Glucocorticoids Promote Na+ Excretion in the Renal Epithelia of Heart Failure Rats by Suppressing Transporter Proteins Involved in Acute Sodium Loading

Shuyu Li, MD,* Yaomeng Huang, MD,† Tongxin Li, MD,† Xiaoran Zhu, MD,‡ Weimin Li, BS,† Kunshen Liu, MD, PhD,† and Chao Liu, MD, PhD†

Abstract: Glucocorticoid receptors are essential for normal development and stress responses. Their role in H2O and Na+ metabolism, especially in chronic heart failure (CHF), is not well defined. In a previous study, we found that glucocorticoids potentiate urination in CHF and promote H2O excretion by inhibiting the vasopressin receptor 2 pathway. The present study examines the effect of glucocorticoids on renal Na+ excretion and the underlying mechanisms in CHF rats with acute sodium loading. CHF was induced by left coronary artery ligation for 8 weeks. Rats were randomly assigned to 5 groups: control, CHF, dexamethasone (DEX)-administered CHF, DEX-administered CHF treated with RU486 (mifepristone, a glucocorticoid receptor antagonist), and RU486-treated CHF. An acute sodium loading test was performed 6 hours after DEX administration. Blood and urine samples were collected, and hemodynamics were measured. The expression and localization of Na+ transporter proteins were determined by immunoblotting and immunohistochemistry. DEX increased the urine volume and urinary sodium and improved cardiac function and the estimatedglomerular filtration rate in CHF rats. The upregulation of the epithelial sodium channel β and γ subunits, Na-K-2Cl cotransporter, serum glucocorticoid-regulated kinase 1 (SGK1), and Na+/K+/ATPase in the renal epithelium of CHF rats was downregulated by DEX. These beneficial effects were abolished by RU486. The expression of natriuretic peptide receptor A was opposite that of the above proteins. Glucocorticoids might induce profound natriuresis in CHF rats during acute sodium loading, which is associated with downregulating some Na+ transporter proteins in the renal epithelium and improving intrarenal hemodynamics.

Key Words: glucocorticoids, Na+ transporter proteins, epithelial sodium channel, Na-K-2Cl cotransporter, Na+/K+/ATPase, serum glucocorticoid regulated kinase 1, natriuretic peptide receptor A

J Cardiovasc Pharmacol 2022;80:453–463

INTRODUCTION

Glucocorticoid receptors (GRs) are ubiquitously expressed in the body and are essential for normal development and stress responses.1 Systemic administration of glucocorticoids therefore has many effects, including altering body fluid and electrolyte metabolism, cardiac output, and systemic vascular resistance.2 However, their role in H2O and Na+ metabolism, especially in the context of diseases with fluid or sodium overload, such as chronic heart failure (CHF), is not well defined. Tubular reabsorption of filtered H2O and Na+ is tightly controlled to maintain body volume homeostasis. Most H2O and Na+ reabsorption is performed by the proximal tubule, loop of Henle, and distal convoluted tubule (DCT). The function of these terminal renal tubules is highly controlled to achieve H2O and Na+ balance, relying on aquaporins (AQPs), and segment-specific apical sodium transporters. We previously demonstrated that glucocorticoids could reverse dilutional hyponatremia (promote H2O excretion) through inhibiting the arginine vasopressin (AVP)–Vasopressin-Receptor 2 (V2R)–AQPs pathway in CHF rats during acute water loading.3 However, the effects of systemic glucocorticoid administration on renal sodium handling in CHF rats involving acute sodium loading remain unclear. In the clinical setting, several clinical trials have indicated that glucocorticoids can enhance renal sodium excretion and reverse diuretic resistance to relieve symptoms in patients with CHF. Mechanisms contributing to the improvement of the natriuretic response are related to a reduction in renal tubular sodium reabsorption or an improvement in intrarenal hemodynamics which can be shown by estimated glomerular filtration rate (eGFR) or both.4 In previous studies in clinical studies, we found that administration of the prednisone to systolic heart failure patients resulted in natriuresis accompanied by increased GFR.5–7 Therefore, we hypothesized that glucocorticoids may promote sodium excretion by suppressing Na+ transporters, such as the luminal Na/H exchanger (NHE3), the Na-K-2Cl cotransporter (NKCC), the Na-Cl cotransporter (NCC), the epithelial sodium transporter (ENaC), and the Na+/K+/ATPase across the renal epithelium in CHF with sodium overload. Then, we carried out a series...
of tests to verify this hypothesis and clarify the mechanisms involved.

MATERIAL AND METHODS

This experiment was conducted at the First Hospital of Hebei Medical University with approvals granted by the Experimental Animal Welfare Ethics Committee of Hebei Medical University.

