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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.

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Hypertension is a major cardiovascular disease. Excessive dietary salt has long been considered a risk factor in hypertension.[1] Approximately 50% of essential hypertension cases are salt sensitive, but the underlying mechanisms are poorly understood.[2,3] Atrial natriuretic peptide (ANP) regulates blood pressure and salt–water balance.[4] In mice, plasma ANP levels were elevated upon high-salt diets,[5] and deficiency in ANP or its receptor causes hypertension.[6,7] To date, however, it is unclear whether a defective ANP pathway alters the response of blood pressure to dietary salts. In ANP-deficient mice, salt-sensitive hypertension was observed initially[6] but not confirmed in a later study.[8] In ANP receptor knockout mice, elevated blood pressure was unchanged upon dietary salt loading in an early study[7] but appeared to be increased in later studies.[9,10] The reasons for the apparent differences may be due to different experimental protocols (conscious vs. anesthetized conditions)[11] and methods for measuring blood pressure, which are notoriously challenging if tail-cuff methods are used.

Corin is a cardiac membrane serine protease.[12,13] It promotes the natriuretic system by activating ANP.[14,15] In mice, corin deficiency prevents pro-ANP to ANP conversion, causing hypertension and cardiac hypertrophy.[16,17] Corin deficiency may also contribute to hypertensive disease in humans. In African Americans, single-nucleotide polymorphisms that impaired corin function were found in patients with hypertension, cardiac hypertrophy, and heart failure.[18–21] Recent studies detected soluble corin in human plasma[22–25] and showed that plasma corin levels were reduced in patients with heart failure.[22,26]

In a previous study, hypertension in corin knockout ($Cor^{-/-}$) mice was exacerbated upon an 8% NaCl diet challenge.[16] The same treatment, however, also elevated blood pressure in control wild-type (WT) mice. It is difficult, therefore, to conclude whether corin deficiency altered the response of blood pressure to dietary salts. In this study, we examined the effect of 2 and 4% high-salt diets on blood pressure in $Cor^{-/-}$ mice and investigated the possible mechanisms underlying hypertension in these mice. Our data indicated that corin deficiency impaired renal sodium excretion upon dietary salt challenges, which contributed to salt-sensitive hypertension in these mice.

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 net changes in systolic blood pressure were examined, the effect was significantly greater in Cor$^{-/-}$ mice than in WT controls (Figure 1b), indicating that blood pressure was more sensitive to dietary salt loading in Cor$^{-/-}$ than WT mice.

Metabolic and electrolyte analyses

To understand the mechanism underlying the observed salt-sensitive hypertension in Cor$^{-/-}$ mice, we conducted metabolic studies in WT and Cor$^{-/-}$ mice. Similar food and water intakes were found with the normal-salt diet (Figure 2a). On the high-salt diet, food intakes remained unchanged, but water intakes and urine volumes increased similarly in WT and Cor$^{-/-}$ mice during the first week and remained at high levels in weeks 2 and 3. Levels of plasma Na^+, Cl^-, and K^+ were unchanged in these mice on the high-salt diet (Figure 2b). Apparently, plasma electrolyte balance was maintained by increased urinary Na^+/Cl^- excretion (Figure 2c). Compared with WT mice, however, Cor$^{-/-}$ mice had reduced Na^+/Cl^- excretion during the first week of high-salt diet. The difference in Na^+/Cl^- excretion between WT and Cor$^{-/-}$ mice narrowed in the following weeks (Figure 2c), indicating that a steady state of salt–water balance was reached gradually in Cor$^{-/-}$ mice. In contrast, levels of urinary K^+ excretion were similar in Cor$^{-/-}$ and WT mice (Figure 2c).

In Cor$^{-/-}$ mice on the high-salt diet, the impaired urinary Na^+/Cl^- excretion was accompanied by increased body weight compared with that in WT mice (Figure 3a). Such a difference in body weight gain between WT and Cor$^{-/-}$ mice was not observed on the normal-salt diet (Figure 3b).

