Glucocorticoids Improve Renal Responsiveness to Atrial Natriuretic Peptide by Up-Regulating Natriuretic Peptide Receptor-A Expression in the Renal Inner Medullary Collecting Duct in Decompensated Heart Failure

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ABSTRACT

In heart failure, the renal responsiveness to exogenous and endogenous atrial natriuretic peptide (ANP) is blunted. The mechanisms of renal hyporesponsiveness to ANP are complex, but one potential mechanism is decreased expression of natriuretic peptide receptor-A (NPR-A) in inner medullary collecting duct (IMCD) cells. Newly emerging evidence shows that glucocorticoids could produce potent diuresis and natriuresis in patients with heart failure, but the precise mechanism is unclear. In the present study, we found dexamethasone (Dex) dramatically increased the expression of NPR-A in IMCD cells in vitro. The NPR-A overexpression induced by Dex presented in a time- and dose-dependent manner, which emerged after 12 h and peaked after 48 h. The cultured IMCD cells were then stimulated with exogenous rat ANP. Consistent with the findings with NPR-A expression, Dex greatly increased cGMP (the second messenger for the ANP) generation in IMCD cells, which presented in a time- and dose-dependent manner as well. In rats with decompensated heart failure, Dex dramatically increased NPR-A expression in inner renal medulla, which was accompanied by a remarkable increase in renal cGMP generation, urine flow rate, and renal sodium excretion. It is noteworthy that Dex dramatically lowered plasma ANP, cGMP levels, and left ventricular end diastolic pressure. These favorable effects induced by Dex were glucocorticoid receptor (GR)-mediated and abolished by the GR antagonist 17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estra-4,9-dien-3-one (RU486). Collectively, glucocorticoids could improve renal responsiveness to ANP by up-regulating NPR-A expression in the IMCD and induce a potent diuretic action in rats with decompensated heart failure.

Introduction

Atrial natriuretic peptide (ANP), a peptide hormone secreted by the atrial myocytes in response to volume expansion, provides a potent mechanism counterbalancing the salt-and water-retaining actions of the rennin-angiotensin-aldosterone system in body fluid control. The ANP and its primary receptor, natriuretic peptide receptor A (NPR-A), are found in the kidney and hypothalamus. Systemic ANP administration activates renal and hypothalamic NPR-A. Renal NPR-A activation produces a potent diuresis and natriuresis, whereas hypothalamic NPR-A activation inhibits water drinking and salt intake (Antunes-Rodrigues et al., 2004; Potter et al., 2006). Volume depletion ensues. It was predicted that ANP and its analogs would be useful in the treatment of congestive heart failure. However, their favorable diuretic and natriuretic effects were blunted in heart failure (Cody et al., 1986; Münzel et al., 1991; Schrier and Abraham, 1999). The failure of natriuretic peptides to reverse salt and water retention in heart failure is very likely caused by the tonic effect of counter-regulatory systems (Tsutamoto et al., 1993; Schrier and Abraham, 1999; Potter et al., 2006). The discovery of ANP-sensitizing reagents is anticipated and will be of extraordinary importance in the treatment of heart failure (Potter et al., 2006).

ABBREVIATIONS: ANP, atrial natriuretic peptide; NPR-A, natriuretic peptide receptor-A; IMCD, inner medullary collecting duct; Dex, dexamethasone; GR, glucocorticoid receptor; CHF, congestive heart failure; GFR, glomerular filtration rate; LVEDP, left ventricular end diastolic pressure; RU486, 17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estra-4,9-dien-3-one.
Traditional teaching indicates that glucocorticoids cause hypertension by sodium and water retention. However, emerging evidence overthrew this notion and demonstrated that the rise in blood pressure is very likely caused by the increased pressor responsiveness induced by glucocorticoids (Turner et al., 1996; Wallerath et al., 1999; Whitworth et al., 2000). Contrary to the notion that glucocorticoids cause renal water and sodium retention, evidence showed glucocorticoid administration could produce potent diuresis and natriuresis in rats (Thunhorst et al., 2007; Liu et al., 2010). It has also been found, in clinical observations, that chronic glucocorticoid administration could produce potent diuresis and restore body fluid homeostasis in patients with heart failure and severe fluid overload (Liu et al., 2006, 2007; Zhang et al., 2008; Massari et al., 2011). Previous evidence showed that glucocorticoids could up-regulate ANP mRNA expression in the atrial myocytes and increase ANP levels in the circulation (Gardner et al., 1988; Dananberg and Grekin, 1992). But it cannot account for the potent diuretic effect induced by glucocorticoids in decompensated congestive heart failure (CHF), because ANP loses its favorable effects in this situation (Cody et al., 1986; Schrier and Abraham, 1999; Cotter et al., 2008). Recently, we demonstrated that glucocorticoids not only produced a potent diuretic effect, but inhibited dehydration-induced water intake and sodium depletion-induced sodium intake by up-regulating the expression of NPR-A in the hypothalamus, resulting in an overt volume depletion (Liu et al., 2010). It is reasonable to assume that the natriuretic action of glucocorticoids in the kidney is also mediated by NPR-A activation, which is matched physiologically by hypothalamic NPR-A activation to produce the volume-depleting effect. The inner medullary collecting duct (IMCD), as the terminal nephron segment of the kidney, is the target of the natriuretic peptide system that promotes sodium excretion (Koseki et al., 1986; Light et al., 1990). We raised a hypothesis that glucocorticoids may up-regulate NPR-A expression in IMCD cells, thus producing a potent diuresis and natriuresis. To elucidate the precise mechanism underlying this protective renal effect we designed this study.

