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Vascular contractile reactivity in hypotension due to reduced renal reabsorption of Na⁺ and restricted dietary Na⁺

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Abstract

Reduced renal Na⁺ reabsorption along with restricted dietary Na⁺ depletes intravascular plasma volume which can then result in hypotension. Whether hypotension occurs and the magnitude of hypotension depends in part on compensatory angiotensin II-mediated increased vascular resistance. We investigated whether the ability of vascular resistance to mitigate the hypotension was compromised by decreased contractile reactivity. In vitro reactivity was investigated in aorta from mouse models of reduced renal Na⁺ reabsorption and restricted dietary Na⁺ associated with considerable hypotension and renin-angiotensin system activation: (1) the Na⁺-Cl⁻-Co-transporter (NCC) knockout (KO) with Na⁺ restricted diet (0.1%, 2 weeks) and (2) the relatively more severe pendrin (apical chloride/bicarbonate exchanger) and NCC double KO. Contractile sensitivity to KCl, phenylephrine, and/or U46619 remained unaltered in aorta from both models. Maximal KCl and phenylephrine contraction expressed as force/aorta length from NCC KO with Na⁺-restricted diet remained unaltered, while in pendrin/NCC double KO were reduced to 49 and 64%, respectively. Wet weight of aorta from NCC KO with Na⁺-restricted diet remained unaltered, while pendrin/NCC double KO was reduced to 67%, consistent with decreased medial width determined with Verhoeff-Van Gieson stain. These findings suggest that hypotension associated with severe intravascular volume depletion, as the result of decreased renal Na⁺ reabsorption, may in part be due to decreased contractile reactivity as a consequence of reduced vascular hypertrophy.

Keywords

Hypotension; Intravascular volume depletion; Knockout; Pendrin; Na-Cl cotransporter; Contraction; Aorta; Na⁺ reabsorption; Renal

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Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethical standards All procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati and were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council USA.

Introduction

A major regulator of arterial pressure is intravascular plasma volume, with increased and decreased volume causing elevated and lowered pressure, respectively. The intravascular plasma volume is regulated by the amount of Na^+ absorption by the kidney. Thus, decreased Na^+ reabsorption and restricted dietary Na^+ represent mechanisms whereby intravascular plasma volume is depleted, thereby lowering arterial pressure. The extent of lowered arterial pressure depends on several factors including the magnitudes of intravascular volume depletion and the compensatory increase in vascular resistance by elevated plasma levels of angiotension II.

Indeed, several mouse models of decreased Na^+ reabsorption with and without restricted Na^+ diet are associated with hypotension despite substantial activation of the renin-angiotensin system (Schultheis et al. 1998; Wall et al. 2004; Kim et al. 2007; Pech et al. 2010; Soleimani et al. 2012; Lazo-Fernandez et al. 2015; Alshahrani et al. 2016; Sinning et al. 2016). Furthermore, possible altered contractile reactivity was investigated in the aorta from the pendrin knockout (KO; Sutliff et al. 2014). This model is associated with hypotension, albeit at somewhat variable levels (5–17 mmHg; Kim et al. 2007; Pech et al. 2010; Lazo-Fernandez et al. 2015) and plasma renin activity is increased twofold (Kim et al. 2007). In the pendrin KO, maximal force expressed per cross-sectional area of the aorta was non-selectively increased by ~25%, suggesting possible mitigation of the associated hypotension (Sutliff et al. 2014). Although, since aorta wet weight and cross-sectional area decreased by ~20 and ~35%, respectively (Sutliff et al. 2014), one could also consider that force generated per vessel length actually remained unchanged, i.e., despite the decreased hypertrophy.

