Hyperaldosteronism, hypervolemia, and increased blood pressure in mice expressing defective APC

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Bhandaru M, Kempe DS, Rotte A, Rexhepaj R, Kuhl D, Lang F. Hyperaldosteronism, hypervolemia, and increased blood pressure in mice expressing defective APC. Am J Physiol Regul Integr Comp Physiol 297: R571–R575, 2009. First published June 3, 2009; doi:10.1152/ajpregu.00070.2009.—Adenomatous polyposis coli (APC) fosters degradation of β-catenin, a multifunctional protein upregulating the serum- and glucocorticoid-inducible kinase (SGK1). SGK1 regulates a wide variety of renal transport processes. The present study explored the possibility that APC influences renal function. To this end, metabolic cage experiments were performed in mice carrying a loss-of-function mutation in the APC gene (apcMin+/-), their wild-type littermates (apc+/-), and apcMin+/- mice lacking functional SGK1 (apcMin+/-/sgk1-/-). As a result, mean body weight, food intake, fluid intake, salt appetite, urinary flow, as well as plasma Na+ and K+ concentrations were similar in apcMin+/- mice, apc+/- mice, and apcMin+/-/sgk1-/- mice. Glomerular filtration rate and absolute renal Na+ excretion were decreased, and fractional urinary K+ excretion was enhanced in apcMin+/- mice. The hyperaldosteronism of apcMin+/- mice was paralleled by significantly elevated plasma volume and blood pressure. The experiments reveal an influence of defective APC on adrenal hormone release and renal function, effects partially but not completely explained by APC dependence of SGK1 expression.

adenomatous polyposis coli; serum and glucocorticoid inducible kinase; aldosterone; corticosterone

The adenomatous polyposis coli (APC) protein binds the multifunctional protein β-catenin and fosters its degradation (10, 13, 17, 21). Lack of APC leads to accumulation of β-catenin, which enters into the nucleus and triggers the expression of several genes (11, 29), including the serum- and glucocorticoid-inducible kinase (SGK1) (8, 19). Among other targets, SGK1 phosphorylates glycogen synthase kinase 3 (GSK3) (24), which in turn phosphorylates β-catenin and is thus involved in APC-dependent regulation of cellular functions (5, 31, 35). SGK1 is a stimulator of a wide variety of renal transport proteins (15). Moreover, lack of SGK1 leads to an increase of plasma mineralocorticoid concentration (33).

Mice carrying a mutation in the APC gene (apcMin+/-) leading to truncation of the gene product at amino acid 850 develop multiple intestinal tumors (18). As shown in gastric glands (23), SGK1 expression may indeed be enhanced in those mice. The present study was thus performed to elucidate whether adrenal hormone levels and/or electrolyte homeostasis are altered in apcMin+/- mice and, if so, whether the difference may be dependent on the presence of SGK1. To this end, metabolic cage experiments were performed in apcMin+/- mice, in their wild-type littermates (apc+/-) and in apcMin+/- mice lacking in addition SGK1 (apcMin+/-/sgk1-/-).

Materials and Methods

Mice with mutated APC (apcMin+/-) and their wild-type littermates (apc+/-) with a mixed (C57/Bl-6-Sv129J) background were generated by breeding of male apcMin+/- mice initially obtained from the Jackson Laboratory (Bar Harbor, ME). Where indicated, additional mice were generated by crossing breeding apcMin+/- with gene-targeted mice lacking functional SGK1 (sgk1-/-) (33) to generate mice carrying the mutant APC and simultaneously lacking SGK1 (apcMin+/-/ sgk1-/-). Sex and age-matched mice 3 mo of age were used for the experiments. All animal experiments were conducted according to the guidelines of the American Physiological Society and were approved by local authorities according to the German law for the care and use of laboratory animals.

Mice (13–16 mice in each group, age 12 wk) were fed a control diet (cat. no. C1314, NaCl content 0.49%; Altromin, Lage, Germany) and had access to drinking water ad libitum. In one series of experiments the animals had free access to both plain tap water and 1% NaCl solution.

