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PCSK9-deficiency does not alter blood pressure and sodium balance in mouse models of hypertension

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ABSTRACT

Objective: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is highly expressed in the kidney, where its function remains unclear. *In vitro* data suggested that PCSK9 could impair the trafficking of the epithelial Na channel (ENaC). Here, we aimed at determining the consequences of PCSK9-deficiency on blood pressure, sodium balance and ENaC function *in vivo* in mice.
Methods: Blood pressure was measured using non-invasive tail-cuff system or radiotelemetry under basal conditions in male and female PCSK9$^{+/+}$ and PCSK9$^{-/-}$ mice, as well as in models of hypertension: L-NAME (2 mg/kg/day), angiotensin II (1 mg/kg/day) and deoxycorticosterone acetate (DOCA)-salt in male mice only. Plasma and urine electrolytes (Na^+, K^+, Cl^-) were collected under basal conditions, after DOCA-salt and amiloride treatment. Renal expression of ENaC subunits was assessed by western blotting.
Results: PCSK9-deficiency did not alter both basal blood pressure and its increase in salt-insensitive (L-NAME) and salt-sensitive (Ang-II and DOCA-salt) hypertension models. Plasma PCSK9 concentrations were increased by 2.8 fold in DOCA-salt-induced hypertension. The relative expression of the cleaved, active, 30-kDa αENaC subunit was significantly increased by 32% in kidneys of PCSK9$^{-/-}$ mice under basal, but not under high-Na^+ diet or DOCA-salt conditions. Amiloride increased urinary Na^+ excretion to similar level in both genotypes, indicating that ENaC activity was not affected by PCSK9-deficiency.
Conclusions: Despite an increase of cleaved αENaC under basal condition, PCSK9$^{-/-}$ mice display normal sodium balance and blood pressure regulation. Altogether, these data are reassuring regarding the development of PCSK9 inhibitors in hypercholesterolemia.

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1. Introduction

PCSK9 (Proprotein Convertase Subtilisin Kexin type 9) is the ninth member of the proprotein convertase family [1]. PCSK9 is secreted by the liver [2], and acts as a natural inhibitor of the LDL receptor (LDLR) pathway, by targeting the receptor to lysosomes for degradation [3,4]. Beside the liver, PCSK9 it also expressed at high levels in the intestine and the kidney [1], where its function remains unclear. The characterization of hepatocyte-specific PCSK9 knockout mice demonstrated that the liver is responsible for two thirds of the hypocholesterolemic phenotype of total PCSK9 knockout mice [2]. Thus, it is reasonable to hypothesize that other organs are involved in the physiological action of PCSK9. PCSK9 seems to regulate intestinal lipoprotein metabolism by controlling both the post-prandial hyperlipidemia [5] and the transintestinal cholesterol excretion (TICE) [6].

The role of PCSK9 in the kidney is less clear. It has been suggested recently that PCSK9 decreases the expression of the amiloride sensitive epithelial Na^+ channel (ENaC) at the protein level [7]. As previously described for the regulation of LDLR, the catalytic

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activity of PCSK9 is not required for reducing ENaC cell surface expression. *In vitro* studies in transfected HEK 293 cells indicated that PCSK9 interacts with the three subunits (α, β and γ) of ENaC, thereby enhancing their degradation in the proteasome pathway [7]. ENaC is expressed in the distal segments of the nephron and it forms a highly regulated pathway for the reabsorption of Na^+ from urine (for review see [8]). The three genes encoding the α, β and γ subunits have been found to harbor mutations or polymorphisms related to gain or loss of function of the channel, increased or decreased sodium reabsorption in the terminal part of the nephron, and high or low blood pressure [9].

Since dietary Na^+ has a significant effect on blood pressure [10], it could be envisaged that PCSK9 activity may impact Na^+ renal handling and blood pressure. This question is of critical importance since PCSK9 inhibitors are currently tested in phase III clinical trials in patients with high cardiovascular (CV) risk [11]. Monoclonal anti-PCSK9 antibodies are able to bind free circulating PCSK9 in the plasma and thus to prevent the degradation of hepatic LDLR. As a result, LDL-cholesterol was found to be reduced by ≈ 60% compared to baseline in phase II studies [12,13].

The aim of the present study was to assess the physiological consequences of PCSK9-deficiency on blood pressure and electrolytes homeostasis *in vivo* in mice. We used the $PCSK9^{-/-}$ mouse model [14] to measure blood pressure and sodium balance in baseline conditions as well as in both salt-sensitive (DOCA-salt, angiotensin II) and salt-insensitive (L-NAME) hypertension models. We also determined the expression of ENaC in the kidney of $PCSK9^{-/-}$ mice. Our data show that PCSK9-deficiency has a neutral effect on blood pressure regulation in hypertension mouse models and ENaC activity in response to amiloride treatment.

