Impaired sodium excretion and salt-sensitive hypertension in corin-deficient mice

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Corin is a protease that activates atrial natriuretic peptide, a cardiac hormone important in the control of blood pressure and salt–water balance. Here we examined the role of corin in regulating blood pressure and sodium homeostasis upon dietary salt challenge. Radiotelemetry-tracked blood pressure in corin knockout mice on a high-salt diet (4% sodium chloride) was significantly increased; however, there was no such change in similarly treated wild-type mice. In the knockout mice on the high-salt diet there was an impairment of urinary sodium excretion and an increase in body weight, but no elevation of plasma renin or serum aldosterone levels. When the knockout mice on the high-salt diet were treated with amiloride, an epithelial sodium channel blocker that inhibits renal sodium reabsorption, the impaired urinary sodium excretion and increased body weight were normalized. Amiloride treatment also reduced high blood pressure caused by the high-salt diet in these mice. Thus, the lack of corin in mice impairs their adaptive renal response to high dietary salt, suggesting that corin deficiency may represent an important mechanism underlying salt-sensitive hypertension.
RESULTS

Salt-sensitive hypertension in Cor−/− mice

We examined the effect of high-salt diets on blood pressure in Cor−/− and WT mice. No significant changes were detected when Cor−/− or WT mice were treated with a medium-high-salt (2% NaCl) diet for 3 weeks (data not shown). After 1 week on a higher-salt (4% NaCl) diet, systolic blood pressure increased in Cor−/− mice (from 125.7 ± 3.2 to 133 ± 2.9 mm Hg, P < 0.01; Figure 1a). Similar increases in diastolic blood pressure and mean arterial blood pressure were also observed (data not shown). In contrast, no significant increase in blood pressure was observed in similarly treated WT mice (Figure 1a). After 3 weeks on 4% NaCl salt diet, Cor−/− mice were switched to a normal-salt (0.3% NaCl) diet, and their blood pressure remained high for 2 more weeks (Figure 1a). When switched to a normal-salt (0.3% NaCl) diet, and their blood pressure remained high for 2 more weeks (Figure 1a). When

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Figure 1  |  Salt-sensitive hypertension in corin knockout (Cor−/−) mice. (a) Cor−/− and wild-type (WT) mice on a 0.3% NaCl diet (basal) were switched to a 4% NaCl diet for 3 weeks (w) and back to the 0.3% NaCl diet for 3 w. Blood pressure was measured by radiotelemetry. Systolic blood pressure (SBP) in Cor−/− and WT mice is shown, n = 6 mice per group. **P < 0.01 vs. WT mice of the same group; †P < 0.01 vs. Cor−/− mice of the basal group by two-way analysis of variance (ANOVA). (b) Changes in SBP from the basal group of the same genotype were calculated, n = 6 mice per group. *P < 0.05 vs. WT mice of the same group by two-way ANOVA.
gain in Cor−/− mice. No significant changes in urinary Na+ excretion were observed in WT and Cor−/− mice on normal-salt diet upon amiloride treatment. The treatment, however, corrected impaired Na+ excretion in Cor−/− mice on the high-salt diet (Figure 6a). Consistently, Cor−/− mice on the high-salt diet and treated with amiloride had body weight gains comparable to those in WT mice (Figure 6b).

We measured blood pressure in mice treated with amiloride (Figure 7a). No significant changes in blood pressure were observed in Cor−/− mice on the normal-salt diet after amiloride treatment. The treatment reduced elevated blood pressure in Cor−/− mice on the high-salt diet. In WT mice on the normal- or high-salt diet, no significant changes in blood pressure were observed after amiloride treatment (Figure 7a). The net effect of amiloride on systolic blood pressure was significantly greater in Cor−/− mice than in WT mice on the high-salt diet (Figure 7b), indicating that inhibiting ENaC activity increased urinary Na+ excretion and ameliorated hypertension in Cor−/− mice.