To further investigate whether vascular reactivity is compromised under conditions of hypotension associated with decreased Na^+ reabsorption, we investigated whether aorta contractile reactivity was decreased in the pendrin/NCC double KO and Na^+ diet-restricted NCC KO (Schultheis et al. 1998; Soleimani et al. 2012; Alshahrani et al. 2016). These models demonstrate considerable hypotension, with decreases of 20 and 13 mmHg, respectively, and increased renin amounts in the kidney analyzed by Western blot, with seemingly much greater renin in the pendrin/NCC double KO as compared to the NCC KO kidney (Soleimani et al. 2012; S.A. et al., manuscript in preparation). Some of these results have been reported in abstract form (Alshahrani et al. 2015).

Methods and materials

Animals

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati and were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council USA. NCC KO on normal (1%) and reduced Na^+ diet (0.1%, 2 weeks), and pendrin/NCC double KO and wild-type mice (Soleimani et al. 2012) of 4–6 months of either sex were used.

Vascular reactivity

Contractile experiments were performed as previously described (Basford et al. 2009). Under a dissecting microscope, thoracic aorta was exposed in situ and cleaned of surrounding tissue. Following placement of the aorta in physiologic salt solution (mmol/L: NaCl 118, KCl 4.73, MgCl₂ 1.2, EDTA 0.026, KH₂PO₄ 1.2, CaCl₂ 2.5, 25 NaH₂CO₃, and glucose 5.5; pH 7.4 with 95% O₂/5% CO₂ bubbling at 37 °C), each vessel was divided into two, ~5 mm ring segments, with the two segments serving as duplicates. Aorta segments from NCC KO with and without reduced Na⁺ diet, and segments from pendrin/NCC double KO and wild type, were tested in parallel.

Segments were placed in an isometric contractile apparatus. This procedure involved placement of two wires through the vessel lumen, with one wire stationary and the other movable, such that the distance between the wires could be adjusted and the resultant applied resting tension monitored via a transducer. Optimal 3 g-force resting tension was determined by repeated 50 mM KCl challenge of segments from the four mouse types. After each KCl challenge and wash, as well as following wash of other agonists, resting tension was readjusted to 3 g-force.

After repeated challenge with 50 mM KCl, segments were exposed to cumulative phenylephrine and U46619 concentrations, with the final concentrations at near maximal, i.e., the EC₅₀s should also be considered as approximate. Angiotensin II challenge was performed with a single concentration, 0.1 μM, to avoid desensitization by prior angiotensin II exposure. Wet weight along with length was determined in a number of these vessel segments and also in some additional segments not subjected to tension.

Histology

Aorta was removed, fixed in formaldehyde solution (4 °C, overnight), and transferred to 70% ethanol, fixed in paraffin and 5 μm sections prepared. Hematoxylin-eosin, Verhoeff-Van Gieson, and Masson's trichrome staining along with immunohistochemistry for smooth muscle actin were then performed. Evaluation included blinded observer.

Statistical analysis

Contraction was calculated as force in millinewton/millimeter aorta length and concentration-contraction curves calculated as percent contraction achieved with the maximally applied agonist concentration. EC₅₀s were determined with GraphPad Prism. Mean ± SE of contractile response and EC₅₀s, expressed as pD₂ values, were determined. "n" Represents the number of mice. Statistical significance between means was determined with Student's unpaired, two-tailed *t* test and significance accepted at *p* < .05.

Materials

Phenylephrine and angiotensin II were from Sigma-Aldrich and U46619 from Cayman Chemical.

Results

Contraction

NCC KO with reduced Na⁺ diet—Contractile sensitivity of aorta to phenylephrine was not different in NCC KO with and without reduced Na⁺ diet ($pD_2 = 7.30 \pm 0.06$ and 7.17 ± 0.06 , respectively; $n = 4$ in each case; Fig. 1). Contractions (millinewton/millimeter length) to 1 μ M phenylephrine and 50 mM KCl were not different in aorta from NCC KO with and without reduced Na⁺ diet (Fig. 1).

Pendrin/NCC double KO—Contractile sensitivity to phenylephrine and U46619 (thromboxane A₂ receptor antagonist) was not different in aorta from pendrin/NCC double KO compared to wild type (phenylephrine $pD_2 = 7.13 \pm 0.08$ and 7.11 ± 0.06 , respectively; $n = 6$ each case; U46619 $pD_2 = 8.03 \pm 0.07$ and 8.21 ± 0.08 , respectively; $n = 5$ in each case; Fig. 2).