Materials and Methods

Chemicals. Dexamethasone (Dex), mifepristone/17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estradiol-3-one (RU486), ANP 127-150 rat (Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-Oh), collagenase used to digest inner medullary tissue, 3-isobutyryl(1-methyl)-xanthine (Sigma-Aldrich) for 10 min to inhibit phosphodiesterase activity, and then stimulated with increasing concentrations of rat ANP (10^-3 to 10^-7 M) for 10 min at 37°C under constant shaking. The incubations were terminated by the removal of medium and the addition of lysis buffer (cGMP HTS Immunoassay Kit; Millipore Corporation, Billerica, MA). The plates were kept at room temperature for 10 min to allow intracellular cyclic nucleotides to be extracted. cGMP levels in supernatant that was obtained after centrifugation at 4°C were then assayed.

Western Blotting Analysis. Western blotting measurements were performed using the method described previously (Santos-Araújo et al., 2009). The NPR-A antibody was provided by Abcam Inc. (Cambridge, MA). Protein bands were digitally analyzed with Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Hercules, CA). The densities were expressed as a relative value compared with the average density measured in the vehicle-treated rats. β-Actin was used as a loading control.

Surgical Procedure. The postinfarction rat model of heart failure used in the study was prepared by the standard method described previously (Trueblood et al., 2005). In brief, rats were anesthetized, incubated, and ventilated with a rodent ventilator. A left thoracotomy was performed in the fourth intercostal space to expose the heart, and the pericardial sac was gently removed. The left coronary artery was visualized and ligated between the left atrial appendage and the right ventricular outflow tract with a 6-0 silk suture. Ligation of the coronary artery was confirmed by noting the pallor of the left ventricular wall. The chest wall was then closed and sutured in layers, and the air was evacuated by slight lateral pressure of the thorax. The rats that survived were raised for 12 weeks to have decompensated CHF (Supplemental Table 1).

Analysis of Blood and Urine Samples. Plasma ANP and cGMP were measured by radioimmunoassay. Blood samples were collected into chilled plastic tubes containing EDTA (1.5 mg/ml) and aprotinin (500 kilo IU/ml). Blood samples were centrifuged at 4°C, and the plasma samples were separated and stored at -70°C until assayed for these peptides by using radioimmunoassay. One milliliter of plasma was extracted on a C18 SEP Pak column for measurement for ANP. The extracts were dried down, reconstituted in 1.0 ml of buffer, and measured by radioimmunoassay with use of ANP radioimmunoassay kits (Beijing North Institute of Biological Technology).

Plasma cGMP was measured with a radioimmunoassay kit (Shanghai University of Traditional Chinese Medicine, Shanghai, China) after ethanol extraction. Urinary cGMP was determined directly using the radioimmunoassay kit after 1.3000 dilution.

Hemodynamic Study. Rats were anesthetized, and the left carotid artery was exposed and cannulated with a fluid-filled polyethylene catheter that was connected to a pressure transducer. The

Isolation and Culture of IMCD Cells. The IMCD cells from Wistar rats were isolated and cultured using the method described previously (Ye et al., 2003). In brief, adult Wistar male rats were killed by cervical dislocation after CO2 narcosis. The inner medullary tissue from each kidney was dissected free from the outer medulla, minced, and digested with 1 mg/ml of collagenase at 37°C with gentle agitation during each 30-min cycle. These IMCD cells were enriched in the preparation using hypotonic lysis as described previously (Grenier et al., 1981). The cells were resuspended in Dulbecco’s modified Eagle’s medium and seeded onto culture plates. After 24 h, the cells were placed in Dulbecco’s modified Eagle’s medium and cultured for 5 to 7 days.