For evaluation of renal excretion, the mice were placed individually in metabolic cages (Techniplast, Hohenpeißenberg, Germany) for 24-h urine collection as described previously (30). They were allowed a 3-day habituation period when food and water intake, urinary flow, urinary excretion of salt, fecal excretion, and body weight were recorded every day to ascertain that the mice were adapted to the new environment. Subsequently 24-h collection of urine was performed for three consecutive days to obtain the urinary parameters. To assure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil.

To obtain blood specimen, animals were lightly anesthetized with diethylether (Roth, Karlsruhe, Germany), and about 150 μl of blood was withdrawn into heparinized capillaries by puncturing the retrobulbar plexus. The animals were allowed to recover for 2 wk.

Plasma and urinary concentrations of Na+ and K+ were measured by flame photometry (model AFM 5051; Eppendorf, Hamburg, Germany). Plasma creatinine concentrations were measured using an enzymatic colorimetric method (creatinine PAP; Lehmann, Berlin, Germany). Urinary creatinine concentrations were measured using the Jaffe method (31a).

Plasma aldosterone concentrations were determined using a commercial RIA kit (Demeditec, Kiel, Germany), and plasma corticosterone concentrations were determined using an ELISA kit (DRG Instruments, Marburg, Germany). Plasma leptin levels were measured using a commercial ELISA kit (Crystal Chemical, Houston, TX).

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Plasma volume was assessed by dye dilution using Evans Blue (Sigma, Taufkirchen, Germany). Mice were anesthetized with diethylether and 30–50 μl of an Evans Blue stock solution (3 mg/ml in 0.9% NaCl) was injected intravenously into the left retroorbital plexus using a 30-gauge insulin syringe (microfine; BD, Heidelberg, Germany). The exact applied volume was determined by weighing the syringe before and after injection. Blood samples (20–25 μl) were drawn from the right retroorbital plexus during superficial diethylether anesthesia after 10, 30, 60, and 120 min, which each time yielded a volume of 10 μl plasma after centrifugation. Absorbance was measured at 620 nm against blank mouse serum after recovery in 10 μl PBS tablets (Invitrogen, Karslruhe, Germany). Plasma concentrations of Evans Blue were calculated using the stock solution dissolved in mouse serum as a standard. Division of the applied dose of Evans Blue (in milligrams) by the y-intercept (in mg/ml) resulted in the distribution volume of Evans Blue which was normalized for body weight.

Systolic arterial blood pressure was determined by the tail-cuff method. As reviewed previously (16), the tail cuff approach to determine arterial blood pressure requires certain precautions to reduce the stress of the animals, including appropriate training of the mouse over multiple days, prewarming to an ambient temperature of 29°C, measurement in a quiet, semidarkened and clean environment, and performance of the measurements by one person and during a defined daytime, when blood pressure is stable (between 1–3 PM). All these precautions were taken in the present study.

Data are provided as means ± SE; n represents the number of independent experiments. All data were tested for significance using ANOVA, and only results with P < 0.05 were considered statistically significant.

RESULTS

The body weight was similar in apcMin/+ mice, apc+/- mice, and apcMin/+/sgk1-/- mice (Table 1). Similarly, food intake and fluid intake were not significantly different between the genotypes. Urine flow tended to be lower in apcMin/+ mice than in apc+/- mice and apcMin/+/sgk1-/- mice; a difference, however, not reaching statistical significance. Mean fractional urinary excretion of K⁺ was significantly higher in apcMin/+ and apcMin/+/sgk1-/- mice than in apc+/- mice. In contrast, absolute urinary excretion of Na⁺ was significantly lower in apcMin/+ mice than in apc+/- mice. The absolute Na⁺ excretion in apcMin/+/sgk1-/- mice tended to be higher than in apcMin/+ mice and was not significantly different from the respective value in apc+/- mice (Table 1). Thus, lack of SGK1 appeared to abrogate the decrease of renal Na⁺ excretion in mice carrying the mutant APC.

Fecal excretion was studied to further clarify the electrolyte homeostasis. As shown in Table 1, fecal dry weight was not different between the genotypes. Fecal sodium and potassium excretion was significantly higher in apcMin/+ mice than in apc+/- mice. The additional lack of SGK1 reversed the difference in fecal sodium excretion but had no significant effect on fecal potassium excretion (Table 1).

