mammals [5]. CRF family members exert their physiological effects through binding to two G protein-coupled

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receptors (CRFR), CREF-1 and -2, to modulate endocrine, autonomic, and behavioral responses to stress. Altogether three types of UCN, *i.e.* UCN1, UCN2 and UCN3, have been identified. CRFR1 has a strong affinity for CRF and UCN1 peptides while UCN2 and UCN3 only show specific binding to CRFR2. The use of stepwise methodologies have led to the development of CRFR1 agonist stressin 1, the long-acting antagonists astressin-2B (AST-2B) which is specific for CRFR2; and astressin B, which binds to both CRFR1 and CRFR2. This analog has potential for the treatment of CRF-dependent diseases in the periphery [6,7]. As a new small molecular active peptide, UCN confers protective effects *via* autocrine and/or paracrine pathways [4,8]. Specifically, the UCNs are identified as endogenous vasoactive peptides that can exert powerful beneficial neurohormonal, hemodynamic, and renal actions in experimental heart failure [9]. A recent study showed that UCN2 has much more potent effects on the cardiovascular system than CRF [10]. It can enhance cardiac contractility, coronary blood flow, heart rates and cardiac output. Some *in vitro* and *ex vivo* investigations showed that in the cases of ischemia/reperfusion (I/R), atherosclerosis and hypertension, UCN2 protect cardiac cells from severe injury [11]. Pretreatment of cardiac myocytes with UCN2 could can improve myocytes' condition *via* mitogen-activated protein kinase (MAPK) pathway, protein kinase C, ATP sensitive K^+ (PKC/K_{ATP}) and calcium channels, *etc.* [12,13]. Our *in vivo* study demonstrated that UCN2 could reduce cardiac damage in an animal model of myocardial I/R injury. Moreover, we also found that UCN2 could inhibit the proliferation of the vascular smooth muscle cells, highly suggesting that UCN2 might play a role in reversing cardiovascular remodeling [14]. However, these data were all obtained from *in vitro* studies. Whether UCN2 promotes or reverses cardiovascular remodeling needs to be further investigated *in vivo*. In addition, as an important aspect of electrical cardiac remodeling, it leaves to be determined whether calcium channel can be influenced by UCNs. In the present study, we aim to observe the effects and mechanism of UCN2 *in vivo* on the remodeling of LV and the hemodynamics in SHR. Our results provide novel evidence showing that UCN2 exerts protective effects on hypertensive myocardium through mechanisms that are dependent on inhibition of the calcium channel and its binding to CRFR2.

Materials and methods

Animals experimental groups

Animal care and experimental Protocols were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996) and approved by the ethics committee of Liaoning medical university. Thirty male SHR and 6 male WKY rats (weight, 250–300 g; age, 20–23 wk, purchased from Experimental Animal Center of Shanghai Animal Institute). These animals were randomly divided into the following groups with 6 animals in each: 1. WKY group (WKY rat, $n = 6$); 2. SHR control group (SHR); 3. UCN2a, urocortin 2 low dose group (1 μg/kg/d); 4. UCN2b, urocortin 2 middle dosage group (3.5 μg/kg/d); 5. UCN2c, urocortin 2 high dose group (7 μg/kg/d); 6. Verapamil treatment group (VER group was the positive control group, 10 mg/kg/d).

Administration of medication

UCN2 (purchased from Sigma-Aldrich, 1 μg/kg/d, 3.5 μg/kg/d, 7 μg/kg/d) was intravenously injected for 2 weeks in the low, middle, high dose groups, respectively, and the tail arterial pressure was tested every day. VER (provided from Sigma-Aldrich, 10 mg/kg/d) was dissolved in distilled water and intravenously injected for 2 weeks. The SHR control group and WKY group were given normal saline.

Hemodynamic measurements

Two weeks after the initiation of therapy, the hemodynamic studies were performed in each group using the Power Lab system. After the rats were weighed and anesthetized with intraperitoneal urethane (1 g/kg), the left carotid artery was exposed and separated, a plastic tube, was inserted into LV to record left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), maximal rates of ventricular pressure rise and fall ($+dp/dt_{max}$, $-dp/dt_{max}$), the heart rates (HR), and arterial mean pressure (AMP) were also recorded simultaneously.

Cell isolation and test the concentration of fluorescence calcium

After the hemodynamic studies, the thorax was rapidly opened, the heart was excised and washed in 0.01 mol l^{-1} PBS, being mounted onto the Langendorff perfusion apparatus by cannulating the aorta, the heart was retrogradely perfused at constant temperature (37 °C) and constant pressure (50 cm H_2O) with the Ca^{2+}-free Tyrode's solution for 5–7 min, secondly, nominally Ca^{2+}-free Tyrode's solution supplemented with collagenase (0.02 g/100 ml, Worthington type 2) for 35–40 min, and finally the heart was rinsed with Ca^{2+}-free Tyrode's solution for 3–5 min to stop the digestion. After the perfusion, the ventricles were taken at the atrioventricular border from the perfusion apparatus and cut out in small pieces of 2–3 mm in size with ophthalmic scissors in KB solution. The minced tissue was gently agitated at 37 °C for about 5 min to dissociate the cells and then filtered through 200 μmol/l mesh nylon gauze to eliminate large superfluous cell material. The isolated myocytes were separated by their gravity in KB solution after settled for 45 min at room temperature and added into 800 μl PBS solution, centrifuged 5 min, 2 times, then added 10 μl Furo 3, used flow cytometry to test the concentration of fluorescence calcium. The effect of the CRF2R selective antagonists astressin-2B (10^{-6} mol/l) was added in isolated myocytes of UCN2b group to observe the effect of UCN2.

Patch-clamp recordings

Single ventricular myocytes were dissociated enzymatically as we reported previously. The currents were recorded by a patch clamp amplifier (Axon 200B, Inc.), Digidata 1322A, and p-Clamp software 8.2 (Axon Instrument, Foster City, CA, USA). Borosilicate glass electrodes were pulled using a level puller (Sutter Instruments, Model P-97) and had a resistance of 2–5 MΩ when filled with the electrode internal solution. After gigaseal and membrane rupture, the electrode resistance in series to the cell membrane was compensated, and the $I_{Ca,L}$ currents were recorded in a voltage-clamp mode. Cell capacitance was measured by integrating the area of the capacitive transient. Data acquisition, storage and analysis running on a personal computer were accomplished with p-Clamp 8.2 (Axon Instrument). Currents of $I_{Ca,L}$ were elicited by a 200 ms-long depolarizing step pulse from the holding potential of −90 mV to 0 mV at the frequency of 1 Hz. Pulses from −40 mV to 60 mV in 10 mV increments were used to elicit the membrane currents for obtaining I–V curve.

Statistical analysis

Statistical analysis was performed with SPSS version 14.0 statistics software package and values were expressed as means ± standard deviation (SD). Differences between groups were evaluated as follows: one-way ANOVA analysis of variance was used,