Animal Preparation

Healthy male Wistar rats weighing 180–220 g were provided by the Hebei Medical University. All animal procedures followed the guidelines for the care and handling of laboratory animals established by the Institutional Animal Care and Use Committee of the Hebei Medical University. The rats were housed in cages, kept in a temperature (20–24°C) and humidity-controlled (45%–65%) environment on a 12-hour:12-hour light/dark cycle, and given ad libitum access to normal sodium rat-pellet diet and distilled water. After acclimatization for 7 days, rats were assigned randomly to 1 of 5 groups: the control group (CON) (n = 12), the CHF group (CHF) (n = 24), the dexamethasone (DEX, 1 mg/kg, intramuscular (im) injection as a bolus)-administered CHF (CHF+DEX) group (n = 24), the group of CHF + DEX rats treated with RU486 (100 mg/kg mifepristone, hypodermic (ih) injection, 1 hour before DEX administration) (CHF+DEX+RU486) (n = 22), and the RU486-treated CHF (CHF+RU486) group (n = 20).

Chronic Heart Failure Model

Chronic heart failure was induced by ligation of the left coronary artery (LAD) as previously described. Briefly, the rats were anesthetized with ketamine and xylazine [100 mg/kg and 10 mg/kg, respectively, intraperitoneal (ip) injection]; an oral endotracheal tube was inserted, and a rodent mechanical ventilation with room air was instituted. Via a left thoracotomy, the heart was exposed. The LAD was ligated between the pulmonary outflow tract and the left atrium with a 6-0 suture. The thorax was closed in layers as air was removed. Penicillin (30,000 U, im) was administered to prevent infection. After recovery from anesthesia and removal from the ventilator, rats were returned to individual metabolism cages with free access to normal sodium rat-pellet diet and distilled water. The rats serving as CON underwent the same procedure without ligation of LAD. The survival rate of the CHF group, the CHF + DEX group, the CHF + DEX + RU486 group, and the CHF + RU486 group were 62.5%, 66.67%, 68.18% and 65%, respectively. The degree of left ventricular dysfunction and heart failure were determined by echocardiography-Vevo 2100 Imaging System (Vevo 2100; FUJIFILM Visual Sonics. Inc. Canada). Rats with left ventricular ejection fraction less than 45% were considered to be in CHF. Comparison of heart size and echocardiography analysis of heart in normal and heart failure rats are detailed in the supplementary material (see File, Supplemental Digital Content 2, http://links.lww.com/JCVP/A840). The success number of making CHF were 13, 12, 13, and 11, respectively.

Experimental Protocol

After 56 days, the rats weighing 280–310 g were placed in metabolic cages for environmental acclimatization 3 days before the start of the experiment. The study design is outlined in Figure 1. Fifty-nine days after the surgery, the rats in the CHF + DEX + RU486 and CHF + RU486 groups received ih injection of RU486 1 hour before DEX administration. The experimental rats were water deprived from the beginning of DEX intramuscular injection to the end of the experiment. Six hours after DEX administration, isotonic saline (4 mL/100 g body weight) was injected into the abdomen over 20 minutes. Six hours after acute sodium loading, blood and urine samples were collected from the rats of the 5 experimental groups (n = 5/group randomly) for physiologically measurement, and then the kidneys were removed and processed for membrane fractionation, western blot, and immunohistochemistry. Urine volume was measured volumetrically, and serum and urinary sodium concentration were measured with direct ion-selective electrode methods. Serum and urinary creatinine were measured by sarcosine oxidase method, and the freezing point depression method was used to measure serum and urinary osmolality. Another cohort of animals from the 5 experimental groups (n = 6/group randomly) were used for the left ventricular function analysis.

Pressure–Volume (P-V) Loop Analyses

We investigated left ventricular function in rats with a Millar P-V catheter (SPR- 838; Millar Instruments, Houston, TX, USA) and an MPVS P-V conductance system (Millar Instruments) coupled to a Powerlab A/D converter (PL3508, ADInstruments, New South Wales, Australia). The P-V loop data were analyzed by the cardiac pressure–volume analysis module PV loop (Labchart 8, AD Instruments, Australia). In short, the rats were anesthetized with urethane (1.5 g/kg, ip) and subjected to endotracheal intubation, and breathing was assisted by a ventilator. The animals were maintained at 37°C with a heated surgical pad. The P-V catheter was inserted into the right carotid artery and then into the left ventricle (LV) to record baseline hemodynamics. All P-V parameters were recorded and calculated, and we measured these representative