2. Material & methods

2.1. Animals

$PCSK9^{+/-}$ mice were purchased from Jackson Laboratories (Maine, USA) to produce PCSK9 $^{+/+}$ and $PCSK9^{-/-}$ mice. Mice had free access to food and water under a 12-h light/12-h dark cycle in a temperature-controlled environment. Experiments were conducted on 10–11 and 30–32 week-old male mice, as well as on 16–18 week-old female mice. All the experiments were approved by the Ethic Committee for Animal Experimentation of Pays de la Loire.

2.2. Arterial pressure measurements

Blood pressure was measured in conscious, unrestrained mice using a radiotelemetry system as described previously (PA-C10 and Dataquest software, Data sciences International) [15] or by computerized tail cuff plethysmography in conscious mice using the BP 2000 analysis system (Visitech Systems) [16].

2.3. Angiotensin II-induced hypertension

$PCSK9^{+/+}$ and $PCSK9^{-/-}$ mice were chronically treated for 21 days with angiotensin II (Ang II) via osmotic minipumps (Alzet, model 2004) placed subcutaneously and filled with saline solution set to deliver Ang II (Sigma) at 1 mg per kilogram of body weight per day.

2.4. L-NAME-induced hypertension

$PCSK9^{+/+}$ and $PCSK9^{-/-}$ mice were given L-NAME (Sigma) in drinking water (300 mg/kg/day) for 17 days.

2.5. DOCA-salt-induced hypertension

$PCSK9^{+/+}$ and $PCSK9^{-/-}$ mice underwent a left kidney nephrectomy or a sham surgery under isoflurane anesthesia (2% isoflurane, 1 L 0_2). Nephrectomised mice received a subcutaneous implantation of a 50 mg pellet of deoxycorticosterone acetate (DOCA) with 21-day release (Innovative Research of America). Mice received 1 day before and 4 days after the surgery ibuprofen (0.2 mg/ml) in drinking water. Both groups received water containing 1% NaCl. Blood pressure was registered by telemetry.

2.6. Plasma and urine metabolites measurement

Urine excretion and collection, food and water intake were measured using metabolic cages (Tecniplast). Mice were acclimatized for 48 h, and measurements were performed during the following 48 h. Plasma creatinine and sodium concentrations were determined respectively using a commercially available kit (Randox) and a potentiometric method with ion-selective electrode (Spotchem E-plate). Urine creatinine sodium, chlorine and potassium were measured respectively using a commercially available kit (Randox), an ion-selective electrode (sodium, chlorine) (IDEXX Vetlyte) and reflectometry (potassium) (Roche Reflovet K). Plasma PCSK9 concentrations were assayed in triplicates using a commercially available quantitative sandwich ELISA assay and following the manufacturer instructions (Circulex CY-8078, CycLex Co, Nagano, Japan).

2.7. Amiloride experiment

After a 3-days adaptation period in metabolic cages, urine was collected every 12 h for 24 h. Amiloride (4.8 nmol/kg, Selleck Chemicals) was then injected intraperitoneally (i.p.) in mice and urine was collected 6 and 24 h after the injection. Urine electrolyte levels were determined as described above.

2.8. Western blots

Renal cortex was separate from total kidney and snaps frozen at −80 °C. Cortex protein extract and immunoblotting was performed as described [17]. The polyclonal antibodies raised α, β and γ ENaC were generous gifts of J. Loffing. Quantification of each band was performed by densitometry using the mini-LAS imaging system and software (Fuji).

2.9. Statistics

All results are reported as means ± SEM. Statistical significance was analyzed using a non-parametric Mann–Whitney test. The values of $p < 0.05$ were considered as significant.

3. Results

3.1. Baseline blood pressure and urinary electrolyte homeostasis in PCSK9-deficient mice

Effect of PCSK9-deficiency on renal function and electrolytes (Na^+, K^+, Cl^-) homeostasis was first assessed in 10–11 week-old male under basal conditions (*i.e.* regular chow diet). Both food and drinking intake, as well as urine excretion volume, were comparable between $PCSK9^{-/-}$ and $PCSK9^{+/+}$ mice (Fig. 1A–C). After adjustment for food intake, the ratio of urine excretion of sodium, potassium and chloride did not vary in $PCSK9^{-/-}$ mice compared to wild-type mice (Fig. 1D–F). Systolic blood pressure profiles measured over 24 h were similar between $PCSK9^{-/-}$ and

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