

Original Article

Relationship and mechanism of Kv2.1 expression to ADH secretion in rats with heart failure

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Abstract: Objective: To explore the mechanisms of Kv2.1 on the secretion of ADH in rats with heart failure. Methods: In the animal study, 70 healthy male SD rats were selected. Ligation of coronary heart failure model surgery was performed in 60 rats and sham surgery was performed in the other 10 rats. Q-PCR was used to detect the mRNA expression of Kv2.1 in hypothalamus and heart. The protein expression of Kv2.1 and ADH was detected by western blot. In the cell culture study, hypothalamic neurons were cultured and divided into 7 groups. The mRNA expression of Kv2.1 and ADH was detected by Q-PCR. The protein expression of Kv2.1, CamKII, phosphorylation SynapsinI, dephosphorylation SynapsinI and ADH was detected by western blot. Results: Compared with the control group of heart failure, LVEDD, LVESD, LVEDV and LVESV were significantly decreased ($P < 0.01$), and LVEF and LVFS were significantly increased ($P < 0.01$) in the Kv2.1 agonist group; in the Kv2.1 inhibitor group, LVEDD, LVESD, LVEDV and LVESV were significantly increased ($P < 0.01$), and LVEF and LVFS were significantly decreased ($P < 0.01$). In cell culture study, after the different concentrations of Kv2.1 inhibitor gradient down the expression of Kv2.1, intracellular Ca^{2+} concentration gradient increased ($P < 0.01$), CamKII and phosphorylation of SynapsinI protein expression gradient increased ($P < 0.01$), dephosphorylation of SynapsinI protein expression gradient decreased ($P < 0.01$), and the ADH mRNA and protein expression of gradient increased ($P < 0.01$). Conclusions: Kv2.1 agonist can prevent the calcium overload by reducing the intracellular Ca^{2+} concentration, so that the phosphorylation of SynapsinI reduces and exocytosis in hypothalamic neurons is inhibited, which ease the secretion of ADH.

Keywords: Heart failure, vasopressin, Kv2.1, CaMKII, SynapsinI

Introduction

Dilution hyponatremia is one of the most important complications in patients with heart failure, which has a serious impact on the prognosis of the disease. Fluid retention in the process of heart failure is often accompanied by a decrease in serum sodium levels [1-5]. The mechanism of the occurrence and development of hyponatremia in patients with heart failure has not been fully clarified. A large number of studies have indicated that the excessive release of ADH may be the main reason [6-9].

In recent years, most of the studies on the diluted hyponatremia have selected ADH as the target, and mainly focused on the downstream V_2 receptor and V_{1a} receptor antagonism. In the early 1970s, the first peptide V_2 receptor antagonist was proved to be effective in animal

experiments [10]. In the year of 1993, Ohnishi et al. found that oral non peptide V_2 receptor selective antagonist produces drainage in healthy subjects [11]. So far, a small number of ADH receptor antagonists have been approved for clinical use. Conivaptan, made in injection type, was approved by FDA in December 2005, but only limited to the use of short-term hospital [12]. Although ADH receptor antagonism has made some progress in the treatment of diluted hyponatremia, it is limited by the application of antagonistic therapy, such as thirst, negative feedback and poor long-term effect. To study the secretion, release and regulation of ADH will be a promising subject.

In our research group, we choose the Kv2.1 potassium current of the hypothalamic neurons as the target to explore whether the Kv2.1 is regulated by the secretion of ADH. Our previous

Kv2.1 expression and ADH secretion

study found that Kv2.1 and ADH were co-expressed in the hypothalamic supraoptic nucleus (SON); the decrease of Kv2.1 expression in hypothalamus of heart failure rats was accompanied by an increase of ADH secretion; and inhibited Kv2.1 function can promote the secretion of ADH from the hypothalamus neurons at the cellular level.

In this study, Kv2.1 agonist and antagonist intervention were given to the rats through the lateral ventricle puncture catheter. The effect of Kv2.1 expression on the secretion of ADH in heart failure rats was studied, and the relationship between ADH secretion, cardiac function deterioration and refractory hyponatremia in the development of heart failure was explored. At the same time, we cultured neurons in the hypothalamus in different concentrations of Kv2.1 activation agent and inhibitor intervention. We observed intracellular Ca^{2+} concentration and the changes in the expression of CamKII, phosphorylation of SynapsinI and dephosphorylation of SynapsinI and their relations with ADH secretion. The mechanism of Kv2.1 effect on ADH secretion was explored.

Materials and methods

Animal experiments

Seventy adult male SD rats with similar age and body weight (200-250 g) were selected (purchased from the experimental animal center of the North Campus of Sun Yat-sen University), 60 of which were under the coronary artery ligation and heart failure model and 10 of which were under the sham operation group. Echocardiography was performed for all animals two weeks after ligation of the coronary artery. One week after the echocardiography, lateral ventricle puncture was conducted for all the rats. At last, ten rats were grouped in the sham operation group. The 45 survival rats after the success of heart failure model and ventricle puncture were randomly grouped into control group of heart failure (n=15), Kv2.1 agonist group (n=15), and Kv2.1 inhibitor group (n=15).

Kv2.1 mRNA expression in hypothalamus and heart was detected by Q-PCR method. Kv2.1 and ADH protein expression in hypothalamus and heart was determined by Western blot method. ImageJ analysis software was used to measure the optical density of the bands, with

GAPDH bands as the reference bands. The expression levels of Kv2.1 and ADH were expressed in the ratio of Kv2.1 bands and ADH bands optical density with GAPDH bands optical density.

Culture of cells

Hypothalamus was selected according to the stereotaxic atlas of the rat brain and was made into 1 mm size tissue block. Trypsin was digested into single cell suspension. The cells were counted under the microscope and inoculated in a culture dish coated with poly lysine. Experimental intervention drugs were Memantine and Stromatoxin-1, which were purchased from sigma, and were dissolved in PBS with PH of 7.2-7.4.