FIGURE 1. Study design. Ih, hypodermic injection; im, intramuscular injection. 4% isotonic saline loading means 4% body weight isotonic saline intraperitoneal injection over 20 minutes.
hemodynamic parameters, including blood pressure (BP), left ventricular end-diastolic pressure (LVEDP), stroke volume (SV), left ventricular ejection fraction (LVEF), end-systolic pressure–volume relationship (ESPVR), and the maximal slope of systolic pressure increment (dP/dtmax). LVEDP is an important measure of LV preload and compliance, which represents the cardiac filling pressure. It can also help determine whether the left ventricle has reached the decompensated stage in acute sodium loading. SV refers to the volume of blood ejected per beat from the left ventricle, which may be calculated as the difference between the left ventricular end-diastolic volume and the left ventricular end-systolic volume. As a hemodynamic parameter that can assess the cardiac pump function and organ perfusion, SV is better than others, because it is subject to less influence from compensatory mechanisms. Cardiologists usually use stroke volume when assessing cardiac dysfunction in patients with congestive heart failure. LVEF also reflects the cardiac pump function, which is the percentage of blood pumped by the LV with each contraction. LVEF is considered a central component in the assessment of both systolic heart failure. ESPVR represents the slope of the end-systolic P-V relationship, which is considered the most reliable index of ventricular contractility. The dP/dtmax occurs in the early stage of isovolumetric contraction and is determined by myocardial contractility and the loading conditions on the ventricle.

Western Blot Analysis

All the kidneys on right were quickly removed and sectioned into cortex and medulla. Plasma membrane protein was purified using a plasma membrane isolation kit (Minute, Invent Biotechnologies, USA). The purity of the plasma membrane fractions was assessed with Na⁺/K⁺-ATPase (Abcam, USA, ab76020) and GAPDH (Proteintech, Wuhan, China) antibodies (see File, Supplemental Digital Content 4, http://links.lww.com/JCVP/A840). Protein concentration was measured by the BCA protein assay reagent kit (Solarbio, Beijing, China). The samples were denatured in warm water at 70°C for 10 minutes, electrophoresed on a 10% Bio-Rad stain-free sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then transferred to PVDF membranes (Millipore, Bedford, MA). The samples were blocked for 1.5 hours with 5% milk at room temperature and incubated with primary antibody overnight at 4°C. After overnight incubation, secondary HRP-conjugated antibody was added for 1 hour at a dilution of 1:10000 in TBST. Details on the primary antibodies and secondary antibodies can be found in the supplementary material (see File, Supplemental Digital Content 1, http://links.lww.com/JCVP/A840). The protein bands were visualized and analyzed by the Odyssey system (LICOR, Lincoln, NE) and ImageJ. Band densities were normalized to the total plasma membrane protein density for quantification (BioRad, Hercules, CA).

Immunohistochemistry

The localization of membrane proteins was determined by immunohistochemical analysis of paraffin sections with immunohistochemical kit (ZSGB-BIO SP-9001, Beijing, China). In brief, all the kidneys on left were fixed with 4% paraformaldehyde for 48–72 hours at 4°C and dehydrated through a serial alcohol gradient. Then, dehydrated tissues were embedded in paraffin and sectioned in to 5-µm-thick tissue. After rehydrating, kidney tissues were unmasked by water-bath heating (100°C) for 5 minutes and then incubated with 3% H₂O₂ for 20 minutes at room temperature to block the endogenous peroxidase activity. Kidney tissues were incubated in PBST plus 5% horse serum for 30 minutes and then stained with primary antibody overnight at 4°C. Details on the primary antibodies can be found in the supplemental data. After incubation with the secondary biotin-conjugated antibody and subsequently with streptavidin solution, color development was performed using DAB. The sections were counterstained using hematoxylin and then dehydrated. The sections were observed with a DP73 microscope (Olympus, Tokyo, Japan).

Statistics

All statistical analyses were performed using IBM SPSS statistics 23. The results are expressed as the mean ± SD. Comparisons were performed by 1-way analysis of variance followed by the LSD post hoc test. Differences were evaluated using 2-sided significant test at the 0.05 level.

RESULTS

DEX Improved Cardiac Function and Organ Perfusion in CHF

As shown in Figure 2, cardiac function was impaired in the CHF group compared with the CON group. A characteristic consecutive right and up shift in the P-V loop and a decrease in ESPVR. This indicated greater left ventricular volume due to dilation of the chamber, an increase LVEDP in acute sodium loading, and a decrease in contractility after myocardial infarction (MI) (Fig. 2B). DEX dramatically reduced LVEDP and increased contractility, and the effect of DEX was abolished by RU486 (Figs. 2C, D). There was a discernible level of cardiac dysfunction, as noted by significant declines in several of the load-dependent parameters of systolic function, such as stroke volume (SV), left ventricular ejection fraction (LVEF), ESPVR, and dP/dtmax, in the CHF group compared with the CON group (Figs. 2H–K). The decline in SV, LVEF, ESPVR, and dP/dtmax indicated a decrease in contractility after myocardial infarction (MI). The decrease of SV not only reflected the decrease in myocardial contractility but also the hypoperfusion of peripheral tissues and organs, such as kidney. In contrast, there was a significant increase of LVEDP (represents the preload of LV) in the CHF group compared with CON group (Fig. 2G). We also observed that these cardiac parameters were improved in the CHF + DEX group, and the effect of DEX was blocked by RU486 (Fig. 2G–K). There was no difference between the CHF and CHF + RU486 groups. This means that DEX has ability to increase the cardiac systolic and diastolic function and renal perfusion in CHF rats. There was no difference in MABP among the 5 groups (Fig. 2F).