ANP Stimulation Test. Natriuretic peptides elicit their physiological responses through synthesis of cGMP when binding to their receptors. Therefore, cGMP levels in renal cells serves as a useful biological marker for the renal activity of ANP in vitro (Ballermann et al., 1985; Cole et al., 1989). The ANP stimulation test was performed by the method described previously (Kanda et al., 1989). In brief, the IMCD cells were cultured with 96-well cell culture plates. The number of cells ranged between 8 × 10^4 and 10 × 10^4 cells/well. The cells were preincubated with 2.0 mM 3-isobutyryl(1-methyl)-xanthine (Sigma-Aldrich) for 10 min to inhibit phosphodiesterase activity, and then stimulated with increasing concentrations of rat ANP (10^-3 to 10^-7 M) for 10 min at 37°C under constant shaking. The incubations were terminated by the removal of medium and the addition of lysis buffer (cGMP HTS Immunoassay Kit; Millipore Corporation, Billerica, MA). The plates were kept at room temperature for 10 min to allow intracellular cyclic nucleotides to be extracted. cGMP levels in supernatant that was obtained after centrifugation at 4°C were then assayed.

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Hemodynamic Study. Rats were anesthetized, and the left carotid artery was exposed and cannulated with a fluid-filled polyethylene catheter that was connected to a pressure transducer. The
fluid-filled catheter was advanced through the carotid artery across the aortic valve into the left ventricular chamber. Hemodynamic data were analyzed off-line with PowerLab (model ML 870; ADInstruments Pty Ltd., Castle Hill, Australia).

Measurement of GFR. The right femoral artery was cannulated for infusion of 8% inulin in isotonic saline solution, at a rate of 2 ml/h, as described previously (Zhang et al., 1994). Urine samples were analyzed by spectrophotometry for calculation of GFR. Renal cGMP production was calculated as follows: renal cGMP generation = (urinary flow \times \text{urinary cGMP concentration}) − (GFR \times \text{plasma cGMP concentration}) (Stevens et al., 1994).

Statistical Analyses. All data were express as mean ± S.E.M. Differences between groups were evaluated with the standard t test, the Wilcoxon rank sum test, as indicated in the figure legends. Data in the ANP stimulation test were analyzed by two-way, repeated-measures analysis of variance, followed by a Student-Newman-Keuls test for multiple comparisons.

Results

The Effect of Glucocorticoids on NPR-A Expression in IMCD Cells. Treatment with Dex (Sigma-Aldrich) for 48 h dramatically up-regulated NPR-A expression in IMCD cells in a concentration-dependent manner (Fig. 1A). The NPR-A expression in IMCD cells was dramatically increased after 24-h Dex treatment (10^{-7} M) and peaked at 48 h (Fig. 1B). To test whether the effect of Dex on NPR-A was mediated by glucocorticoid receptor (GR), IMCD cells were treated with mifepristone/RU486 (Sigma-Aldrich) alone or coadministration with Dex. RU486 alone had no effect on NPR-A expression in the IMCD cells (Fig. 1C). The effect of Dex (10^{-7} M) on NPR-A expression in the IMCD cells was completely inhibited by the GR antagonist RU486 (10^{-6} M), indicating it was GR-mediated (Fig. 1C).

The Effect of Glucocorticoids on cGMP Content in IMCD Cells. To test whether the up-regulation in NPR-A expression induced by Dex was associated with an increase in its action, we conducted the ANP stimulation test. After 48-h treatment, IMCD cells were stimulated by rat ANP (Sigma-Aldrich), and cGMP levels in IMCD cells were measured. It showed that cGMP levels were well correlated with the NPR-A expression induced by Dex in IMCD cells, presenting in a dose- and time-dependent manner as well.

After stimulation with increasing concentrations of ANP, the IMCD cells treated with higher concentrations of Dex had much higher levels of cGMP than did the IMCD cells treated with lower concentrations of Dex or vehicle (n = 5 in each point; P < 0.01; Fig. 2A). Then, the IMCD cells with increasing time periods of Dex (10^{-7} M) treatment were stimulated with ANP (10^{-7} M). The data showed that cGMP content in IMCD cells dramatically increased with Dex-treated time and peaked after 48-h Dex treatment (n = 8 in each group; P < 0.01; Fig. 2B). Finally, RU486 (10^{-6} M) completely abolished the effect of 48-h Dex (10^{-7} M) treatment on cGMP content in IMCD cells (n = 8 in each group; Fig. 2C).