Contractions (millinewton/millimeter length) to 1 μ M phenylephrine and 50 mM KCl in aorta from pendrin/NCC double KO were 64 and 49%, respectively, of wild type (Fig. 2). Contraction to 0.1 μ M U46619 tended to be reduced (46% of wild type), but the reduction was not statistically significant (Fig. 2).

Contraction to 0.1 μ M angiotensin II of aorta from pendrin/NCC double KO and wild type was only 0.04 ± 0.04 and 0.03 ± 0.02 millinewton/millimeter length, respectively ($n = 3$ in each case).

Wet weight and protein

Wet weight/aorta length of segments from NCC KO with Na⁺ restricted diet and pendrin/NCC double KO remained unchanged and was 67% of wild type, respectively (Fig. 3). In an additional experiment in which milligram protein was determined in aorta segments (segments combined to increase protein measurement detection), milligram protein/millimeter vessel length was 20% less in aorta from pendrin/NCC double KO as compared to aorta from wild type.

Histology

Verhoeff-Van Gieson staining revealed reduced medial extracellular matrix and greater convolution of laminae in aorta from pendrin/NCC double KO as compared to wild type (Fig. 4). Although these differences were difficult to discern in hematoxylin-eosin and Masson's trichrome stained sections (not shown). Reduction of smooth muscle actin assessed with immunohistochemistry was also difficult to discern in pendrin/NCC double KO (Fig. 4). Differences were not observed in extracellular matrix and laminae in aorta from NCC KO with and without reduced Na⁺ diet as assessed with Verhoeff-Van Gieson staining (data not shown).

Discussion

The present findings suggest that decreased vascular contractile reactivity may limit the ability of vasoconstrictors to reverse the hypertension associated with intravascular volume

depletion caused by severely reduced Na^+ reabsorption. This suggestion is supported by the decreased force per length of aorta from pendrin/NCC double KO. Whether this suggestion is in conflict with the essentially lack of decreased force of aorta from pendrin/NCC double KO when accounting for the accompanying reduced aorta wet weight should also be considered.

An alternative explanation for the decreased contraction of aorta from pendrin/NCC double KO is the depressed overall growth in this model, i.e., body weight is decreased to 60% (Soleimani et al. 2012). However, 7% Na^+ diet for 2 weeks reversed the hypotension, as well as reduced the elevated renin mRNA levels in the kidney, but only slightly increased body weight (Soleimani et al. 2012; S.A., unpublished observation). Thus, it is likely that the decreased contractile reactivity was due to depleted intravascular volume rather than depressed overall growth.

The reduced contraction in aorta from pendrin/NCC double KO appears to result from decreased amounts of media, based on histological and protein determinations. These findings are consistent with the observed decreased contractile efficacy and lack of effect on sensitivity.

Interestingly, the decreased hypertrophy of aorta from pendrin/NCC double KO may be unexpected based on the considerable activation of the renin-angiotension II system in this model (Soleimani et al. 2012; S.A. et al., manuscript in preparation) and the well-known ability of angiotensin II to increase hypertrophy (Berk 2001). A speculation regarding the mechanism underlying the decreased hypertrophy in aorta from the pendrin/NCC double KO is that angiotensin II under hypotensive conditions interacts with additional factors to decrease hypertrophy. Although the effects of hypotension on hypertrophy have not been studied (to our knowledge), also of possible relevance is that elevated pressure elicits hypertrophy (Anwar et al. 2012).