DEX Promoted Natriuresis and Increased eGFR in CHF

In the acute sodium loading test, cumulative urinary sodium excretion, urine volume, and eGFR (represented by
creatinine clearance rate) was significantly less in the CHF group than in the CON group (Figs. 3A–C). The change of body weight (body weight at the end of experiment – body weight at the start of experiment) was significantly heavier in the CHF group than in the CON group (Fig. 3D). Compared with the CHF group, the natriuretic response of the CHF + DEX group increased significantly (the cumulative urinary sodium excretion was 673.0% more in the CHF + DEX group than in the CHF group, $P < 0.05$), and this increase was accompanied by an increase in urine volume and eGFR and

**FIGURE 2.** DEX improved cardiac function and organ perfusion in CHF (n = 6). Representative LV P-V loops from the CON (A), the CHF (B), the CHF + DEX (C), the CHF + DEX+RU486 (D), and the CHF+RU486 (E) groups following inferior vena cava occlusion. F, Effect of DEX on MABP. G, Effect of DEX on LVEDP. H, Effect of DEX on SV. I, Effect of DEX on LVEF. J, Effect of DEX on ESPVR. K, Effect of DEX on dP/dtmax. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; NS, no statistical difference. We calculated and compared the hemodynamic parameters and derivative parameters of different groups.
a decrease in change of body weight. The beneficial effects of DEX administration were abolished by RU486, a GR antagonist, indicating that these effects are mediated by GR. The change in eGFR indicated that the restored effect of DEX on renal hypoperfusion and function in CHF was antagonized by RU486. We also found that there was a remarkable increase in fractional excretion of sodium (FeNa) in the CHF + DEX group, which was abolished by RU486. However, DEX administration did not affect excretion of potassium (FeK) (see File, Supplemental Digital Content 3, http://links.lww.com/JCVP/A840). DEX administration did not affect serum osmolality or Na+ concentration (Figs. 3E, F). DEX did not produce negative sodium balance but promoted to excrete excess sodium in vivo. However, urinary osmolality in the CHF + DEX group was reduced compared with that in the CHF and CHF + RU486 groups (Fig. 3G).

**EXPERIMENTAL STUDIES**

**FIGURE 3.** DEX promoted natriuresis and increased eGFR in CHF (n = 5). A, Effect of DEX on urinary sodium. B, Effect of DEX on cumulative urine volume. C, Effect of DEX on eGFR. D, Effect of DEX on change of BW. E, Effect of DEX on serum osmolality. F, Effect of DEX on serum sodium. G, Effect of DEX on urinary osmolality. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; NS, no statistical difference. eGFR, estimated glomerular filtration (total urinary creatinine × urine volume/serum creatinine × urine collection time). Change of BW, change of body weight (body weight at the end of experiment − body weight at the start of experiment).

**DEX Downregulated SGK1 and ENaC Expression in the Medulla in CHF**

Compared with that in the CON group, ENaC-β and ENaC-γ expression was upregulated in the CHF group, and this upregulation was accompanied by increased expression of serum glucocorticoid-regulated kinase 1 (SGK1). SGK 1 activity is necessary for ENaC-mediated Na+ transport (Figs. 4B–D). In contrast, the expression of ENaC-β, ENaC–γ, and SGK 1 was decreased in the CHF + DEX group. The effects of DEX on ENaC-β, ENaC–γ, and SGK 1 were abolished by RU486, indicating that these effects were mediated by GR. There was no difference in ENaC and SGK1 expression between the CHF and CHF + RU486 groups. There was no significant difference in ENaC-α expression among 5 experimental groups (Fig. 4A). In this study, DEX
improved Na⁺ excretion partly through downregulating ENaC-β and ENaC-γ expression in a GR-mediated manner.

**DEX Downregulated NKCC and Na⁺/K⁺-ATPase and Upregulated NPR-A Expression in the Medulla in CHF**

NKCC and Na⁺/K⁺-ATPase expressions were higher in the CHF group than in the CON group and were downregulated by DEX. This effect of DEX was distinctly abrogated by RU486 (Figs. 5A, B). In contrast to NKCC and Na⁺/K⁺-ATPase expression, the expression of natriuretic peptide receptor A [NPR-A, the receptor for natriuretic peptides (NPs)] was reduced in the CHF group. However, it could be upregulated by DEX (Fig. 5C). This finding indicates that renal responsiveness to NPs was blunted by CHF and could be restored by DEX. The restorative effect of DEX was abolished by RU486. There was no difference in NKCC, Na⁺/K⁺-ATPase and NPR-A expression between the CHF and the CHF+RU486 groups.