Table 1. Body weight, food and fluid intake, urinary flow, fecal dry weight, creatinine clearance, absolute and fractional urinary Na⁺, K⁺ excretion, plasma and fecal Na⁺, K⁺ concentrations and plasma leptin concentration in apc+/- mice, apcMin/+ mice and apcMin/+/sgk1-/- mice

<table>
<thead>
<tr>
<th></th>
<th>apc+/-</th>
<th>apcMin/+</th>
<th>apcMin/+/sgk1-/-</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>25.6±1.0</td>
<td>25.0±0.9</td>
<td>24.3±0.7</td>
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<td>Food intake, mg/24 h</td>
<td>4.0±0.2</td>
<td>3.9±0.2</td>
<td>3.8±0.2</td>
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<td>Fluid intake, ml/24 h</td>
<td>6.1±0.4</td>
<td>5.5±0.2</td>
<td>5.5±0.3</td>
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<td>Urinary flow, μl/24 h⁻¹·g body wt⁻¹</td>
<td>38.9±3.9</td>
<td>26.2±4.7</td>
<td>34.2±4.3</td>
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<tr>
<td>Fecal dry weight, mg/24 h⁻¹·g body wt⁻¹</td>
<td>4.8±5</td>
<td>54±3</td>
<td>52±3</td>
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<td>[Creatinine]plasma, mg/dl</td>
<td>0.30±0.02</td>
<td>0.43±0.04*</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Urinary creatinine, μg/24 h⁻¹·g body wt⁻¹</td>
<td>27±2</td>
<td>22±2</td>
<td>21±2</td>
</tr>
<tr>
<td>Creatinine clearance, μl/min·g body wt⁻¹</td>
<td>6.6±0.6</td>
<td>4.0±0.5*</td>
<td>4.4±0.6*</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion, μmol/24 h</td>
<td>134±14</td>
<td>79±14*</td>
<td>123±17</td>
</tr>
<tr>
<td>Urinary K⁺ excretion, μmol/24 h</td>
<td>509±26</td>
<td>431±45</td>
<td>448±50</td>
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<tr>
<td>Plasma Na⁺ concentration, mM</td>
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<tr>
<td>Plasma K⁺ concentration, mM</td>
<td>3.82±0.10</td>
<td>3.76±0.09</td>
<td>3.73±0.08</td>
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<td>Plasma leptin, ng/ml</td>
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<td>0.67±0.16*</td>
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<td>FE Na⁺, %</td>
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<td>0.43±0.07</td>
<td>0.61±0.06</td>
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<td>FE K⁺, %</td>
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<td>Fecal Na⁺, mmol/24 h</td>
<td>148±17</td>
<td>206±14*</td>
<td>120±108†</td>
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<tr>
<td>Fecal K⁺, mmol/24 h</td>
<td>229±15</td>
<td>286±20*</td>
<td>286±19*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 13–16, except leptin n = 6. APC, adenomatous polyposis coli; SGK1, serum- and glucocorticoid-inducible kinase. *Statistically significant (P < 0.05) difference with respect to apc+/- mice; †statistically significant (P < 0.05) difference with respect to apcMin/+ mice.

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were measured to depict differences in body fat. Serum leptin concentrations were significantly lower in both $apc^{Min/+}$ and $apc^{Min/+}/sgk1^{-/-}$ mice than in $apc^{+/+}$ mice (Table 1). Thus, APC deficiency decreased the body fat mass, an effect not sensitive to additional lack of SGK1.

Additional experiments were performed to elucidate whether the altered renal Na$^+$ output was paralleled by enhanced salt appetite. As shown in Fig. 3, neither $apc^{Mut/+}$ mice nor $apc^{-/-}$ mice preferred water or saline, and there were no differences in salt appetite between the two genotypes.

Hyperaldosteronism is known to elevate blood pressure. Thus, blood pressure was determined in an additional series of experiments. As illustrated in Fig. 4, blood pressure was indeed significantly higher in $apc^{Min/+}$ mice than in $apc^{+/+}$ mice. The difference was partially reversed by additional lack of SGK1 ($apc^{Min/+}/sgk1^{-/-}$).

