Impairment of Sodium Balance in Mice Deficient in Renal Principal Cell Mineralocorticoid Receptor

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Germline inactivation of the mineralocorticoid receptor (MR) gene in mice results in postnatal lethality as a result of massive loss of sodium and water. The knockout mice show impaired epithelial sodium channel (ENaC) activity in kidney and colon. For determination of the role of renal MR in aldosterone-driven ENaC-mediated sodium reabsorption, mice with principal cell MR deficiency were generated using the Cre-loxP system. For driving Cre recombinase expression in principal cells, the regulatory elements of the mouse aquaporin 2 (AQP2) gene were used. Mutant mice (MR<sup>AQP2Cre</sup>) were obtained by crossing AQP2Cre mice with mice that carried a conditional MR allele. Under standard diet, MRAQP2Cre mice develop normally and CNT can compensate to a large extent deficient ENaC-mediated sodium reabsorption in late CNT and CD. Mutant mice show preserved renal ENaC activity, this study provides evidence that the late distal convoluted tubule and early CD and late CNT can be compensated under standard diet but no longer when sodium supply is limited. Because the mutant mice show preserved renal ENaC activity, this study provides evidence that the late distal convoluted tubule and early CNT can compensate to a large extent deficient ENaC-mediated sodium reabsorption in late CNT and CD.

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Control of sodium reabsorption in kidney is essential for maintaining sodium balance and requires tight regulation of the activity of several transport proteins, which are differentially expressed in distinct functional segments of the renal tubules (1,2). One important regulator of sodium reabsorption is the mineralocorticoid hormone aldosterone, which activates the transcription factor mineralocorticoid receptor (MR) in its target cells (3). MR binds mineralocorticoids as well as glucocorticoids with the same affinity. However, in aldosterone target cells, MR is protected from glucocorticoids as well as glucocorticoids with the same affinity. Moreover, in aldosterone target cells, MR is protected from glucocorticoids as well as glucocorticoids with the same affinity.

Sodium reabsorption in the aldosterone-responsive distal tubular segments (Figure 1A) is mediated by the amiloride-sensitive epithelial sodium channel (ENaC) that is present in the principal cells of the collecting duct (CD) and connecting tubule (CNT), together with the thiazide-sensitive sodium-chloride co-transporter (TSC) in the distal convoluted tubule (DCT). In mice, ENaC and TSC are coexpressed in the late part of the DCT (7). Aldosterone-regulated ENaC activity is also found in epithelial cells of the distal colon and the ducts of the salivary and sweat glands (8).

ENaC is a heteromultimeric channel composed of three subunits (α, β, and γ) (9). Heterologous expression of single ENaC subunits in Xenopus oocytes revealed that only α-ENaC can by itself generate a sodium current (10). However, β-ENaC and γ-ENaC greatly potentiate sodium current when coexpressed with α-ENaC (9). In mice, impairment of α-ENaC translocation to the apical membrane is thought to disturb the trafficking of β-ENaC and γ-ENaC (11).

The importance of α-ENaC in sodium homeostasis in humans is highlighted by a homozygous loss-of-function mutation in the gene that encodes the α-ENaC subunit, causing pseudohypoaldosteronism type 1 (PHA1), which is characterized by renal salt wasting and high plasma aldosterone levels (12,13). Gene targeting in mice confirmed the crucial role of α-ENaC for sodium homeostasis (14).