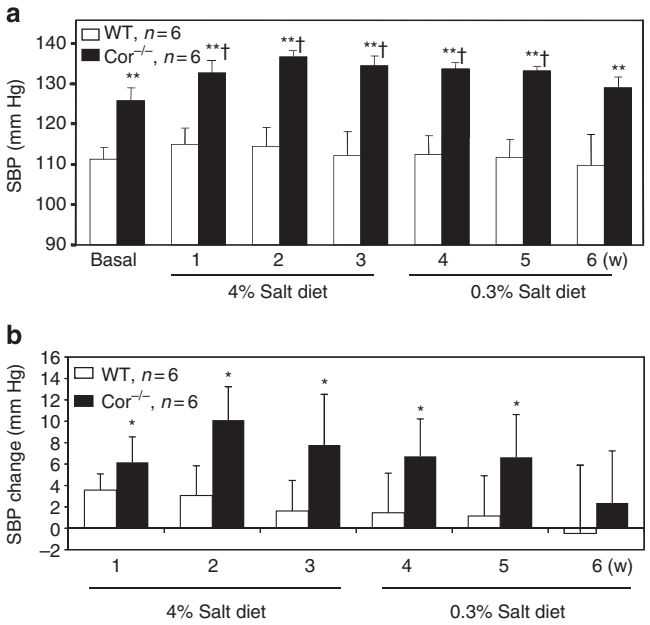

Figure 1 | Salt-sensitive hypertension in corin knockout (Cor$^{-/-}$) mice. (**a**) Cor$^{-/-}$ and wild-type (WT) mice on a 0.3% NaCl diet (basal) were switched to a 4% NaCl diet for 3 weeks (w) and back to the 0.3% NaCl diet for 3 w. Blood pressure was measured by radiotelemetry. Systolic blood pressure (SBP) in Cor$^{-/-}$ and WT mice is shown. $n=6$ mice per group. $^{**}P<0.01$ vs. WT mice of the same group; $^{\dagger}P<0.01$ 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.

Levels of plasma renin and serum aldosterone

To examine whether an enhanced renin–angiotensin (Ang) –aldosterone system (RAAS) may be responsible for hypertension and sodium–water retention in Cor$^{-/-}$ mice, we measured plasma renin concentration and serum aldosterone. Similar plasma renin levels were found in WT and Cor$^{-/-}$ mice on the normal-salt diet (Figure 4a). On the high-salt diet, both groups had significantly lower plasma renin concentration, indicating that renin levels were suppressed. There was no significant difference in plasma renin concentration between the two groups (Figure 4a). Serum aldosterone levels were found to be lower in Cor$^{-/-}$ than in WT mice on both the normal- and high-salt diets (Figure 4b). The results indicated that an enhanced RAAS was unlikely to be present in Cor$^{-/-}$ mice.

Losartan treatment

To further examine the potential role of RAAS, we treated Cor$^{-/-}$ mice with losartan, an Ang II type 1 receptor blocker. Losartan reduced blood pressure in WT and Cor$^{-/-}$ mice on both the normal- and high-salt diets (Figure 5a and c). The extent of reduction was similar in both groups (Figure 5b and d). The treatment did not lower blood pressure in Cor$^{-/-}$ mice to similar levels in WT mice. The results supported that mechanisms other than an enhanced RAAS were likely involved in hypertension in Cor$^{-/-}$ mice.

Amiloride treatment

Epithelial sodium channel (ENaC) mediates renal Na^+ reabsorption, thereby reducing Na^+ excretion.[27,28] A recent report showed that the corin/ANP pathway may regulate renal ENaC expression and/or activity.[29] To determine whether the impaired urinary Na^+ excretion in Cor$^{-/-}$ mice on high-salt diets was due to an enhanced ENaC activity, we tested the hypothesis that amiloride, an ENaC blocker, might increase urinary Na^+ excretion and decrease body weight