Cultured cells were divided into 7 groups: (1) control group (0.9% NaCl); (2) 2 μ mol Memantine group; (3) 10 μ mol Memantine group; (4) 50 μ mol Memantine group; (5) 10 nM Stromatoxin-1 group; (6) 40 nM Stromatoxin-1 group; and (7) 100 nM Stromatoxin-1 group. Drug interventions were given according to the groups in the second day after medium changed. After 24 h of continuous intervention, the trypsin digested the cells. The intracellular Ca^{2+} concentration was measured by calcium fluorescent indicator method, and the mRNA and protein of the cells were extracted. The expression of Kv2.1 and mRNA ADH in hypothalamic neurons was detected by Q-PCR. The expressions of Kv2.1, CamKII, SynapsinI phosphorylation, SynapsinI dephosphorylation, and ADH protein in hypothalamic neurons were determined by Western blot. ImageJ analysis software was used to measure the optical density of the bands, with GAPDH bands as the reference. The expression levels of Kv2.1, CamKII, SynapsinI phosphorylation, SynapsinI dephosphorylation, and ADH were expressed as the ratio of Kv2.1, CamKII, SynapsinI phosphorylation, SynapsinI dephosphorylation, and ADH optical density with GAPDH band optical density.

Statistical methods

The experiment were repeated for 3 times, and the mean value was expressed as mean \pm standard deviation (SD). T-test was used to compare the difference between the two groups and Pearson correlation analysis was used to analyze the correlation between the two groups.

Kv2.1 expression and ADH secretion

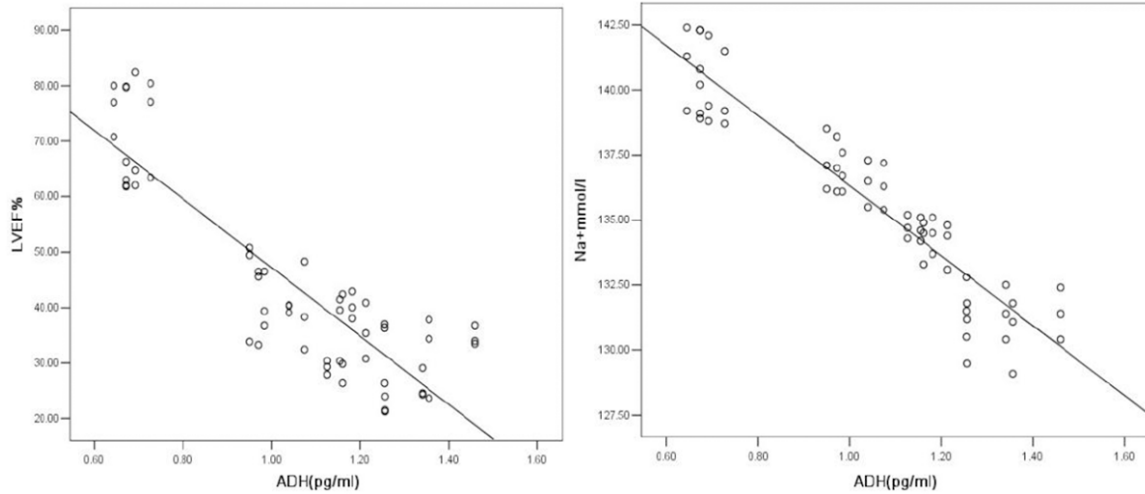


Figure 1. Correlations between ADH and LVEF, and Na^+ and ADH in rat serum plasma.

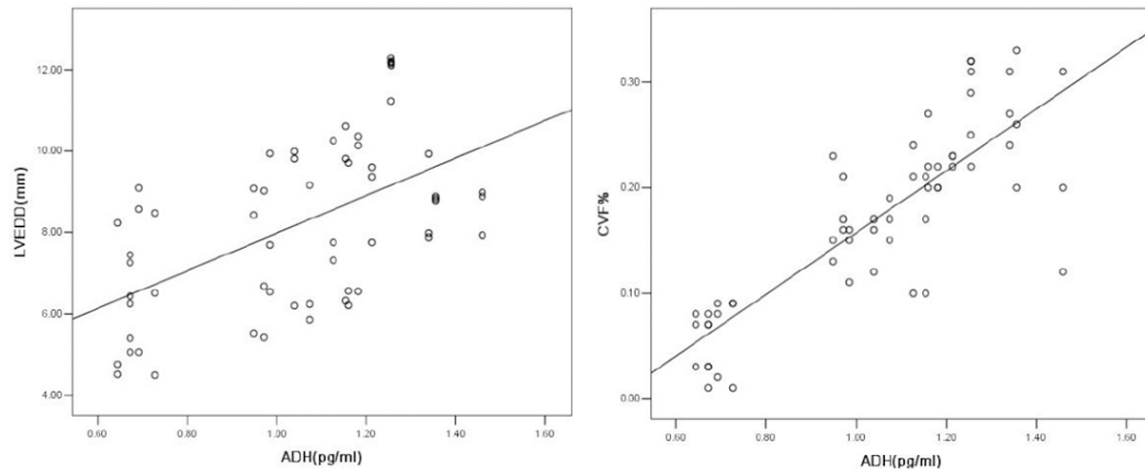


Figure 2. Correlations between ADH and LVEDD, and ADH and CVF% in rat serum plasma.

$P < 0.05$ was considered as statistically significant.

Results

Animal experiments

The level of ADH in rat plasma was negatively correlated with LVEF ($r=-0.761$), and the concentration of Na^+ was negatively correlated with the level of ADH ($r=-0.881$) (**Figure 1**). The level of ADH was positively correlated with LVEDD ($r=0.298$) and CVF% ($r=0.696$) (**Figure 2**).

The results of the echocardiography taken the day before death showed that LVEDD, LVESD, LVEDV and LVESV were significantly increased

($P < 0.01$), LVEF and LVFS were significantly decreased ($P < 0.01$), compared control group of heart failure with sham operated group. Compared with the control group of heart failure, LVEDD, LVESD, LVEDV and LVESV were significantly decreased ($P < 0.01$), and LVEF and LVFS were significantly increased ($P < 0.01$) in the Kv2.1 agonist group; in the Kv2.1 inhibitor group, LVEDD, LVESD, LVEDV and LVESV were significantly increased ($P < 0.01$), and LVEF and LVFS were significantly decreased ($P < 0.01$). The results are shown in **Table 1**.