**Effects of DEX on NCC and NHE3 Expression in the Cortex in CHF**

There was no significant difference in the expression of NCC and NHE3 in 5 experimental groups according to the western blot and immunohistochemistry results (Figs. 6A, B).

We also performed the experiments about the effect of dexamethasone on SGLT1, SGLT2, and NaPi-2a. We have added these data and results to the supplemental material (see File, Supplemental Digital Content 6D, http://links.lww.com/JCVP/A840).

**DISCUSSION**

The major finding of this study is that glucocorticoids therapy significantly improved the ability of the kidneys in CHF rats to excrete sodium, resulting in a strong natriuretic effect during acute sodium loading. This protective effect of glucocorticoids was associated with the suppression of Na⁺ transport proteins in the renal epithelium in CHF. Also, we once again showed that glucocorticoids could improve renal function, which was also associated with increased sodium excretion.

In systolic CHF, a reduced stroke volume accompanied by decreased systemic arterial pressure and renal perfusion causes the activation of the renin–angiotensin–aldosterone system (RAAS), the sympathetic nervous system and the AVP system, and desensitizes the kidneys to NPs. Consequently, the sodium-handling ability of the kidneys is reduced in CHF. The systemic balance of sodium is achieved in coordination with the regulation of water reabsorption in DCT. In this study, DEX improved the renal natriuretic response in CHF rats implicated in acute isotonic saline loading. This effect was linked to a decrease in renal tubular sodium reabsorption. Na⁺ transporters within nephrin segments, as an important regulator to influence sodium reabsorption in vivo, unquestionably play crucial roles in this process.

ENaC, a rate-limiting protein involved in Na⁺ reabsorption located in the intracellular vesicles and apical membrane of the principal cells in the connecting duct and collecting tubules of the kidney, is a highly selective nonvoltage-gated Na⁺ channel. ENaC, which is composed of α-, β-, and γ-subunits in a ratio of 1:1:1, plays a crucial role in Na⁺ equilibrium. Trafficking of the β- and γ-subunits to the plasma membrane is required for functional ENaC, whereas the α-subunit determines the stability of ENaC. Recently, proteolytic release of inhibitory tract within the γ-subunits has been recognized as a form of activation related to ENaC. Volk et al found that γ-subunit is more important for ENaC surface expression than the other subunits. Aldosterone-MR signaling can activate SGK1;21,22 reduce the affinity of neural precursor cell expressed developmentally downregulated protein 4-2 (Nedd4-2) which promotes ENaC ubiquitination and degradation; inhibit WNK (reducing ENaC activation); and thus increase ENaC expression at the plasma membrane. To some extent, DEX, as a synthetic glucocorticoid, theoretically has the same characteristics as mineralocorticoids, leading to fluid and sodium retention contributing to decompensation in CHF patients. In vitro studies showed that DEX upregulates SGK1 expression in rat inner medullary collecting duct cells.27,28 In the present study, CHF rats exhibited increased expression of SGK1, ENaC-β, and ENaC-γ during acute sodium loading. However, these sodium retention effects were reversed by DEX administration. The above paradoxical effects are likely due to the specificity of MR for aldosterone in vivo. The in vivo specificity of GR and MR for their cognate ligands is a property conferred by the prereceptor metabolism of glucocorticoids by 11β-hydroxysteroid dehydrogenase isozymes (11β-HSDs). 11β-HSD type 2 can affect MR affinity for cortisol; cortisol activates MR, whereas cortisone that was converted from cortisol by 11β-HSD type 2 does not.29 Our study findings that the effects of DEX on renal biophysiology were abolished by RU486, indicating that these favorable effects induced by DEX on the kidneys are mediated by GR activation and thus supporting the above postulation.

NKCC expressed in the thick ascending limb of Henle (TAL), and macula densa is mainly involved in Na⁺ reabsorption and tubuloglomerular feedback. NKCC is primarily regulated by the AVP system.30 In the distal nephron, the AVP-V2R pathway not only participates in fluid retention but also activates epithelial Na⁺ channels, causing Na⁺ reabsorption.31 AVP-V2R pathway activation inhibits Nedd4-2 and promotes the actions of ENaC and NKCC. It has been observed that chronic administration of AVP to Brattleboro rats can enhance Na⁺ transport and concentration.30,35 DEX binds to GR and can affect the transcription of target genes such as AVP. This effect is mainly caused by upregulating glucocorticoid response element and downregulating the transcription factor activating protein 2 (AP2). Glucocorticoid response element present within the AVP promoter plays a negative role in regulating transcription.36 In contrast, adrenal insufficiency can cause elevation of plasma AVP levels and upregulation of ENaC and NKCC expression in adrenocorticotomized mice.31,32,37 Besides genomic regulation, DEX may be involved in nonnongenomic membrane receptor-mediated response, but this remains of uncertain physiological relevance and needs further research to explore.38
of AVP-V2R induced by DEX may account for the reduced expression of renal tubular ENaC-β, ENaC-γ, and NKCC.