In contrast to autosomal recessive PHA1, autosomal dominant forms of PHA1 are caused by inactivating mutations in the human MR gene (15,16). Germline inactivation of the MR gene in mice results in early postnatal lethality as a result of massive...
loss of sodium and water. These MR knockout mice show strongly impaired ENaC activity in kidney and colon (17). To overcome postnatal lethality and to address the role of MR in renal ENaC-mediated sodium reabsorption, we generated mice that lack MR in renal principal cells using the Cre-loxP recombination system that allows somatic cell-specific gene inactivation. To drive the expression of the Cre recombinase, we used the regulatory elements of the mouse aquaporin 2 (AQP2) gene. AQP2 is an apical water channel, which is coexpressed with ENaC in principal cells of the CD and CNT (7). AQP2Cre transgenic mice were bred with mice that carried a conditional MR allele (18) to generate mutant mice (MR<sub>AQP2Cre</sub>). Under standard diet, MR<sub>AQP2Cre</sub> mice grow and develop normally and show unaltered renal sodium excretion. When challenged with a low-sodium diet, MR<sub>AQP2Cre</sub> mice show increased renal sodium and water excretion that is associated with a continuous loss of body weight but, surprisingly, preserved renal ENaC activity. Analysis of protein expression revealed that the loss of MR and apical α-ENaC is restricted to the CD and late CNT. MR<sub>AQP2Cre</sub> mice exhibit strongly increased plasma aldosterone levels under low-sodium as well as standard diet. We conclude that targeted inactivation of MR in CD and late CNT causes renal sodium and water loss, which can be compensated by increased aldosterone levels acting on upstream renal tubular segments under standard diet but no longer when sodium supply is limited.

**Materials and Methods**

**Generation of Transgenic Mice**

A mouse genomic PAC library (RPCI21; Deutsches Resourcenzentrum für Genomforschung, Berlin, Germany) was screened with a probe for the AQP2 gene. A PAC harboring a 125-kb 5′ upstream region and a 31-kb 3′ downstream region of the AQP2 gene was modified by ET-recombination (19) to insert a cassette that contained sequences that encode a codon-improved Cre recombinase (20) (figure 1B). The modified genomic fragment that contained the Cre knock-in at the ATG of the AQP2 gene was microinjected into the pronucleus of FVB/N mouse oocytes (21). Founder mice were bred to C57Bl/6 mice to generate Cre-transgenic (AQP2Cre) mice. A two-copy line was chosen to generate MR mutant mice. The mutant MR<sub>flox/flox</sub>AQP2Cre (MR<sub>AQP2Cre</sub>) mice and their control MR<sub>flox/flox</sub> littermates were obtained by breeding MR<sub>flox/flox</sub> with MR<sub>flox/wt</sub>AQP2Cre mice. The conditional MR allele (MR<sub>flox</sub>) was generated as reported previously (18). The genetic background of the mice that were used in this study was a mixture of FVB/N and C57Bl/6. All experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD).

**Immunohistochemistry**

Kidney paraffin sections were incubated with a mouse monoclonal MR antibody (1:20) (22), followed by incubation with a goat anti-mouse biotinylated antibody (1:150; Dianova-Jackson Laboratories, Hamburg, Germany). Staining was visualized using a horseradish peroxidase–streptavidin complex (1:200; Vector Laboratories, Burlingame, CA). A goat polyclonal AQP2 antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) was used with the same method as the MR antibody, except that sections were incubated with a swine anti-goat biotinylated antibody (1:80; Cedarline Laboratories, Hornby, ON, Canada) and staining was visualized using an alkaline phosphatase–streptavidin complex (1:200; Vector Laboratories).

**Immunofluorescence**

Kidneys were dissected and processed after removal of the inner medullary part as described previously (11). Serial cryosections were incubated with mouse anti-MR (1:20), mouse anti-calbindin D28k (1:1000; Dianova-Jackson Laboratories, Hamburg, Germany), and AQP2 antibody (1:80; Dianova-Jackson Laboratories, Hamburg, Germany) followed by incubation with a swine anti-mouse biotinylated antibody (1:150; Dianova-Jackson Laboratories, Hamburg, Germany) and staining with an alkaline phosphatase–streptavidin complex (1:200; Vector Laboratories).
mice that expressed Cre recombinase under the control of the