LVESP and $\pm dp/dt$ were significantly decreased ($P < 0.01$), and LVEDP was significantly increased ($P < 0.01$) in the control group compared with sham operation group. Compared

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Table 1. Baseline levels and echocardiographic data after drug therapy

Parameters	Sham operation group	Control group of heart failure	Kv2.1 agonist group	Kv2.1 inhibitor group
LVEF (%)				
Baseline	80.43±8.13	44.73±8.45 ^a	45.67±11.97 ^a	45.30±12.87 ^a
After	71.37±9.77	35.05±7.23 ^a	41.07±11.46 ^{a,b}	29.64±10.69 ^{a,b}
LVFS (%)				
Baseline	45.72±5.42	26.28±3.59 ^a	26.66±5.89 ^a	26.13±9.43 ^a
After	37.15±4.73	18.78±5.64 ^a	24.76±3.06 ^{a,b}	14.06±3.39 ^{a,b}
LVEDV (dl)				
Baseline	303.09±16.99	426.60±18.87 ^a	426.97±18.40 ^a	429.14±20.29 ^a
After	314.43±18.32	501.17±18.43 ^a	448.04±18.39 ^{a,b}	571.45±14.08 ^{a,b}
LVESV (dl)				
Baseline	59.32±14.17	235.90±15.07 ^a	231.98±11.08 ^a	234.80±13.20 ^a
After	89.92±13.83	325.46±11.64 ^a	264.11±13.52 ^{a,b}	401.98±18.80 ^{a,b}
LVEDD (mm)				
Baseline	5.69±0.93	6.37±0.72 ^a	6.40±0.74 ^a	6.42±0.59 ^a
After	6.50±0.85	8.56±0.91 ^a	7.71±1.22 ^{a,b}	10.02±1.08 ^{a,b}
LVESD (mm)				
Baseline	3.08±0.73	4.68±0.76 ^a	4.69±0.74 ^a	4.74±0.85 ^a
After	4.08±0.73	6.95±0.69 ^a	5.80±0.83 ^{a,b}	8.62±0.76 ^{a,b}

LVESD = left ventricular systolic diameter, LVEDD = left ventricular diastolic diameter, LVESV = left ventricular systolic volume, LVEDV = left ventricular diastolic volume, LVEF = left ventricular ejection fraction, LVFS = left ventricular short axis shortening rate. Baseline: two weeks after heart failure model. After: one day before animals were killed. ^aP < 0.05 compared with sham operation group. ^bP < 0.05 compared with control group of heart failure.

Table 2. Hemodynamic data

Parameters	Sham operation group	Control group of heart failure	Kv2.1 agonist group	Kv2.1 inhibitor group
LVEFP (mmHg)	116.17±7.41	92.46±6.07 ^a	102.20±8.95 ^{a,b}	84.03±3.23 ^{a,b}
LVEDP (mmHg)	-4.08±2.93	7.80±1.43 ^a	3.37±1.77 ^{a,b}	13.40±2.14 ^{a,b}
+dp/dt (mmHg)	2864.39±139.13	1958.56±192.02 ^a	2416.44±104.17 ^{a,b}	1371.35±151.23 ^{a,b}
-dp/dt (mmHg)	-2902.56±257.60	-2009.16±198.96 ^a	-2421.25±149.49 ^{a,b}	-1485.97±201.15 ^{a,b}

LVEFP = left ventricular end systolic pressure, LVEDP = left ventricular end diastolic pressure, +dp/dt = maximum rise rate of left ventricular pressure, -dp/dt = maximum left ventricular pressure drop rate. ^aP < 0.05 compared with sham operation group. ^bP < 0.05 compared with control group of heart failure.

Table 3. Plasma ADH concentrations and electrolyte levels

Parameters	Sham operation group	Control group of heart failure	Kv2.1 agonist group	Kv2.1 inhibitor group
ADH (pg/ml)	0.68±0.01	1.17±0.01 ^a	1.00±0.01 ^{a,b}	1.33±0.02 ^{a,b}
(Na ⁺) (mmol/l)	140.21±1.72	134.29±1.78 ^a	136.49±1.74 ^{a,b}	131.81±1.75 ^{a,b}
(Cl ⁻) (mmol/l)	98.71±1.13	98.48±1.46	98.49±1.42	98.20±1.18
(K ⁺) (mmol/l)	4.72±0.31	4.61±0.38	4.49±0.35	4.52±0.30

^aP < 0.05 compared with sham operation group. ^bP < 0.05 compared with control group of heart failure.

with the control group of heart failure, LVEFP and ±dp/dt were increased (P < 0.01), and LVEDP were decreased (P < 0.01) in the Kv2.1 agonist group; LVEFP and +dp/dt were decreased (P < 0.01), and LVEDP was increased (P < 0.01) in the Kv2.1 inhibitor group. The results are shown in **Table 2**.

Compared with sham operated group, the plasma ADH level was significantly higher (P < 0.01), and the concentration of Na⁺ was significantly lower (P < 0.01) in the control group of heart failure. Compared with the control group of heart failure, the level of ADH was lower (P < 0.01) and concentration of Na⁺ was higher (P <

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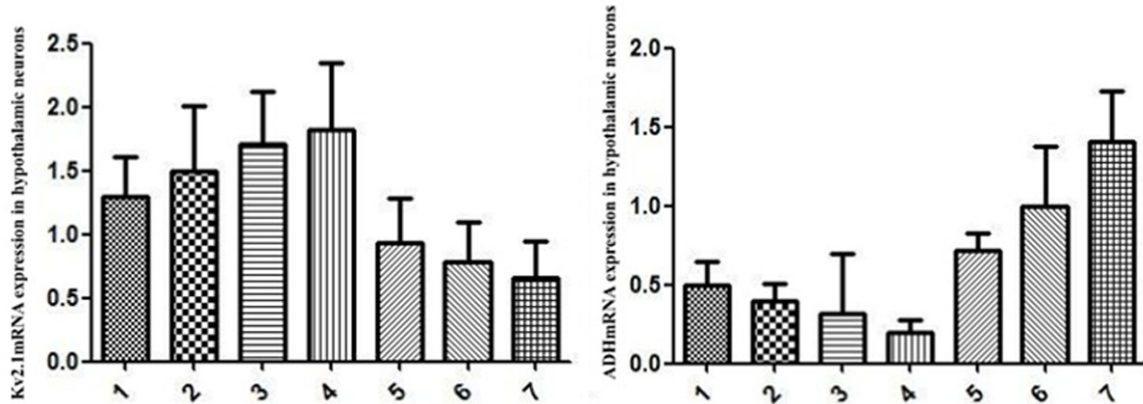


Figure 3. Expression levels of Kv2.1 and ADH mRNA in hypothalamic neurons. 1: control group; 2: Kv2.1 agonist 2 μmol group; 3: Kv2.1 agonist 10 μmol group; 4: Kv2.1 agonist 50 μmol group; 5: Kv2.1 inhibitor 10 nM group; 6: Kv2.1 inhibitor 40 nM group; 7: Kv2.1 inhibitor 100 nM group.