Renal NPR-A activation can inhibit renin–angiotensin–aldosterone system and V2R activation, and it responds positively to diuresis and natriuresis, thus reducing preload and improving cardiac function. In the present study, the renal expression of NPR-A in the CHF group was significantly downregulated, indicating that renal responsiveness or sensitivity to NPs was blunted in the CHF group. However, DEX remarkably normalized the expression of NPR-A in the kidney and restored renal sensitivity to NPs. NPR-A overexpression is presumably the main reason for the reduction of NKCC expression induced by DEX.
Additionally, glucocorticoid-induced NPR-A overexpression may be one of the mechanisms of decreased expression of ENaC-β, ENaC-γ, and NKCC in the kidneys in CHF.

Na+/K+-ATPase is ubiquitously expressed along the basolateral membrane of nephrons.40 Under physiological conditions, Na+/K+-ATPase exchanges 3 intercellular Na+ ions and 2 extracellular K+ ions at the expense of 1 ATP molecule. This process is the final stage of Na+ reabsorption in the kidney. Renal epithelial cells display highly coordinated apical and basolateral sodium transport rates to prevent the variations in sodium intracellular concentration and cell volume.34 Therefore, the electrochemical driving force of Na+ entry through ENaC, NCC/ NKCC, and NHE3 is provided by Na+/K+-ATPase.41 Aldosterone, AVP, and insulin have stimulatory effects on Na+/K+-ATPase that can be counteracted by several negative modulators, such as ANP, BNP, and Na+ in renal tubules.42–44 Along the nephron, ANP can inhibit Na+/K+-ATPase and decrease NKCC expression.45 In the present study, DEX decreased the expression of Na+/K+-ATPase, likely by upregulating NPR-A expression and downregulating V2R expression. The result also displayed that the change of Na+/K+-ATPase proteins were consistent with that of ENaC and NKCC to maintain the balance of sodium entry and exit in kidney tubules. The favorable effect of DEX on Na+/K+-ATPase was abolished by RU486, suggesting that this effect was mediated by GR activation.
NCC is located at the apical membrane of the renal DCT and can reabsorb 5%–10% of sodium.21,46 Its activity is modulated by multiple pathways that involve SGK1, potassium, chloride, insulin, and ENaC.47,48 NHE3 is the most important Na⁺ transporter in the renal proximal tubule epithelium.49 These proteins are expressed nearly exclusively in the DCT and proximal tubule, respectively, which are located in the renal cortex. The present study did not show that DEX affected renal tubular NCC and NHE3 expression. Actually, the collecting duct is the rate-limiting step in sodium reabsorption in marked contrast with proximal and distal convoluted tubules. Thus, the result of DEX on NCC and NHE3 did not influence the overall effect of DEX on sodium excretion.

Finally, the positive inotropic actions of glucocorticoids may play an important role in renal sodium handling in rats with CHF during acute sodium loading. Our investigation, once again, demonstrated that DEX increased myocardial contractility, stroke volume, and eGFR in CHF rats. This result support that this favorable effect might increase the renal fraction of cardiac output or favorably influence renal hemodynamics, such changes would contribute to restore the renal function and increased renal sodium excretory ability in CHF. Glucocorticoids were observed clinically to have positive inotropic actions as early as the 1970s.50,51 However, the mechanisms by which corticosteroids stimulate cardiac muscle are not well understood. Animal studies of various disease models have indicated that multiple mechanisms may be involved in glucocorticoid-induced enhancement of myocardial contractility.52,53 First, this influence might be mediated indirectly by the release and/or potentiation of endogenous catecholamines.50 Second, it could be a result of upregulation of β adrenergic receptor expression in the myocardium.54,55 Third, it may be a consequence of inflammatory suppression in CHF. Finally, the cardiovascular protective effects of glucocorticoids could be mediated by nontranscriptional activation of endothelial nitric oxide synthase.56 The role of glucocorticoids in myocardial contractility is supported by the recent finding that deleting GR in male mouse hearts leads to a profound dysregulation of the expression of genes involved in calcium handling that are implicated in the progression of CHF.52 Also, it cannot be ruled out that cardiac function improvement is a result of natriuresis and diuresis induced by glucocorticoids. That is to say, glucocorticoids can transform the vicious circle of cardio-renal syndrome into a virtuous circle.