The Effect of Glucocorticoids on NPR-A Expression in Inner Renal Medulla, Renal cGMP Generation, Plasma ANP, and Plasma cGMP. To determine whether glucocorticoids can potentiate ANP’s action in the kidney in rats with heart failure by up-regulation of the NPR-A expression in the kidney, Wistar rats with decompensated CHF were randomized to receive dexamethasone sodium phosphate (1.0 mg/kg), vehicle, RU486 (100 mg/kg) alone, or RU486 (100 mg/kg) alone.

Consistent with the findings in vitro, Western blotting analysis showed Dex dramatically increased NPR-A expression in the inner renal medulla after 24-h treatment (Fig. 3A). Consequently, Dex greatly increased renal cGMP generation (n = 6–7 in each group; P < 0.01; Fig. 3B); whereas Dex remarkably reduced plasma ANP levels and plasma cGMP levels (n = 6–7 in each group; P < 0.01; Fig. 3, C and D). The effects of Dex on renal cGMP generation, plasma cGMP, and plasma ANP levels were abolished by RU486 (100 mg/kg) (Fig. 3, B–D).

The Effect of Glucocorticoids on Urine Flow Rate, Renal Sodium Excretion, and GFR. After 24-h treatment, Dex

![Fig. 1](image-url). The effect of glucocorticoids on NPR-A expression in IMCD cells. A, concentration-dependent effect of Dex on NPR-A expression after 48-h treatment (n = 4 in each group). *, P < 0.05. B, time-dependent effect of Dex (10^{-7} M) on NPR-A expression (n = 4 in each group). *, P < 0.05. C, effect of Dex (10^{-7} M) on NPR-A expression with or without RU486 (10^{-6} M) (n = 4 in each group). *, P < 0.05.
greatly increased urine flow rate (n = 6–7 in each group; P < 0.001; Fig. 4A) and renal sodium excretion (n = 6–7 in each group; P < 0.01; Fig. 4B), whereas it had no significant effect on GFR (n = 6–7 in each group; P = 0.88; Fig. 4C).

The Effect of Glucocorticoids on the Diuretic Effect and Left Ventricular End Diastolic Pressure. To test whether the effect of glucocorticoids on renal NPR-A expression could be translated to a real diuretic action in vivo
rats with decompensated CHF were randomized to receive Dex (1 mg/kg) or vehicle. During the 24-h treatment period, Dex doubled urinary volume and dramatically increased renal sodium excretion in rats with decompensated CHF; whereas they dramatically reduced plasma ANP levels and plasma cGMP levels.

The inner medullary collecting duct is the terminal nephron segment of the kidney and the target of ANP that promotes sodium excretion (Koseki et al., 1986; Light et al., 1990). The ANP, acting through cGMP, reduces Na\(^+\) absorption across the IMCD (Light et al., 1990). In a chronic situation, such as CHF, patients are resistant to the natriuretic effect of ANP. Increased ANP levels are proportional to the severity of heart failure. The increased ANP levels cannot induce potent diuretic effects to excrete the excessively retained sodium and water, and decompensated CHF occurs. The mechanisms of renal hyporesponsiveness to the ANP in CHF are complex, including diminished NPR-A expression in the IMCD segment (Yechieli et al., 1993; Bryan et al., 2007), decreased ANP delivery to the IMCD segment by increased enzymatic degradation of ANP by neutral endopeptidase in the proximal tubule (Margulies et al., 1995), decreased sodium delivery to the collecting duct (Abraham et al., 1995), or increased activity of cGMP phosphodiesterases (Supaporn et al., 1996). Theoretically, any reagent targeting the above mechanisms could partly reverse ANP resistance and produce a potent diuresis in heart failure. It has been reported that neutral endopeptidase inhibition (Wilkins et al., 1990), increasing distal tubular sodium delivery (Abraham et al., 1995), or phosphodiesterase inhibition (Chen et al., 2006) could reverse ANP resistance and produce potent diuresis and natriuresis in heart failure. But, there has been no report that any reagent could increase NPR-A expression in the IMCD segment and produce a potent diuretic effect.

Dexamethasone could up-regulate NPR-A expression in IMCD, improve renal responsiveness to endogenous ANP, resulting in a potent diuresis in decompensated CHF. This is why Dex could decrease plasma ANP and cGMP levels in decompensated CHF. It is without doubt that our finding may provide a new therapeutic option for heart failure.