**MRAQP2Cre Mice Are Able to Maintain Sodium Balance under Standard Diet**

In contrast to MR inactivation in the germline (17), inactivation of the MR gene in renal principal cells did not impair survival, because at 4 wk, a Mendelian distribution of genotypes was observed. When fed with a standard diet, MRAQP2Cre mice grew and developed normally and exhibited unaltered body weight (data not shown). No change in plasma sodium and potassium levels or in urine volume and urinary sodium concentration was observed in MRAQP2Cre mice compared with controls (data not shown). Therefore, absolute sodium excretion was not changed (Figure 2). These results show that MRAQP2Cre mice are able to maintain sodium balance under standard conditions.

**MRAQP2Cre Mice Show Increased Renal Loss of Sodium and Water under Low-Sodium Diet**

Two-month-old mice were challenged with a low-sodium diet. After 10 d under salt restriction, both control and MRAQP2Cre mice significantly decreased the absolute sodium excretion in comparison with standard diet conditions (Figure 2). This indicates that the low-sodium diet was effective and that the mice attempted to conserve sodium by enhancing renal...
sodium reabsorption. However, this attempt was only partially successful in the mutants, because their absolute sodium excretion was 70% higher compared with that of controls (Figure 2). Both genotypes showed no significant difference in food consumption and therefore in sodium intake (controls 0.18 ± 0.01 g food/24 h per g body wt [n = 11]; mutants 0.20 ± 0.01 g food/24 h per g body wt [n = 13]; P = 0.38), as well as in water consumption (controls 0.13 ± 0.01 ml/24 h per g body wt [n = 9]; mutants 0.13 ± 0.03 ml/24 h per g body wt [n = 9]; P = 0.85).

In contrast, MRAQP2Cre mice showed increased urine volume produced per day (controls 1.34 ± 0.05 ml/24 h [n = 15]; mutants 1.85 ± 0.16 ml/24 h [n = 15]; P < 0.05) but unchanged urinary sodium concentration (controls 30.3 ± 1.5 mmol/L [n = 15]; mutants 32.3 ± 1.0 mmol/L [n = 15]; P = 0.25) compared with controls after 10 d on low-sodium diet. This increase was associated with a continuous loss of body weight (Figure 3A) and hyperkalemia (controls 6.48 ± 0.18 mmol/L [n = 9]; mutants 7.62 ± 0.25 mmol/L [n = 7]; P < 0.05). The additional urine excretion in mutants under low-sodium diet did not lead to a significant change in plasma sodium concentration within the observation period of 10 d (standard diet 147.7 ± 1.2 mmol/L [n = 9]; low-sodium diet 149.7 ± 1.3 mmol/L [n = 6]; P = 0.29). The additional renal loss of 0.51 ml of fluid during the 10th day was paralleled by a relative loss of 0.34 g body wt of mutants versus controls. Determination of the absolute urinary potassium excretion using the same urine samples that uncovered increased absolute urinary sodium excretion in mutants revealed no significant difference between mutants and controls (controls 25.3 ± 1.5 μmol/24 h per g body wt [n = 15]; mutants 27.7 ± 2.1 μmol/24 h per g body wt [n = 15]; P = 0.34).