SGLT2 inhibitors have demonstrated to improve myocardial performance and reduce arterial stiffness.57,58 SGLT2 inhibitors also can induce the natriuresis effect,59 but which will disappear under chronic treatment due to sodium absorption being increased by other sodium transporters.60 In fact, the SGLT2 inhibitor, dapagliflozin, will increase the expression of ENaC.61 In this study, we found that DEX upregulated SGLT1 and SGLT2 expression but downregulated ENaC expression in CHF group involving acute sodium loading, which was antagonized by RU486 (see File, Supplemental...
Digital Content 6, http://links.lww.com/JCVP/A840). Future studies are warranted to test the effect of the combined treatment, ie, GC and SGLT2i in HF patients especially in those with diuretic resistance.

However, our study has several limitations. Our findings were based on male rats. Further studies are warranted to examine the role of DEX in Na+ transporter expression in female rats to support our findings. Strictly speaking, eGFR is the best test to measure the level of renal function and determine the stage of renal disease if any, which is only reflect the intrarenal hemodynamics but not accurate enough to reflect the renal perfusion. In future research, radiology, nuclear medical technology, or ultrasonics can be used to assist in observing the exact effects of DEX on renal perfusion. The present study was conducted in short term with single dose of DEX in a rat model of HF. Long-term treatment with corticoids can have untoward adverse effects. Thus, the effect of corticoids could be diminished with prolonged exposure and needs to be tested in the future studies. Nevertheless, our findings are of highly clinical relevance. Glucocorticoid has emerged as an important therapy to manage severe COVID-19 cases. In summary, we have shown that glucocorticoids exert a profound natriuretic effect in CHF rats during acute sodium loading was related to suppression of partial Na+ transporter proteins in the renal epithelium and improvement of intrarenal hemodynamics. The effect of DEX on sodium transporters was associated with inhibiting SGK1 and activating NPR-A.

CONCLUSIONS

In summary, we have shown that glucocorticoids exert a profound natriuretic effect in CHF rats during acute sodium loading was related to suppression of partial Na+ transporter proteins in the renal epithelium and improvement of intrarenal hemodynamics. The effect of DEX on sodium transporters was associated with inhibiting SGK1 and activating NPR-A.

ACKNOWLEDGMENTS

The authors thank the following institutions. The Central Laboratory of the first hospital of the Hebei Medical University—the experimental platform.