In apparent conflict with the suggestion that angiotensin II under hypotensive conditions interacts with additional factors to decrease hypertrophy is the finding that pretreatment (7 days) with an angiotensin₁ receptor antagonist normalized the reduction in cross-sectional area of the aorta from the pendrin KO (Sutliff et al. 2014). However, arterial pressure was not determined in this study (Sutliff et al. 2014) and variable levels of hypotension have been reported in this model (5–17 mmHg; Kim et al. 2007; Pech et al. 2010; Lazo-Fernandez et al. 2015). In any case, reduced hypertrophy was not detected in aorta from the Na^+ diet restricted NCC KO (present results), in which arterial pressure is decreased although not to the level observed in pendrin/NCC double KO (Schultheis et al. 1998; Soleimani et al. 2012; Alshahrani et al. 2016) and in which there is relatively less activation of the renin-angiotensin system (Soleimani et al. 2012; S.A. et al., manuscript in preparation). Thus, possible interaction between angiotensin II and factors associated with hypotension to reduce hypertrophy may require a certain balance between angiotensin II and these factors.

Also in contrast to aorta from pendrin/NCC double KO, force per aorta length was not decreased in the Na^+ diet restricted NCC KO. The unchanged force is consistent with the unaltered medial layer. Thus, the reduced ability of vasoconstrictors to mitigate the

hypotension is limited to higher levels of severity of intravascular volume depletion, i.e., the pendrin/NCC double KO.

Limitations of the in vitro contractile studies include that (1) the aorta is a conduit vessel, negligibly contributing to vascular resistance (although mouse conduit vessels are commonly used in vitro to determine isometric vascular contractility due to their relatively greater mass and, thus, increased force generation) and (2) the effects of angiotensin II, a major constrictor associated with intravascular volume depletion, minimally constricted the aorta. Weak contractile efficacy of the mouse aorta to angiotensin II was previously reported (Russell and Watts 2000), although in another study 0.1 μM angiotensin II elicited 35% of maximal phenylephrine contraction (Sutliff et al. 2014). Likely explanations for the variable angiotensin II constrictor efficacy include the mouse strain. Experiments utilizing resistance-type vessels and, further, which respond with significant contractile efficacy to angiotensin II are warranted.