A detailed time-course analysis of the first 5 d under low-sodium diet revealed that, from day 2 onward, MRAQP2Cre mice show increased renal sodium excretion compared with controls, but within the first 24 h, no genotype effect was detected (Figure 3B). Whereas urinary sodium concentration was unchanged in mutant mice compared with control mice during the whole salt-restriction period, MRAQP2Cre mice showed increased urine volume production, resulting in increased renal sodium excretion (Figure 3C). Both genotypes showed no significant difference in food and water consumption (data not shown). For the time-course analysis, the mice were kept permanently in metabolic cages. Under these conditions, the renal salt wasting of MRAQP2Cre mice was more pronounced than when the mutants and controls were kept for 9 d in their home cage and put only at the 10th day in a metabolic cage for urine collection (Figures 2 and 3B). It is interesting that controls showed reduced absolute urinary sodium excretion (home/metabolic cage 1.91 ± 0.12 μmol/24 h per g body wt [n = 15]; metabolic cage only 1.18 ± 0.10 μmol/24 h per g body wt [n = 5]; P < 0.005) and reduced urine sodium concentration (home/metabolic cage 30.3 ± 1.5 mmol/L [n = 15]; metabolic cage only 17.2 ± 0.5 mmol/L [n = 5]; P < 0.005) when kept permanently in metabolic cages, indicating that in the home cage, their own feces are used as an additional sodium source. In the mutants, increased absolute urinary sodium excretion but no significant reduction of urine sodium concentration was observed (home/metabolic cage 32.3 ± 1.0 mmol/L [n = 15]; metabolic cage only 25.2 ± 3.6 [n = 5]; P = 0.12).

Determination of plasma aldosterone revealed a dramatic increase in MRAQP2Cre mice (Table 1). After 3 d of low-sodium diet, as expected, plasma aldosterone increased in controls.

![Figure 3](image-url)

**Figure 3.** MR<sup>AQP2Cre</sup> mice show a continuous loss of body weight, sodium, and water under low-sodium diet. (A) Body weight variation determined in home cages as the percentage difference with the initial body weight at day 1 in controls (□) and MR<sup>AQP2Cre</sup> (■) mice. MR<sup>AQP2Cre</sup> mice show a continuous loss of body weight, whereas controls gain weight. (B and C) Time course for 24-h urinary sodium excretion (B) and 24-h urine volume (C) determined in metabolic cages. MR<sup>AQP2Cre</sup> mice (■) show a continuously increased urinary sodium excretion and urine production over time from day 2 onward after the switch to low-sodium diet. *P < 0.05; **P < 0.005; n = 10 to 11 per genotype.

**Table 1.** Plasma aldosterone in control and MR<sup>AQP2Cre</sup> mice under standard diet and after 3 or 10 d of low-sodium diet<sup>a</sup>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Plasma Aldosterone (pg/ml)</th>
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<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Standard</td>
<td>430 ± 82 (7)</td>
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<tr>
<td>Low-sodium (3 d)</td>
<td>1083 ± 154 (10)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low-sodium (10 d)</td>
<td>3167 ± 423 (3)&lt;sup&gt;f&lt;/sup&gt;</td>
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<sup>a</sup>Data are means ± SEM (number of observations).
<sup>b</sup>P < 0.005 (mutants versus controls).
<sup>c</sup>P < 0.05 (low-sodium diet versus standard diet).
compared with standard diet. However, an increase was also detected in mutants to the same extent. Under both diets, mutants exhibit a 10-fold elevated plasma aldosterone levels compared with controls. After 10 d of low-sodium diet, plasma aldosterone increased further in both genotypes, and mutants still exhibited six-fold increased levels.

Because the elevated plasma aldosterone that was observed in MRAQP2Cre mice might activate the GR (26) and thereby compensate at least partially for the loss of MR in the targeted cells, MRAQP2Cre mice were treated in parallel to the onset of the low-sodium diet with the GR antagonist RU486 for 5 d. RU486 had no effect on food and water consumption, absolute urinary sodium excretion, and body weight variation in both control and MRAQP2Cre mice (data not shown).