REFERENCES

1. Scherholz ML, Schlesinger N, Androulakis IP. Chronopharmacology of glucocorticoids. Adv Drug Deliv Rev. 2019;151-152:245–261.
2. Liu B, Zhang TN, Knight JK, et al. The glucocorticoid receptor in cardiovascular health and disease. Cells. 2019;8:1227.
3. Zhu X, Huang Y, Li S, et al. Glucocorticoids reverse diluted hypotonemia through inhibiting arginine vasopressin pathway in heart failure rats. J Am Heart Assoc. 2020;9:e014950.
4. DiBona GF, Sawin LL. Effect of metoprolol administration on renal sodium handling in experimental congestive heart failure. Circulation. 1999;100:82–86.
5. Liu C, Li G, Zhou C, et al. Potent diuretic effects of prednisone in heart failure patients with refractory diuretic resistance. Can J Cardiol. 2007;23:3.
6. Liu C, Zhao Q, Zhen Y, et al. Prednisone in urine acid lowering in symptomatic heart failure patients with hyperuricemia (PUSH-path) study. Can J Cardiol. 2013;29:1048–1054.
7. Liu C, Zhen Y, Zhao Q, et al. Prednisone lowers serum uric acid levels in patients with decompensated heart failure by increasing renal uric acid clearance. Can J Physiol Pharmacol. 2016;94:797–800.
32. Eccelberger CA, Kim GH, Terris J, et al. Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney. *Am J Physiol Ren Physiol*. 2000;279:F46–F53.
33. Snyder PM, Olson DR, Kabra R, et al. cAMP and serum and glucocorticoid-inducible kinase (SGK) regulate the epithelial Na(+) channel through convergent phosphorylation of Nedd4-2. *J Biol Chem*. 2004;279:45753–45758.
34. Feraile E, Dizien E. Coordinated control of ENaC and Na(+), K+-ATPase in renal collecting duct. *J Am Soc Nephrol*. 2016;27:2554–2563.
35. Besseghir K, Trimble ME, Stoner L. Action of ADH on isolated medullary thick ascending limb of the Brattleboro rat. *Am J Physiol*. 1986;251:F271–F277.
36. Kim JK, Sumner SN, Wood WM, et al. Role of glucocorticoid hormones in arginine vasopressin gene regulation. *Biochem Biophys Res Commun*. 2001;289:1252–1256.
37. Sasaki S, Imai M. Effects of vasopressin on water and NaCl transport across the in vitro perfused medullary thick ascending limb of Henle’s loop of mouse, rat, and rabbit kidneys. *Pflugers Arch*. 1980;383:215–221.
38. Tasker JG, Di S, Malcher-Lopes R. Minireview: rapid glucocorticoid signaling via membrane-associated receptors. *Endocrinology*. 2006;147:5549–5556.
39. Volpe M, Rubattu S, Burnett J. Natriuretic peptides in cardiovascular diseases: current use and perspectives. *Eur Heart J*. 2013;35:419–425.
40. Takada T, Yamamoto A, Omori K, et al. Quantitative immunogold localization of Na, K-ATPase along rat nephron. *Histochemistry*. 1992;98:183–197.
41. Alvarez de la Rosa D, Gimenez I, Forbush B, et al. SGK1 activates Na(+), K+-ATPase in amphibian renal epithelial cells. *Am J Physiol Cel Physiol*. 2006;290:C492–C499.
42. El Mernissi G, Doucet A. Specific activity of Na-K-ATPase after adrenalectomy and hormone replacement along the rabbit nephron. *Pflugers Arch*. 1984;402:258–263.
43. Vinciguerra M, Mordasini D, Vandewalle A, et al. Hormonal and non-hormonal mechanisms of regulation of the Na-K-pump in collecting duct principal cells. *Semin Nephrol*. 2005;25:312–321.
44. Pearce D, Soundararajan R, Trimpert C, et al. Collecting duct principal cell transport processes and their regulation. *Clin J Am Soc Nephrol*. 2015;10:135–146.
45. Thelig F, Wu Q. ANP-induced signaling cascade and its implications in renal pathophysiology. *Am J Physiol Ren Physiol*. 2015;308:F1047–F1055.
46. Gamba G. Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. *Physiol Rev*. 2005;85:70.
47. Moreno E, de Los Heros P, Plata C, et al. Structure-function relationships in the renal NaCl cotransporter (NCC). *Curr Top Membr*. 2019;83:177–204.
48. Hoorn EJ, Gritter M, Cuevas CA, et al. Regulation of the renal NaCl cotransporter and its role in potassium homeostasis. *Physiol Rev*. 2020;100:321–356.
49. Li XC, Zhu D, Chen X, et al. Proximal tubule-specific deletion of the NHE3 (Na(+) /H(+) exchanger 3) in the kidney attenuates ang II (angiotensin II)-Induced hypertension in mice. *Hypertension*. 2019;74:526–535.
50. Walker BR. Glucocorticoids and Cardiovascular Disease. *Eur J Endocrinol*. 2007;157:545–559.
51. Tecklenberg PL, Mullin EM, Stinson EB, et al. The effects of massive doses of methylprednisolone on myocardial contractility and peripheral vascular resistance. *Am Heart J*. 1973;85:216–226.
52. Cruz-Toptete D, Oakley R, Carroll N, et al. Deletion of the cardiomyocyte glucocorticoid receptor leads to sexually dimorphic changes in cardiac gene expression and progression to heart failure. *J Am Heart Assoc*. 2019;8:e011012.
53. Oakley R, Cruz-Toptete D, He B, et al. Cardiomyocyte glucocorticoid and mineralocorticoid receptors directly and antagonistically regulate heart disease in mice. *Sci Signal*. 2019;4:17.
54. Chao Liu M, Kunshen Liu M. Cardiac outcome prevention effectiveness of glucocorticoids in acute decompensated heart failure: COPE-ADHF study. *J Cardiovasc Pharmacol*. 2014;63:5.
55. Lesuis SL, Timmermans W, Lucasen PJ, et al. Glucocorticoid and β-adrenergic regulation of hippocampal dendritic spines. *J neuroendocrinology*. 2020;32:e12811.
56. Lambden S, Creagh-Brown BC, Hunt J, et al. Definitions and pathophysiology of vasoplegic shock. *Crit Care*. 2018;22:174.
57. García-Ropero A, Vargas-Delgado AP, Santos-Gallego CG, et al. Inhibition of sodium glucose cotransporters improves cardiac performance. *Int J Mol Sci*. 2019;20:3289.
58. Requena-Ibáñez JA, Santos-Gallego CG, Rodriguez-Cordero A, et al. Mechanistic insights of empagliflozin in nondiabetic patients with HFpEF: from the EMPA-TROPISM study. *JACC Heart Fail*. 2021;9:578–593.
59. Nassif ME, Qintar M, Windsor SL, et al. Empagliflozin effects on pulmonary artery pressure in patients with heart failure: results from the EMBRACE-HF trial. *Circulation*. 2021;143:1673–1686.
60. Mordi NA, Mordi IR, Singh JS, et al. Renal and cardiovascular effects of SGLT2 inhibition in combination with loop diuretics in patients with type 2 diabetes and chronic heart failure: the RECEDE-CHF trial. *Circulation*. 2020;142:1713–1724.
61. Ma C, de Baaij JH, Millar PJ, et al. Effect of dapagliflozin treatment on the expression of renal sodium transporters/channels on high-fat diet diabetic mice. *Nephron*. 2019;142:51–60.