0.01) in the Kv2.1 agonist group; the level of ADH was higher ($P < 0.01$) and concentration of Na^+ was lower ($P < 0.01$) in the Kv2.1 inhibitor group. The concentrations of K^+ and Cl^- were not statistically different between the groups. The results are shown in **Table 3**.

Compared with sham operation group, CVF% was significantly higher in control group of heart failure ($P < 0.01$). Compared with the control group of heart failure, CVF% was lower ($P < 0.01$) in the Kv2.1 agonist group and CVF% was higher ($P < 0.01$) in the Kv2.1 inhibitor group (Figures S1, S2).

Compared with sham operation group, the expression of Kv2.1mRNA and protein in hypothalamus and heart were significantly decreased ($P < 0.01$), and the expression of ADH protein was significantly increased ($P < 0.01$) in the control group of heart failure. Compared with the control group of heart failure, the expression level of Kv2.1mRNA and protein was up-regulated in the Kv2.1 agonist group ($P < 0.01$), and the expression level of ADH was decreased ($P < 0.01$). The expression level of Kv2.1mRNA and protein was down regulated in Kv2.1 inhibition group ($P < 0.01$), and the expression level of ADH protein was increased ($P < 0.01$) (Figures S3, S4, S5).

Culture of cells

In rat hypothalamic neurons, after different concentrations of Kv2.1 agonist gradient up regulated, intracellular Ca^{2+} concentration gradient decreased ($P < 0.01$), CamKII and phos-

phorylation of SynapsinI protein expression gradient decreased ($P < 0.01$), dephosphorylation of SynapsinI protein expression gradient increased ($P < 0.01$), and the ADH mRNA and protein expression gradient decreased ($P < 0.01$). After the different concentrations of Kv2.1 inhibitor gradient down the expression of Kv2.1, intracellular Ca^{2+} concentration gradient increased ($P < 0.01$), CamKII and phosphorylation of SynapsinI protein expression gradient increased ($P < 0.01$), dephosphorylation of SynapsinI protein expression gradient decreased ($P < 0.01$), and the ADH mRNA and protein expression of gradient increased ($P < 0.01$). The results are shown in **Figures 3-5**.

Discussion

The aim of current study was to find out the relationship between plasma ADH levels and cardiac function and electrolyte concentration, which were performed in the whole animal study and cell culture. We explored the relationship between Kv2.1 and ADH secretion in heart failure rats, as well as the mechanism of Kv2.1 affecting ADH secretion. In animal experiments, the model of heart failure was made by ligation of the left anterior descending artery. Our results indicated that compared the rats in the coronary artery ligation model to those in the sham operation group, left ventricular systolic diameter and left ventricular diastolic diameter were increased significantly, left ventricular shoot ejection fraction and left ventricular short axis shortening rate were decreased significantly, and CVF% was increased; the expres-

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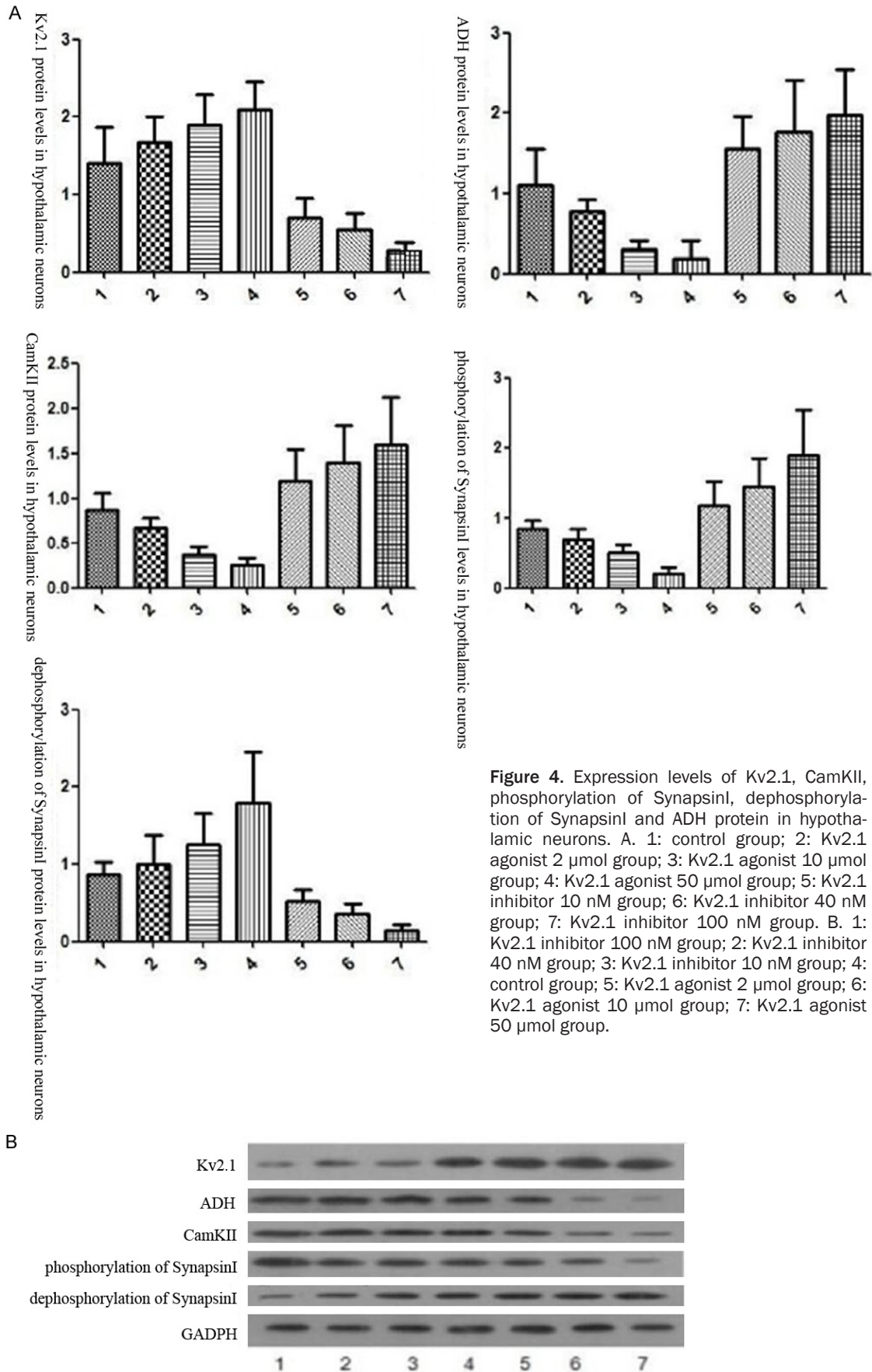