**MRAQP2Cre Mice Show Preserved ENaC Activity**

To examine whether ENaC-mediated renal sodium reabsorption was affected in MRAQP2Cre mice, we determined the effect of amiloride, a specific blocker of ENaC, on the excreted part of the sodium filtered by the kidney (fractional excretion of sodium [FE(Na⁺)]). Because after 10 d of low-sodium diet MRAQP2Cre and control mice show a similar GFR (controls 6.42 ± 0.51 μl/min per g body wt [n = 9]; mutants 6.43 ± 0.46 μl/min per g body wt [n = 10]; P = 0.98), we assessed renal ENaC activity by determination of the amiloride-inhibitable part of the FE(Na⁺). In the absence of amiloride, FE(Na⁺) tended to be higher in MRAQP2Cre mice than in controls, suggesting salt wasting. However, the difference between both genotypes did not reach significance within the 2-h sampling period. As expected, amiloride strongly increased FE(Na⁺) in controls. Surprising, amiloride had a comparable effect on FE(Na⁺) in MRAQP2Cre mice, suggesting preserved renal ENaC activity in the mutants (Figure 4).

**MRAQP2Cre Mice Show Loss of MR and Apical α-ENaC in CD and Late CNT**

Because MRAQP2Cre mice showed preserved amiloride-sensitive renal sodium reabsorption, we assessed renal ENaC expression by immunohistochemistry in control and mutant mice after 10 d of low-sodium diet. As reported previously (27), we detected in controls a weaker apical α-ENaC staining in cortical CD (Figure 5A) compared with CNT (Figure 5C). In MRAQP2Cre mice, both MR and α-ENaC were absent from all principal cells of CD (Figure 5B) and most principal cells of the CNT (Figure 5D), demonstrating efficient targeting of MR in these tubular segments and the importance of MR for apical α-ENaC protein expression. However, some principal cells of the CNT retain MR and therefore apical α-ENaC expression (Figure 5D, arrows). Closer inspection and co-immunostainings with antibodies against calbindin D28k and TSC, established markers for distal tubule subsegments (23), revealed strong expression of

![Figure 4. MR^AQP2Cre^ mice show preserved renal ENaC activity under low-sodium diet. Determination of the effect of amiloride on the fractional excretion of sodium [FE(Na⁺)] in untreated (□) and amiloride-treated (▪) control and MR^AQP2Cre^ mice after 10 d of low-sodium diet. The FE(Na⁺) was calculated as (([urine sodium]/[plasma sodium]) × ([plasma creatinine]/[urine creatinine])). Untreated MR^AQP2Cre^ mice show a higher FE(Na⁺) than the controls, suggesting salt wasting. However, the difference between both genotypes did not reach significance within the 2-h sampling period (controls 0.17 ± 0.04% [n = 5]; mutants 0.24 ± 0.04% [n = 6]; P = 0.20). MR^AQP2Cre^ mice show no significant difference in the amiloride-sensitive portion of the FE(Na⁺). **P < 0.005 (amiloride treated versus untreated). Untreated groups n = 5 to 6; amiloride-treated groups n = 11 to 13.](https://example.com/figure4)

![Figure 5. Loss of MR and apical α-ENaC expression in CD and CNT in MR^AQP2Cre^ mice. CD and CNT from control (A and C) and MR^AQP2Cre^ (B and D) mice that were kept for 10 d on low-sodium diet. Double immunofluorescence on the same cryosection with antibodies against α-ENaC and MR, respectively. CD and CNT were identified on account of their localization in medullary rays and in the cortical labyrinth adjacent to cortical radial vessels, respectively. (A and C) In control, apical α-ENaC is well detectable in MR-expressing cells of CD (A) and CNT (C). In MRAQP2Cre mice, apical α-ENaC and MR immunostainings are absent from principal cells in the CD, which express high levels of Cre as shown by immunofluorescence on a consecutive cryosection (low-magnification insert). (D) In CNT of MR^AQP2Cre^, apical α-ENaC and MR are absent from most of the CNT cells. Only a few CNT cells (arrows) retain MR and apical α-ENaC expression.](https://example.com/figure5)
Figure 6. Within the CNT of MR<sup>AQP2Cre</sup> mice, loss of MR is restricted to the late part of this tubular segment. DCT (D), CNT, and CD from a MR<sup>AQP2Cre</sup> mouse that was kept for 10 d on a low-sodium diet. Immunofluorescence on cryosections with antibodies against Cre, calbindin D28k (CB), TSC, α-ENaC, and MR. (A) Cre expression is strong in CD but weak in CNT. V, cortical radial vessel. (B) The early CNT (†), which can be distinguished from the adjacent DCT (D) by the higher prevalence of CB-negative intercalated cells, lacks visible Cre expression. Cre becomes detectable only in cells (arrows) that are localized further downstream. (C) The early CNT (†), like the adjacent TSC-positive DCT (D), consistently retains expression of MR and apical α-ENaC.