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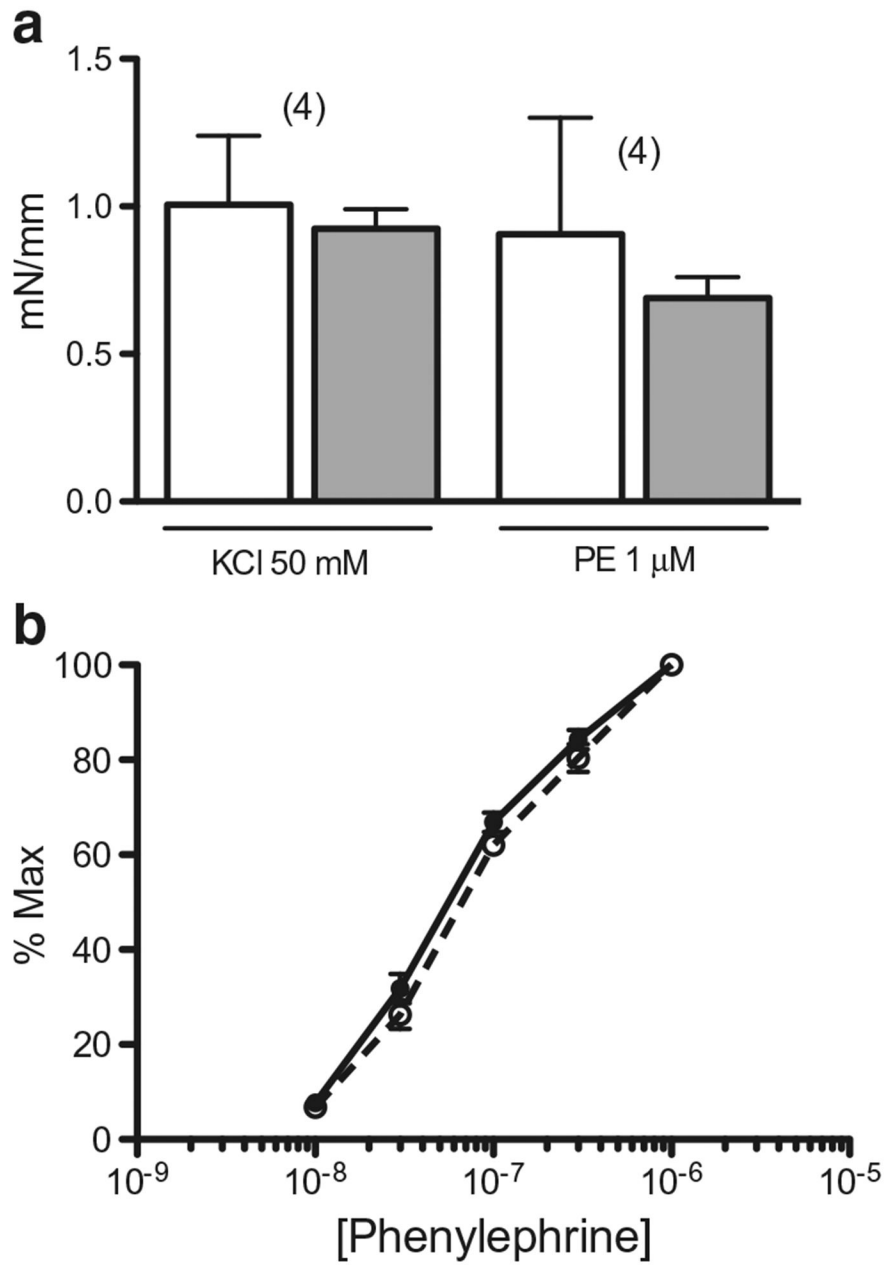
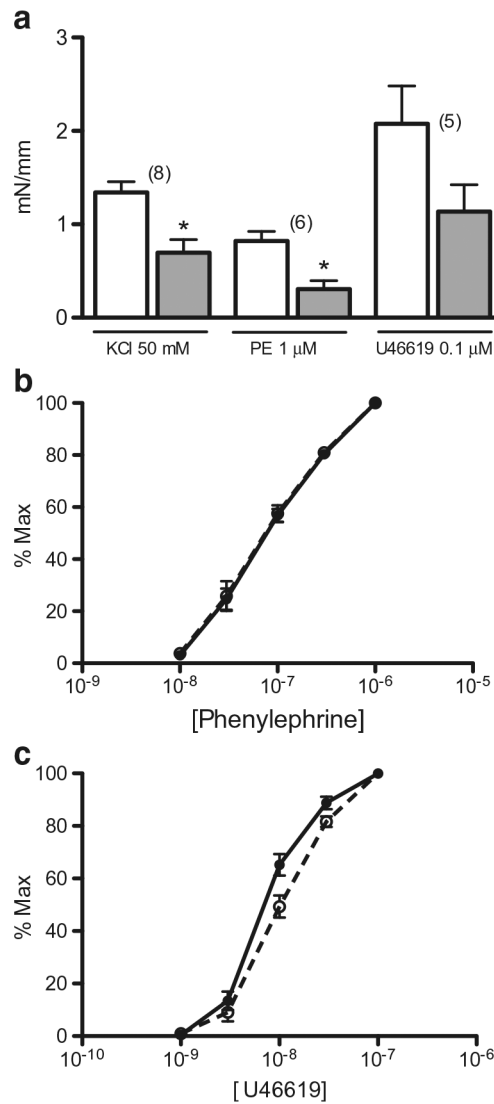


Fig. 1. Effect of NCC KO with reduced Na⁺ diet on agonist-induced aorta contraction. **a** Contractile responses of ring segments from NCC KO with (*closed bar*) and without reduced Na⁺ diet (*open bar*) to 50 mM KCl and 1 μM phenylephrine (PE) are expressed as millinewton/millimeter (mN/mm) vessel length. *Number in parenthesis* represents number of animals in each case. **b** Concentration-contraction curve of aorta to phenylephrine (*n* = 4 in each case) from NCC KO with reduced Na⁺ (*open circle plus dashed line*) and normal diet (*closed circle plus uninterrupted line*) was determined from the experiments in **a** and expressed as percent maximal contraction