Figure 4. Expression levels of Kv2.1, CamKII, phosphorylation of SynapsinI, dephosphorylation of SynapsinI and ADH protein in hypothalamic neurons. A. 1: control group; 2: Kv2.1 agonist 2 μ mol group; 3: Kv2.1 agonist 10 μ mol group; 4: Kv2.1 agonist 50 μ mol group; 5: Kv2.1 inhibitor 10 nM group; 6: Kv2.1 inhibitor 40 nM group; 7: Kv2.1 inhibitor 100 nM group. B. 1: Kv2.1 inhibitor 100 nM group; 2: Kv2.1 inhibitor 40 nM group; 3: Kv2.1 inhibitor 10 nM group; 4: control group; 5: Kv2.1 agonist 2 μ mol group; 6: Kv2.1 agonist 10 μ mol group; 7: Kv2.1 agonist 50 μ mol group.

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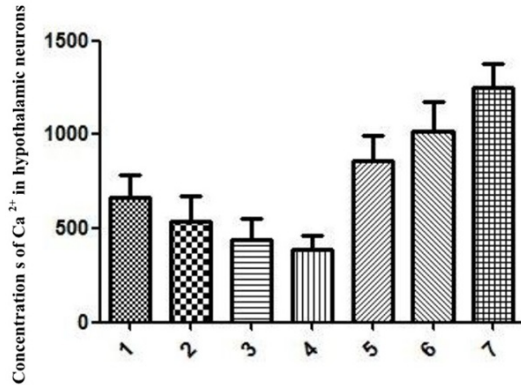


Figure 5. Concentrations of Ca²⁺ in hypothalamic neurons. 1: control group; 2: Kv2.1 agonist 2 μmol group; 3: Kv2.1 agonist 10 μmol group; 4: Kv2.1 agonist 50 μmol group; 5: Kv2.1 inhibitor 10 nM group; 6: Kv2.1 inhibitor 40 nM group; 7: Kv2.1 inhibitor 100 nM group.

sion of ADH protein was significantly increased in hypothalamus and heart, the plasma level of ADH was significantly increased, and Na⁺ concentration was significantly decreased. The plasma level of ADH was negatively correlated with left ventricular ejection fraction, and was positively correlated with left ventricular end diastolic diameter and CVF%. Plasma Na⁺ concentration and ADH level were negatively correlated.

Our study showed that the level of ADH in rat plasma was negatively correlated with cardiac function, which was consistent with Nakamura et al.'s study [13]. The traditional concept believes that when heart failure occurs, decreased heart function and decreased cardiac output make effective circulating blood volume decreases, stimulating left atrial and aortic arch capacity sensor and reducing the carotid sinus and renal afferent baroreceptor stimulation, activating the neuroendocrine system, resulting in ADH non osmotic release [14], and causing dilutional hyponatremia.

Our results showed that the concentration of ADH increased along with the heart failure progression and participate in the occurrence and development of heart failure diluted hyponatremia syndrome and ventricular remodeling. Correcting the excessive secretion of ADH can alleviate the dilution of hyponatremia and ventricular remodeling, which is of great significance in the treatment of heart failure. In 1990, Francis et al. [15]. showed that compared with the symptomatic patients with left ventricular

dysfunction, the plasma ADH concentration was lower in asymptomatic ones, and with the increase of plasma ADH level, heart function of patients deteriorates. SAVE study [16] found that heart failure patients with higher plasma ADH levels have higher one year mortality from cardiovascular disease.

Our animal studies found that the expressions of Kv2.1mRNA and protein in hypothalamus and heart were significantly decreased and ADH secretion was significantly increased in the control group of heart failure compared with the sham operation group. After Kv2.1 agonist intervention, the expressions of Kv2.1mRNA and protein in hypothalamus and heart were increased, and the expression of ADH protein and concentration of plasma ADH were decreased in heart failure rats. After Kv2.1 inhibitor intervention, the expressions of Kv2.1mRNA and protein in hypothalamus and heart were decreased, and the expression of ADH protein and concentration of plasma ADH were increased in heart failure rats. These results indicate that the expression of Kv2.1 decreases and the secretion of ADH increases in the process of heart failure, and Kv2.1 regulates the expression and secretion of ADH negatively in rats with heart failure. At the same time, the results of cell culture showed that Kv2.1 agonist reduced the secretion of ADH in hypothalamic neurons, and Kv2.1 inhibitor increased the secretion of ADH in hypothalamic neurons, which also indicates that Kv2.1 regulates the secretion of ADH negatively in hypothalamic neurons.