Cre in the CD and decreasing levels along the CNT toward the early CNT. In this subsegment, Cre expression was undetectable (Figure 6, A and B). Detailed analysis of MR and α-ENaC expression revealed that the cells that retain MR and apical α-ENaC expression are situated in the early CNT, adjacent to the transition from DCT to CNT, and in the late DCT (Figure 6C). Thus, MR<sup>AQP2Cre</sup> mice exhibit targeting of the MR gene and loss of apical α-ENaC expression in cortical CD and late CNT. The early CNT, however, is not targeted because of undetectable Cre expression. A gross survey revealed that <30% of the CNT principal cells were not targeted.

Immunofluorescence for β-ENaC and γ-ENaC revealed apical expression in cortical CD and CNT of control mice. In MR<sup>AQP2Cre</sup> mice, β-ENaC and γ-ENaC are located in the cytoplasm of CD and late CNT cells, where MR is lost, but are expressed at the apical membrane of early CNT and late DCT cells, where MR and α-ENaC expression are preserved (data not shown).

Investigation of the outer medullary CD using immunofluorescence revealed a complete loss of MR, as already observed by immunohistochemistry. However, apical ENaC staining could not be detected in controls and mutants (data not shown).

**MR<sup>AQP2Cre</sup> Mice Show Increased ENaC Activity in Colon**

To determine whether the elevated plasma aldosterone levels that were observed in MR<sup>AQP2Cre</sup> mice are operative on ENaC-positive epithelial cells that express MR, we assessed ENaC activity in the colon after 10 d of low-sodium diet. As expected, mutants exhibited an increased amiloride-sensitive transepithelial voltage compared with control mice (controls 22.1 ± 4.7 mV [n = 6]; mutants 34.9 ± 4.0 mV [n = 6]; P < 0.05).

**Discussion**

We report the generation of mice with specific inactivation of the MR gene in renal principal cells (MR<sup>AQP2Cre</sup> mice). Whereas the germ line inactivation (knockout) of the mouse MR gene results in early postnatal lethality as a result of a massive renal loss of sodium and water (17), the renal principal cell–specific inactivation of MR did not impair survival. In contrast to the MR knockout mice, not all tight epithelia are targeted in MR<sup>AQP2Cre</sup> mice. The use of the regulatory elements of the mouse AQP2 gene restricts the MR deficiency to the kidney and within the kidney to ENaC-expressing principal cells of the CNT and CD. Therefore, MR<sup>AQP2Cre</sup> mice allow the investigation of the role of these cells in MR-regulated sodium homeostasis.