**Fig. 2.**

Effect of pendrin/NCC double KO on agonist-induced aorta contraction. **a** Contractile responses of ring segments from pendrin/NCC double KO (*closed bar*) and wild type (*open bar*) to 50 mM KCl, 1 μ M phenylephrine (PE), and 0.1 μ M U46619 were expressed as millinewton/millimeter (mN/mm) vessel length. *Number in parenthesis* represents number of mice in each case. **b, c** Concentration-contraction curves of aorta to phenylephrine ($n = 6$ in each case) and U46619 ($n = 5$ in each case), respectively, from pendrin/NCC double KO (*open circle plus dashed line*) and wild type (*closed circle plus uninterrupted line*) were determined from the experiments in **a** and expressed as percent maximal contraction. * $p < .05$

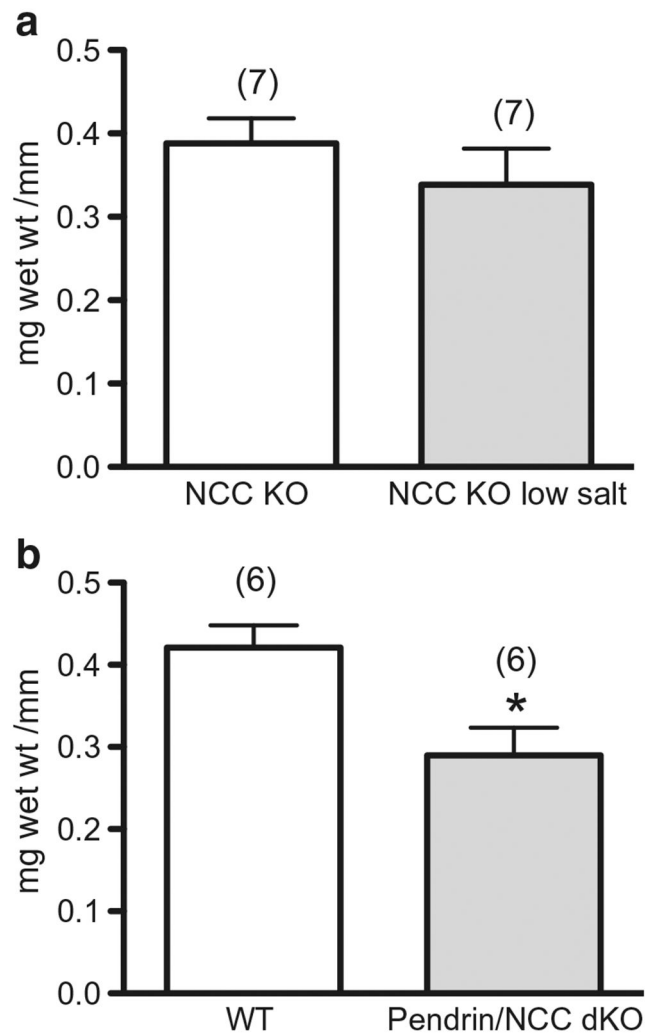


Fig. 3. Wet weight of aorta from NCC KO with reduced Na⁺ diet and pendrin/NCC double KO. Shown are milligram wet weight per millimeter (mg wet wt)/mm ring segment length from **a** NCC KO with (*closed bar*) and without reduced Na⁺ diet (*open bar*) and **b** pendrin/NCC double KO (dKO; *closed bar*) and wild type (*open bar*). *Number in parenthesis* represents number of animals. **p* < .05