Kv2.1 is a member of the Shab family of voltage gated potassium ion channels [17, 18], which is a delayed rectifier potassium current, and plays an important role in cell action potential repolarization phase 3 [19]. The main function of Kv2.1 is to regulate the potassium ions flowing to the outside of the cell, formatting action potential phase 3 descending branch. If the activity of Kv2.1 changes, the cell excitability will be changed. Kv2.1 may adjust the excitable cells and Ca²⁺ currents, effect Ca²⁺ mediated signal transduction, and regulate the secretion of ADH through affect action potential in phase 3 repolarization process. Jacobson et al. found that after knockdown or inhibition of Kv2.1 in pancreatic β cells of rats, the duration of action potential was prolonged, intracellular Ca²⁺ concentration increased, and the insulin secretion increased [20-25]. MacDonald, Tamarina, and

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Herrington also demonstrated that Kv2.1 inhibitor promotes insulin secretion in β cells [22, 23, 25].

Neurotransmitters store in synaptic vesicles. After stimulation of nerve endings, Ca^{2+} influx triggers exocytosis of synaptic vesicle, causing neurotransmitter release. The release of transmitter is often accompanied by phosphorylation or dephosphorylation of the related proteins. It has been proved that the regulation of reversible phosphorylation state of SynapsinI is an important factor to regulate the release of synaptic vesicles during the process of cell vomiting.

Hackett et al. [26]. demonstrated that changes in the phosphorylation state of SynapsinI can affect the number of synaptic vesicles that fuse with the presynaptic membrane, using wave analysis in goldfish neurons. Llinas et al. [27]. injected the dephosphorylation of SynapsinI into giant axon terminals of squid and found a reduction in neurotransmitter release. Lu et al. [28] injected the phosphorylated SynapsinI into cultured neurons of *Xenopus* embryo spinal cord, which enhanced the release of neurotransmitters. Hence, we can see that dephosphorylation of SynapsinI inhibits neurotransmitter release, and phosphorylation of SynapsinI promotes neurotransmitter release.

In order to further clarify the relationship between SynapsinI phosphorylation status and neurotransmitter release, we cultured hypothalamic neurons of neonatal rat, observed the changes in intracellular Ca^{2+} concentration, phosphate SynapsinI, dephosphorylation SynapsinI protein expression and neurotransmitter ADH release after excited and inhibit the expression of Kv2.1. Cell culture results showed that Kv2.1 agonists decrease intracellular Ca^{2+} concentration in the neurons of hypothalamus, decrease the activity of CammKII, decrease the expression of phosphorylation SynapsinI protein, increase the expression of dephosphorylation SynapsinI protein, and decrease the cell spit process inhibit and ADH secretion. Kv2.1 inhibitors increase intracellular Ca^{2+} concentration in the neurons of hypothalamus, increase the activity of CammKII, increase the expression of phosphorylation SynapsinI protein, decrease the expression of dephosphorylation SynapsinI protein, and increase ADH secretion. We believe that Kv2.1 regulates CamKII activity

and phosphorylation state of SynapsinI by affecting the phase 3 complex polarization and intracellular Ca^{2+} concentration, and ultimately regulates exocytosis and the release of ADH.

In conclusion, the effect and mechanism of Kv2.1 on the secretion of ADH were discussed in this study, and it is of great significance to further clarify the mechanism of the occurrence and development of hyponatremia and to develop new drugs. The change of ADH secretion in the brain is of great significance in the development of advanced refractory hyponatremia, ventricular remodeling and cardiac function deterioration in patients with heart failure. Kv2.1 agonist can prevent the calcium overload by reducing the intracellular Ca^{2+} concentration, so that the phosphorylation of SynapsinI reduces and exocytosis in hypothalamic neurons is inhibited, and thus ease the secretion of ADH. Therefore, it is of great value for Kv2.1 agonist in the treatment of heart failure in the future.

Disclosure of conflict of interest

None.

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Kv2.1 expression and ADH secretion