**Amlodipine treatment**

To understand the mechanism underlying hypertension in Cor−/− mice on the normal-salt diet, we examined the effect of amlodipine, a calcium channel blocker, on blood pressure. Amlodipine reduced the blood pressure in Cor−/− and WT mice on the normal-salt diet (Figure 8a). The reduction was greater in Cor−/− than in WT mice when 1.5 mg/kg of amlodipine was used (Figure 8b). Amlodipine also reduced blood pressure in WT and Cor−/− mice on the high-salt diet (Figure 8c). However, the reduction was similar in both groups (Figure 8d). Blood pressure in Cor−/− mice remained significantly higher than that in similarly treated WT mice (Figure 8c).
DISCUSSION

In this study, we used radiotelemetry to monitor blood pressure in Cor−/− mice on normal- and high-salt diets. We found that blood pressure in Cor−/− mice was more sensitive to dietary salts than that in WT mice, as indicated by the exacerbated hypertension in Cor−/− mice on the 4% NaCl diet. We further investigated possible mechanisms underlying the salt-sensitive hypertension in these mice. ANP is known to antagonize the RAAS. Elevated plasma renin activities were reported in ANP knockout mice on high-salt diets, suggesting that hypertension in these mice may result from an enhanced RAAS. However, this hypothesis was not supported by another study, which showed similar plasma Ang II levels and responses to dietary salts in ANP knockout and WT mice. In our study, we did not detect elevated levels of plasma renin or serum aldosterone in Cor−/− mice (Figure 4a and b). Moreover, losartan did not normalize high blood pressure in Cor−/− mice (Figure 5a and c), indicating that an enhanced RAAS is unlikely to be a key determinant for salt-sensitive hypertension in Cor−/− mice.

In the metabolic studies, no major differences were found in food/water intakes, body weight, and plasma electrolyte levels between Cor−/− and WT mice on the normal-salt diet. After 1 week of dietary salt challenge, Cor−/− mice had levels of plasma electrolytes similar to those in WT mice but exhibited a significant reduction in urinary Na+/Cl− excretion. This was accompanied by body weight gains, indicating that water was retained to maintain electrolyte homeostasis. Gradually, a steady state of renal sodium excretion was reached in Cor−/− mice, indicating that corin...
deficiency impaired an adaptive renal response to high dietary salts, leading to salt-sensitive hypertension.

Corin is highly expressed in cardiomyocytes.32,33 Corin mRNA and protein expression was found in the kidney, including the proximal tubule, thick ascending limb, connecting tubule, and collecting duct.13,29 In rat models of proteinuric kidney disease, renal corin expression was markedly reduced, and the reduction was associated with sodium retention, indicating that corin defects may impair sodium homeostasis in nephrotic syndrome.29,34 The result is consistent with our findings of impaired sodium excretion and exacerbated hypertension in Cor−/− mice on high-salt diets.

ENaC is essential for renal sodium reabsorption.27,28 Mutations in the ENaC genes cause hypertension in patients.2 Polzin et al.29 showed that renal β-ENaC expression was increased in Cor−/− mice, suggesting that corin may down-regulate renal ENaC expression and activity.34 Zhao et al.35 also showed that ANP-mediated inhibition of sodium reabsorption in distal nephron segments is critical for promoting natriuresis. These data suggest that impaired sodium excretion in Cor−/− mice may be due to an increased renal ENaC activity. Consistent with this hypothesis, amiloride treatment prevented sodium retention, normalized body weight gain (Figure 6), and reduced high blood pressure (Figure 7) in Cor−/− mice on high-salt diet. These results support the hypothesis that renal corin expression has an important role in regulating ENaC activity. Because corin is the physiological pro-ANP convertase, it is likely that the renal function of corin is mediated by its activation of ANP.

Interestingly, no significant changes in urinary sodium excretion and blood pressure were observed when Cor−/− mice on normal-salt diet were treated with amiloride (Figure 6a and 7a). Moreover, no significant differences in urinary sodium excretion were detected between Cor−/− and WT mice on the normal-salt diet (Figure 2a), suggesting that impaired sodium excretion may not be a major factor in hypertension in Cor−/− mice on normal-salt diet. ANP is known to bind to its receptor on vascular smooth muscles to promote intracellular cyclic guanosine monophosphate production and vasodilation.4 Possibly, lack of corin reduced ANP levels and increased vascular resistance in these mice, contributing to hypertension on normal-salt diets. Consistently, treatment of amlodipine, a calcium channel blocker that relaxes vascular smooth muscles, reduced blood pressure in Cor−/− mice to a level similar to that in WT mice on the normal-salt diet (Figure 8a).

Evolutionarily, natriuretic peptides were developed in primitive vertebrates for their adaptation to survive. In salmon and eels, e.g., natriuretic peptides are critical for maintaining electrolyte homeostasis when these animals migrate from fresh water to salty water during their life cycle.36,37 Here we show that corin deficiency prevents mice
from adapting to dietary salt challenges, indicating that this ancient molecular mechanism is preserved in mammals for similar purposes. Together, our data provide new insights into the role of corin in regulating sodium homeostasis and the mechanisms underlying salt-sensitive hypertension in Cor−/− mice. Genetic variants that impair corin function have been identified in African Americans, a population known for high prevalence of salt-sensitive hypertension.38 Our results suggest that corin defects may represent an important mechanism underlying hypertension in humans.