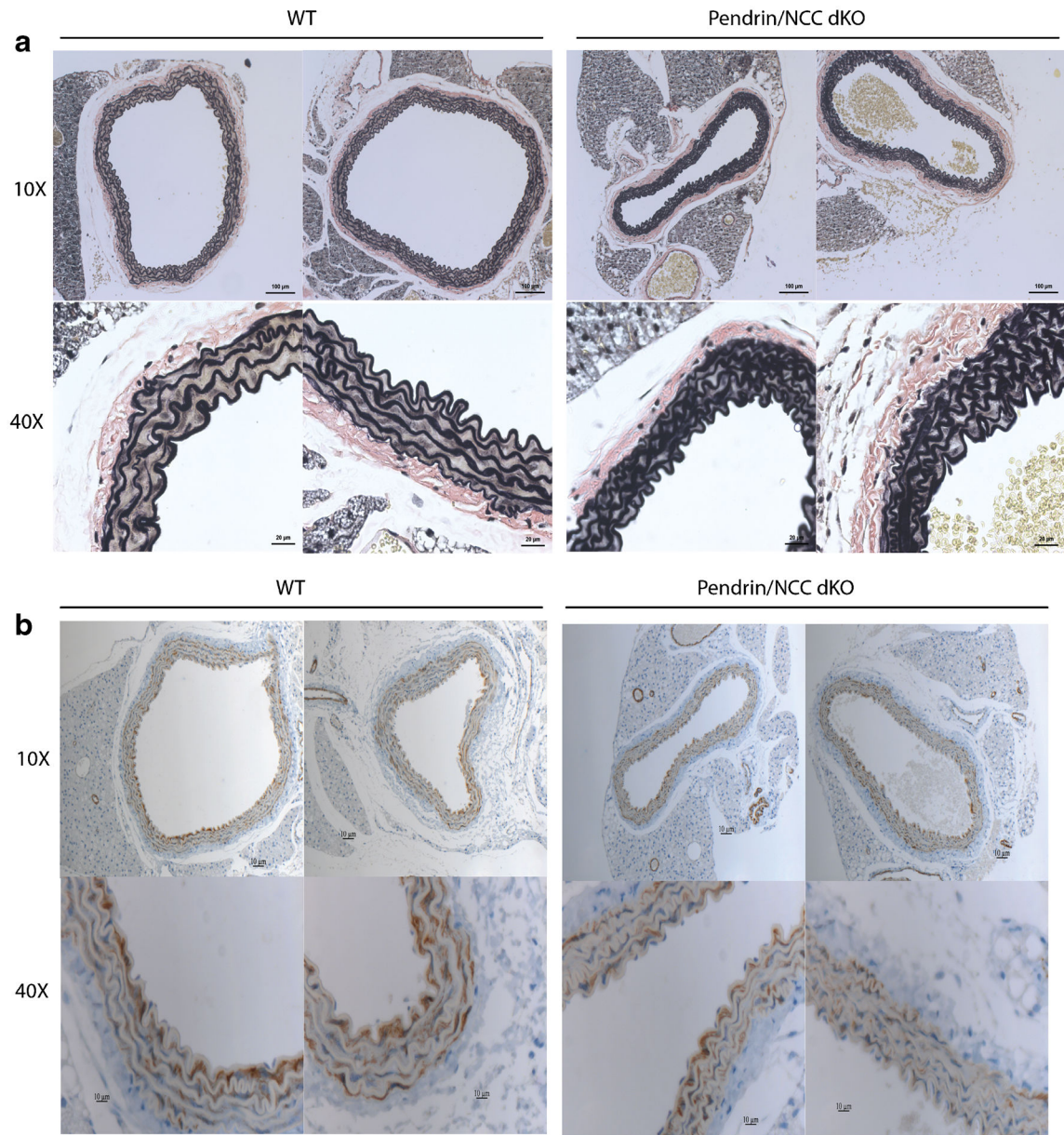


Fig. 4. Verhoeff-Van Gieson and smooth muscle actin staining in aorta from pendrin/NCC double KO. **a** Verhoeff-Van Gieson and **b** smooth muscle actin immunohistochemical stained aorta sections from two pendrin/NCC double KO and wild type (WT) mice at $\times 10$ and $\times 40$ magnification. See text for description