The role of glucocorticoids in body fluid metabolism is provocative. Traditionally, it is assumed that glucocorticoids may cause hypertension by water and sodium retention. But, large amounts of data demonstrated that the rise in blood pressure is caused by the increased pressor responsiveness induced by glucocorticoids (Handa et al., 1984; Turner et al., 1996; Wallerath et al., 1999; Whitworth et al., 2000). There is no relationship between sodium retention and the rise in blood pressure (Whitworth et al., 2000). Moreover, studies from animals and humans have documented the potent diuretic effect induced by glucocorticoids. It was reported that glucocorticoids could potentiate renal responsiveness to acute volume expansion and restore body fluid homeostasis more quickly than did untreated rats by producing a potent depleting effect in rats with heart failure. As a result, LVEDP, a known index of left ventricular end-diastolic filling status, was dramatically lowered (n = 7–8 in each group; P < 0.01; Fig. 5D).

**Discussion**

In summary, glucocorticoids could up-regulate NPR-A expression in IMCD cells both in vitro and in vivo. Consequently, glucocorticoids greatly increased renal cGMP generation, urine flow rate, and renal sodium excretion in rats with decompensated CHF; whereas they dramatically reduced plasma ANP levels and plasma cGMP levels.

![Fig. 4. The effect of glucocorticoids on urine flow rate, renal sodium excretion, and GFR. A, effect of glucocorticoids on urine flow rate (n = 6–7 in each group). Data were analyzed by the standard t test. **, P < 0.01 compared with rats treated with vehicle. B, effect of glucocorticoids on renal sodium excretion (n = 6–7 in each group). Data were analyzed by the standard t test. **, P < 0.01 compared with rats treated with vehicle. C, effect of glucocorticoids on GFR (n = 6–7 in each group). Data were analyzed by Wilcoxon rank sum test.](https://www.aspetjournals.org)
diuresis and natriuresis (Nevskaia et al., 1977). In heart patients with severe fluid retention, glucocorticoids could induced potent diuretic effect and restore fluid hemostasis (Liu et al., 2006, 2007; Zhang et al., 2008; Massari et al., 2011). Recently, we demonstrated that glucocorticoids even produced potent diuresis in rats with overnight water deprivation (Liu et al., 2010). The data presented here coupled with our previous findings suggest glucocorticoids could potentiate ANP’s action in body fluid control by up-regulating NPR-A expression in the hypothalamus and kidney (Liu et al., 2010). The overall effect of glucocorticoids on body fluid is to have the body under systemic volume depletion (Thunhorst et al., 2007; Liu et al., 2010), which is consistent with the physiological effects of NPR-A activation on body fluid control. Our findings were also supported by data from humans. Damjancic and Vierhapper (1990) reported glucocorticoids could potentiate renal responsiveness to ANP in humans with adrenocortical insufficiency, therefore leading to a dramatic increase in natriuresis and diuresis. Massari et al. (2011) reported that methylprednisolone induced potent diuretic effect in a patient with severe heart failure but lowered natriuretic peptide levels in the circulation at the same time, suggesting glucocorticoid might have a role in natriuretic peptide system.

Previous evidence showed that glucocorticoids increased blood pressure and increased GFR. Their effects are also time-dependent, which begin to appear after 1-day Dex treatment (Handa et al., 1984; Aguirre et al., 1999). Our study did show that there was a trend that Dex may slightly raise blood pressure and GFR, but there were no statistically significant differences between Dex-treated rats and vehicle-treated rats because of the short treatment period and small sample size (Supplemental Data).

There are several limitations to this study. First, the focus of the study was on the IMCD that is thought to be the primary site for ANP’s action. However, the role of glucocorticoids on other parts of the nephron remains to be determined by future studies. Second, even though the glucocorticoids’ effect on collecting duct was confirmed by Western blotting analysis and renal cGMP generation study, the inner medulla in heart failure model used to perform Western blotting analysis mainly (but not only) contains collecting duct, which may compromise our findings. Third, our findings were based on the rat model and should not be extrapolated to humans.

Collectively, the data suggested that glucocorticoids could induce a potent diuretic action in the rats with decompensated CHF, which was associated with a dramatic increase in NPR-A expression in IMCD cells. Future studies are warranted to confirm whether glucocorticoids have the same effect on renal NPR-A expression in humans.

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Authorship Contributions

Participated in research design: C. Liu and K. Liu.
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References


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