**MATERIALS AND METHODS**

**Mice**

Cor−/− mice were generated by deleting the exons coding for corin active sites.16,39 Male Cor−/− mice and WT littermates, aged 8–12 weeks old and weighing 20–30 g, were used in this study. Mouse genotypes were not blinded to the persons who performed the experiments. The mice were maintained in a facility with a 12-h light–dark cycle and unrestricted access to food and water. The study was conducted in accordance with the NIH guidelines for the ethical treatment and handling of animals in research, and approved by the Institutional Animal Care and Use Committee at the Cleveland Clinic.

**Blood pressure measurements**

Radiotelemetry was used to monitor blood pressure in conscious and unrestrained mice.16 Mice were anesthetized with ketamine/xylazine on a 37°C warming plate. A TA11PA-C10 telemetry device (Data Science International, St Paul, MN) was inserted into the left common carotid artery under a microscope. After surgery, mice were singly caged and fed with standard diet and water ad libitum. Telemetry receivers (model RPC-1) were used for data acquisition using the Dataquest System (Data Science International). After ~7 days of postoperative recovery, blood pressure was recorded.

**Effect of dietary salt on blood pressure**

Mice were fed with diets containing normal (0.3% NaCl), medium–high (2% NaCl), or high (4% NaCl) salts (Harlan Teklad, Madison, WI) for 3 weeks. Blood pressure was monitored before, during, and after the treatment of different salt diets.

**Metabolic studies**

Mice were placed singly in metabolic cages (Nalgene Labware, Lima, OH) for a 5-day habituation period, during which a normal-salt diet was fed. Next, mice were fed with the normal-salt diet for 3 days, and then switched to a high-salt diet. Body weight, food consumption, and water intake were measured daily. Urine samples were collected in a device covered with water-saturated oil to prevent sample evaporation. Urine volume in a 24-h period was recorded. At different time points, mice were killed by CO₂ inhalation and blood samples were collected in tubes (BD, Franklin Lakes, NJ) containing heparin for electrolyte analysis or ethylenediaminetetraacetic acid for plasma renin concentration assay.

**Plasma and urinary electrolytes**

Plasma and urine samples were analyzed for sodium, potassium, and chloride concentrations using an electrolyte analyzer (Roche AVL9180, Indianapolis, IN).
Effects of sodium or calcium channel blockers or Ang II receptor antagonist

Mice on 0.3 or 4% NaCl diets were treated with amiloride (Alexis Biochemicals, San Diego, CA), an ENaC blocker, or amlodipine (Sigma, St Louis, MO), a calcium channel blocker, or losartan (US Pharmacopeia, Rockville, MD), an Ang II receptor type 1 antagonist, by intraperitoneal injection. The injection volume was ~0.1 ml, and vehicles were used as controls. Blood pressure was monitored by radiotelemetry, starting 7, 5, and 3 days after amiloride, amlodipine, and losartan treatment, respectively. Urinary sodium excretion and body weight change after drug treatment were measured.

Plasma renin and serum aldosterone assays

Active renin concentration in plasma was measured as the amount of Ang I generated from angiotensinogen. Plasma samples were diluted and incubated with excess porcine angiotensinogen (1 μmol/l, Sigma) in a reaction mixture containing sodium acetate (50 mmol/l, pH 6.5), aminoethylbenzene sulfonyl fluoride (2.5 mmol/l), 8-hydroxyquinoline (1 mmol/l), leupeptin (100 μmol/l), and EDTA (1 mmol/l) at 37 °C for 30 min. The reaction was stopped by heating at 100 °C. Generated Ang I was determined by an enzyme immunoassay (Peninsula Labs, San Carlos, CA). Plasma renin concentration was expressed as the amount of Ang I generated per microliter of plasma per hour. As a negative control, the reaction was performed in the presence of pepstatin A (1 μmol/l, Sigma). The value from this reaction was used as a background. Serum aldosterone levels were measured using an ELISA kit from Alpha Diagnostic International (San Antonio, TX), according to the manufacturer’s instructions.

Statistical analysis

Data were analyzed using the Prism software (Graph-Pad, La Jolla, CA). Comparisons between two groups were made using Student’s t-test. Three or more groups were compared using two-way analysis of variance followed by post hoc least significant difference. All data are presented as means ± s.d. A P-value of <0.05 was considered to be statistically significant.

DISCLOSURE

All the authors declared no competing interests.

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