**Renal Principal Cell–Specific Inactivation of MR**

In this study, the AQP2 promoter was used to drive the expression of the Cre recombinase. In contrast to the homeobox B7 (Hoxb7) gene, which is expressed only in CD and which was used by Rubera et al. (11) to inactivate α-ENaC at this site, the AQP2 gene is expressed in principal cells of the CNT and CD (7). The AQP2 promoter was already used for Cre recombinase expression to target renal principal cells. However, a 14-kb human as well as a 9.5- and an 11-kb mouse AQP2 promoter fragment gave variegated patterns of transgene activity (28–30). We used here a PAC transgene that harbors a 156-kb genomic fragment that contains the regulatory elements of the mouse AQP2 gene, with an approximately 125-kb 5′ upstream region. Cre, MR, and α-ENaC expression analysis by immunofluorescence revealed that the Cre expression of the PAC transgene resembles the reported AQP2 expression pattern, with strong expression in CD and decreasing levels along the CNT toward the early CNT (7). Therefore, the AQP2Cre-PAC transgene that we used allowed us to target all principal cells of the CD and late CNT. The targeted cells with ablation of MR expression showed no apical staining for α-, β-, and γ-ENaC. The early CNT and late DCT were not targeted because of absence of Cre expression. In this region, MR and apical α-, β-, and γ-ENaC expression was still observed. However, a pharmacologic study in rats that aimed to ablate MR function by using a specific antagonist showed no effect of MR blockade on apical ENaC expression under low-sodium diet, while observing an effect on the plasma potassium levels (31). We demonstrate, using a genetic approach that leads to complete loss of MR signaling in the targeted cells, that α-, β-, and γ-ENaC trafficking to the cell surface depends on MR function.

**Late CNT Is an Important Site of MR-Regulated ENaC-Mediated Sodium Reabsorption**

MR knockout mice die in the second week after birth as a result of severe sodium and water loss. They showed decreased ENaC-mediated sodium reabsorption in both kidney and colon (17). On a standard diet, MR<sup>AQP2Cre</sup> mice are viable, grow normally, and show unaltered absolute sodium excretion. When challenged with a low-sodium diet, MR<sup>AQP2Cre</sup> mice experience a continuous sodium wasting, as indicated by elevated absolute sodium excretion, and a continuous water loss,
as shown by the increased urine volume that is associated with a continuous loss of body weight.

To counterbalance the loss of MR in the majority of renal principal cells, the RAAS was activated in MR\(^{-}\)AQP2Cre mice under standard and low-sodium diet, as shown by the strongly elevated plasma aldosterone levels. Under standard diet, this aldosterone increase was apparently sufficient to achieve complete compensation. However, when sodium supply was limited, even the strong activation of the RAAS could no longer fully compensate the MR deficiency in MR\(^{-}\)AQP2Cre mice. It was previously reported that mice with \(\alpha\)-ENaC deficiency in the CD show no change in renal sodium and water excretion. Compared with controls, these \(\alpha\)-ENaC-deficient mice showed normal plasma aldosterone levels on both standard and low-sodium diet (11). On the basis of these results, we conclude that the increased renal sodium excretion and the observed elevation of plasma aldosterone levels that were observed in MR\(^{-}\)AQP2Cre mice result primarily from the loss of MR expression in the late CNT. The importance of the CNT in ENaC-mediated sodium reabsorption is highlighted by a recent patch-clamp study that showed that ENaC-mediated sodium transport is several times higher in the CNT than in the CD in aldosterone-treated rats (32). This is consistent with a previous study on isolated perfused nephron segments, which showed that the rate of sodium reabsorption is much higher in the CNT than in the CD (33).

Because in MR knockout mice most of the amiloride effect was abolished (17), we expected to find a clear reduction of ENaC activity in MR\(^{-}\)AQP2Cre mice. However, MR\(^{-}\)AQP2Cre mice show only a tendency to higher FE\((\text{Na}^+)^{\text{a}}\) compared with controls within an acute 2-h sampling procedure, and amiloride strongly increased FE\((\text{Na}^+)^{\text{a}}\) to the same extent in both genotypes. We have shown that the late DCT and early CNT, which are not targeted, still express apical \(\alpha\)-ENaC. Increased colonic ENaC activity in MR\(^{-}\)AQP2Cre mice indicates that the elevated plasma aldosterone levels are operative on tight epithelial cells that still express MR. Therefore, increased sodium transport in the early CNT and late DCT is expected, representing an intrinsic compensatory mechanism that is responsible for the preserved renal ENaC activity that is observed in MR\(^{-}\)AQP2Cre mice.