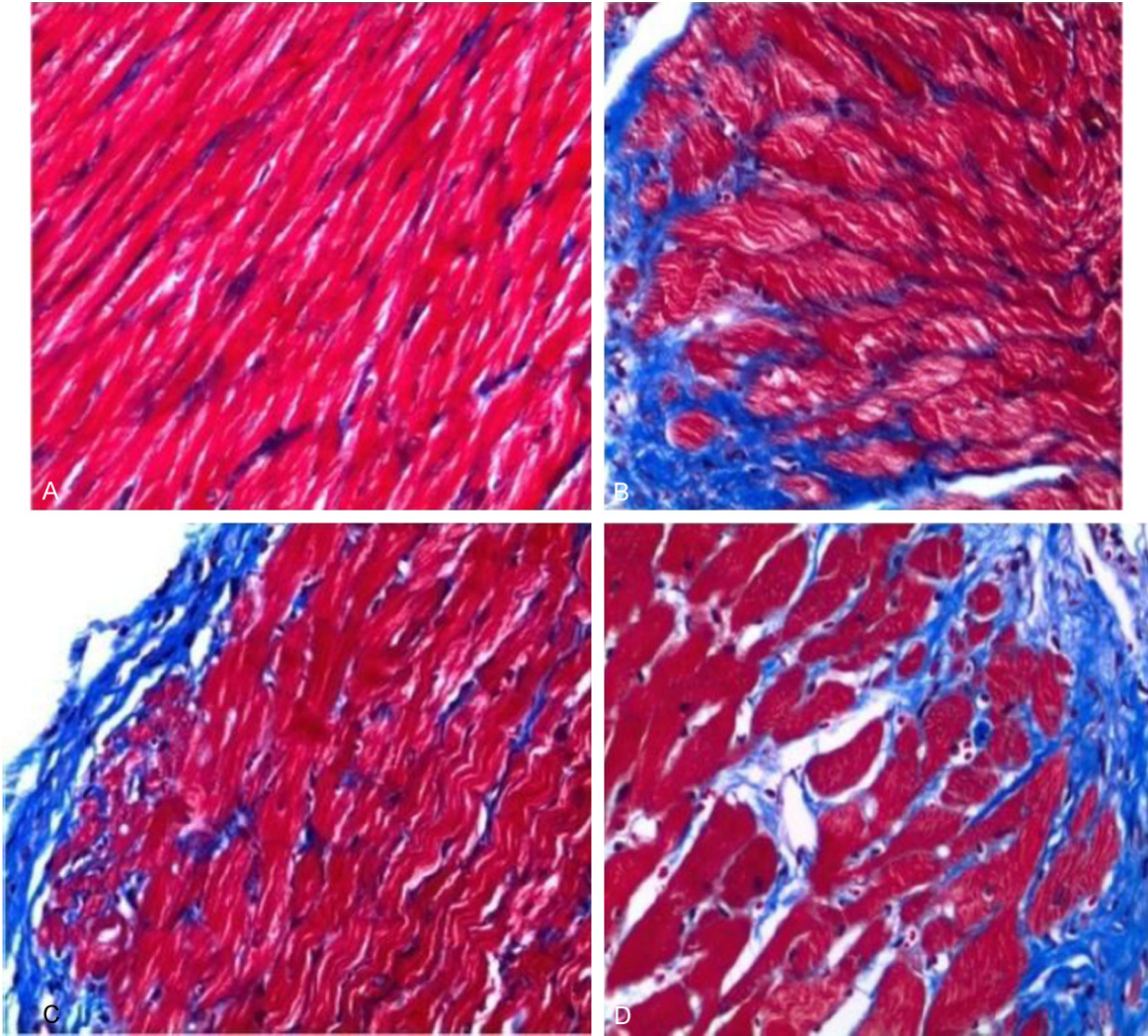


Figure S1. Masson staining of pathological section in the left ventricular non infarct region Blue area represents collagen tissue, and red area represents cardiac muscle tissue.

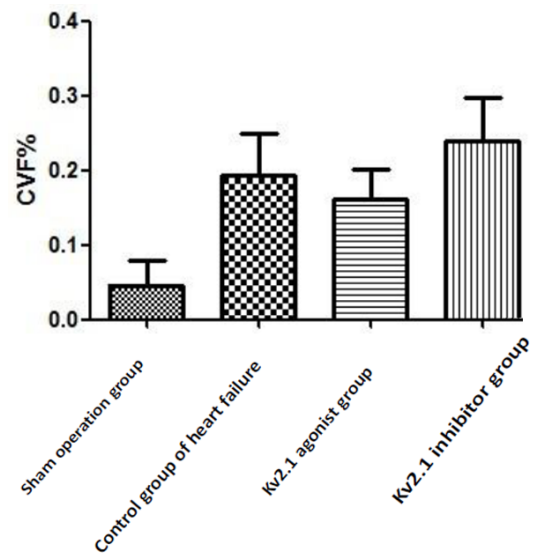


Figure S2. Left ventricular non infarct region CVF%.

Kv2.1 expression and ADH secretion

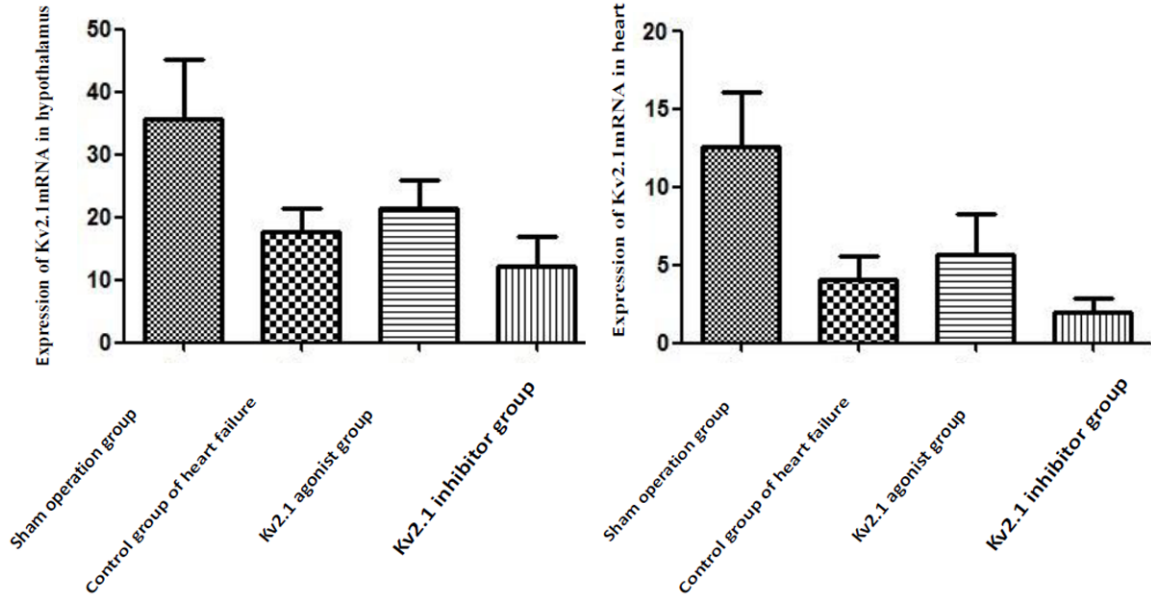


Figure S3. Kv2.1 mRNA expression levels in hypothalamic and heart.