Because it is known that the renal principal cells express GR (34), which bind aldosterone with low affinity (26), and it was shown that in the absence of MR activated GR can induce renal sodium reabsorption (35), we treated MR\(^{-}\)AQP2Cre mice with RU486 for 5 d in parallel with the onset of low-sodium diet to determine whether a possibly activated GR counterbalances the loss of MR. The treatment did not worsen the renal sodium excretion and loss of body weight of MR\(^{-}\)AQP2Cre mice, suggesting that no major GR-driven compensation takes place in the targeted cells.

Unchanged acute FE\((\text{Na}^+)\) in MR\(^{-}\)AQP2Cre mice is not easy to reconcile with the salt-wasting syndrome and the continuous loss of body weight that were observed under limited sodium supply. A possible explanation is that our assessment of acute FE\((\text{Na}^+)\) may allow the detection of only a three-fold increase as measured in heterozygous MR knockout mice (17). Therefore, minor changes that could account for the observed 70% increased absolute sodium excretion over 24 h, as well as minor changes in the amiloride-sensitive part of the FE\((\text{Na}^+)\), might escape this type of analysis. For detection of minimal changes in renal ENaC activity, more sophisticated quantitative analysis using patch-clamp of single cells or nephron segment perfusions are required. However, these types of analysis might be extremely complicated because the boundary of the early and late CNT is visually indistinguishable and can vary from animal to animal.

Other Possible Sites of Compensatory Action by Increased Plasma Aldosterone Levels

We showed that MR loss in CD and late CNT leads to increased plasma aldosterone. Besides activating MR in untargeted principal cells of the early CNT and late DCT, this may activate TSC-mediated sodium reabsorption in the DCT and ENaC-mediated sodium reabsorption in the colon. For the colon, we showed by measuring the amiloride-sensitive rectal transepithelial voltage that MR\(^{-}\)AQP2Cre mice exhibit a 30% increase in colonic ENaC activity. However, the colon is known to reabsorb only approximately 5% of the total amount of absorbed sodium. Moreover, sodium transport in the colon is almost absent after weaning compared with the early lifetime (36). Therefore, it is unlikely that colonic ENaC activity plays a major role in the compensation of MR loss in CD and late CNT. Because we targeted the renal principal cells, we focused our analysis of MR\(^{-}\)AQP2Cre mice on ENaC-mediated sodium reabsorption. Since the amiloride-sensitive portion of the FE\((\text{Na}^+)\) is similar in MR\(^{-}\)AQP2Cre and control mice, a strong compensatory action by other renal sodium reabsorbing systems such as the TSC in DCT is not expected.

Conclusion

We achieved MR deficiency in whole CD and late CNT using an AQP2Cre-PAC transgene in combination with a conditional MR allele. MR\(^{-}\)AQP2Cre mice grow and develop normally under standard diet. The switch to low-sodium diet results in continuous loss of body weight as a result of increased renal loss of sodium and water. The mutant mice show dramatically increased plasma aldosterone levels on both standard and low-sodium diet, which leads to elevated colonic ENaC activity. Therefore, increased ENaC-mediated sodium reabsorption in untargeted principal cells of the late DCT and early CNT is expected. This intrarenal compensatory action could explain the preserved ENaC activity that was observed in mutant mice. Under low-sodium diet, no apical \(\alpha\)-ENaC expression was detectable in the late CNT and cortical CD. In conclusion, we showed that the targeted inactivation of MR in late CNT and CD can be compensated on a standard diet by activation of the RAAS, but this compensation fails when sodium supply is limited. The preserved renal ENaC activity that was observed in the mutant mice indicates that the late DCT and early CNT can compensate to a large extent deficient ENaC-mediated sodium reabsorption in late CNT and CD. In addition, our study demonstrates for the first time using a genetic approach the crucial role of MR for ENaC trafficking in vivo.
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Disclosures
None.

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