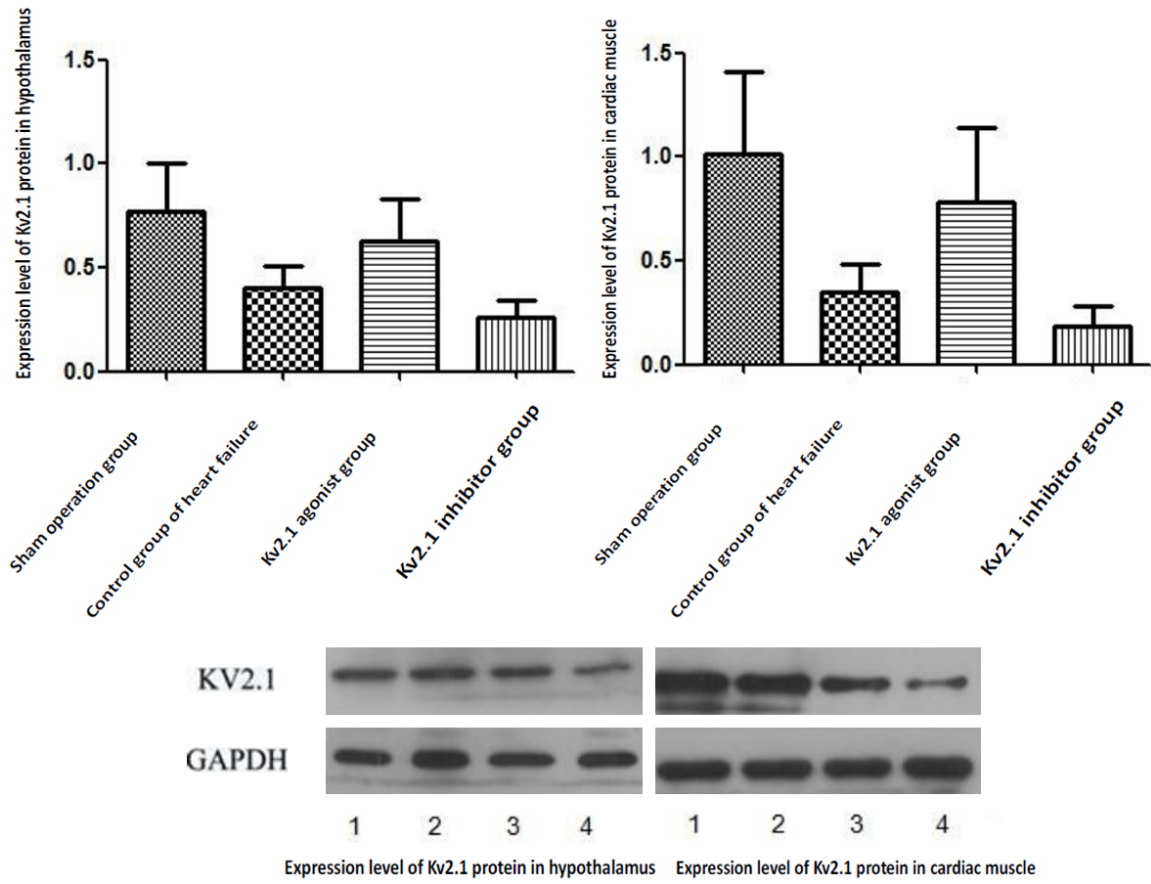


Figure S4. Expression levels of Kv2.1 in hypothalamus and heart. 1: sham operation group, 2: control group of heart failure, 3: Kv2.1 agonist group, 4: Kv2.1 inhibitor group.

Kv2.1 expression and ADH secretion

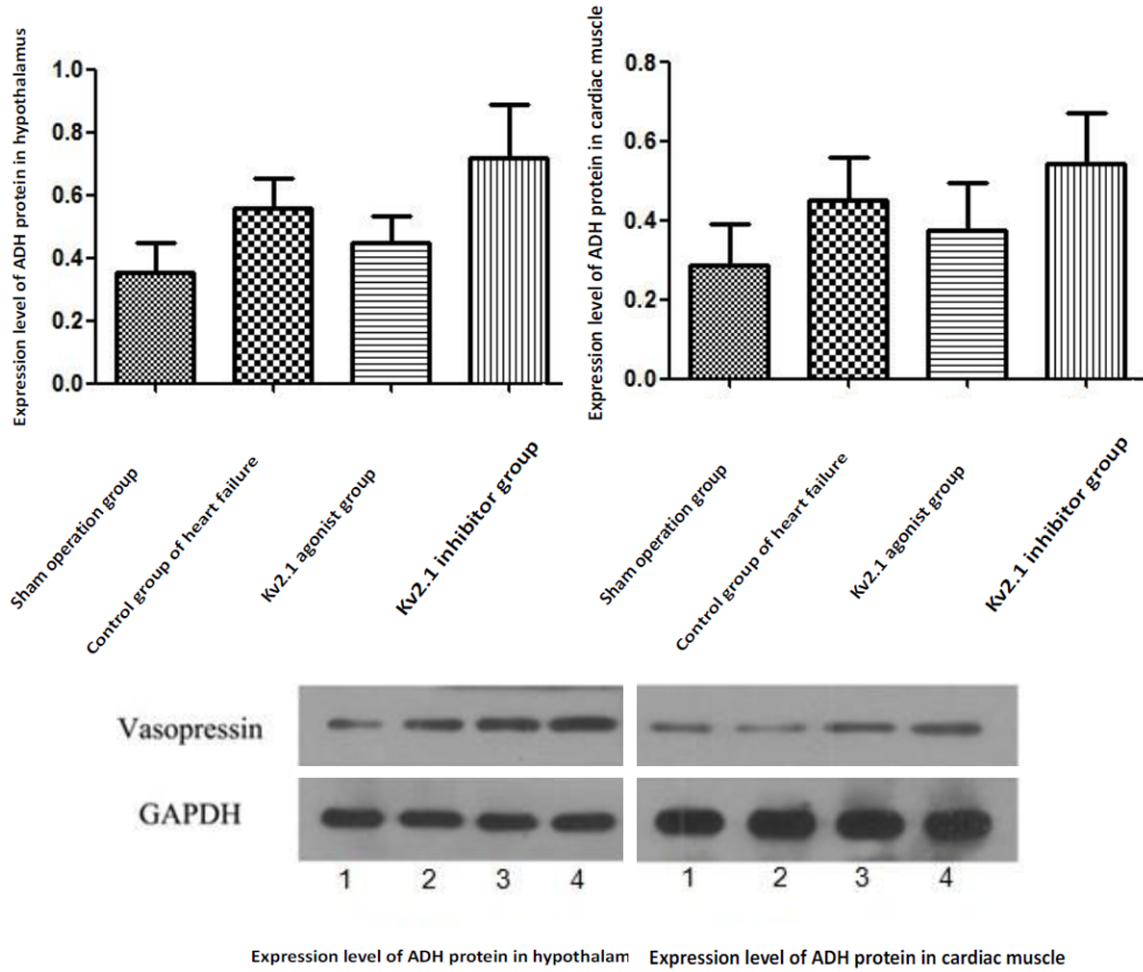


Figure S5. Expression levels of ADH in hypothalamus and heart. 1: sham operation group, 2: control group of heart failure, 3: Kv2.1 agonist group, 4: Kv2.1 inhibitor group.