**DISCUSSION**

The present observations reveal several subtle differences between mice carrying a mutation in the APC gene ($apc^{Min/+}$) and their wild-type littermates ($apc^{+/+}$). Most importantly, the $apc^{Min/+}$ mice had higher plasma aldosterone and plasma corticosterone concentrations than the $apc^{+/+}$ mice.

Lack of APC could, at least in theory, modify electrolyte homeostasis by decreased degradation of β-catenin with subsequent increase of β-catenin levels and stimulation of SGK1 expression. As demonstrated earlier, genes upregulated by β-catenin include SGK1 (8, 19), which in turn phosphorylates glycogen synthase kinase 3 (GSK3) (24) and thus blunts the downregulation of β-catenin by GSK3-dependent phosphorylation (5, 31, 35). SGK1 further stimulates the expression and/or activity of a variety of channels and carriers (1, 3, 4, 6, 10).
agonist was similarly shown to enhance plasma volume (2), an effect, in part, dependent on SGK1.

Hypervolemia would in turn be expected to decrease aldosterone release. The opposite was observed, i.e., plasma aldosterone levels were significantly higher in apcMin/+ mice than in apc+/+ mice. Moreover, the hyperaldosteronism was not reversed but augmented by additional knockout of SGK1. The further increase of plasma aldosterone concentration is in line with the enhanced plasma aldosterone levels observed in SGK1-deficient mice, a result of impaired renal Na+ retention in those animals (9, 33). Clearly, the hyperaldosteronism of apcMin/+ mice was not due to enhanced SGK1 expression and was neither due to volume depletion nor due to hyperkalemia. Instead, APC-dependent signaling may influence aldosterone release more directly. Corticosterone levels were not significantly different between apcMin/+sgk1−/− and apcMin/+ mice. Thus, unlike the increase of aldosterone levels, the increase of corticosterone levels may be dependent on the presence of SGK1.

Although plasma aldosterone levels were even higher in apcMin/+sgk1−/− mice, urinary Na+ excretion was higher in those mice than in apcMin/+ mice, an expected finding in view of the known effect of SGK1 on renal tubular Na+ transport (9, 33). The enhanced plasma aldosterone levels could explain the increased fractional K+ excretion and the elevated blood pressure in apcMin/+ mice than in apc+/+ mice.

In contrast to plasma volume, body weight was not different between apcMin/+ mice and apc+/+ mice. Thus, the possibility was considered that the abundance of body fat was decreased in apcMin/+ mice. As body fat mass is correlated with leptin plasma concentrations (32), plasma leptin levels were determined. As a result, APC deficiency was paralleled by marked decrease of plasma leptin concentrations, pointing to reduced body fat mass. Recent observations indeed point to an inhibitory effect of β-catenin signaling on adipocyte differentiation (7, 14, 20).

Fig. 3. Plain tap water and saline consumption of in apcMin/+ mice and apc+/+ mice. Arithmetic means ± SE (n = 12 each group) of daily drinking volumes of plain tap water (water) and 1% saline (NaCl) (A) and the ratio of drinking volumes saline/water (B) in mice carrying a defective APC (apcMin/+), black bar) and their wild-type littermates (apc+/+, white bar).

12, 15, 25–28, 34) and is thus important for renal Na+ retention (9, 33). Enhanced expression of SGK1 would be expected to foster renal Na+ retention and thus increase plasma volume. Accordingly, urinary Na+ excretion was indeed significantly decreased in apcMin/+ mice, a difference reversed by additional lack of SGK1. Mirror-like changes were observed in fecal Na+ excretion. Unlike renal tubular Na+ reabsorption (9, 33), colonic Na+ reabsorption appears not to depend on SGK1 (22). The fractional urinary Na+ excretion was not significantly different between apcMin/+ mice and apc+/+ mice, as glomerular filtration rate was similarly lower in apcMin/+ mice than in apc+/+ mice, a difference, however, not reversed by additional lack of SGK1. The observations point to a role of APC in the maintenance of glomerular function.

According to the experiments with Evans Blue, plasma volume was significantly larger in apcMin/+ mice, an effect partially blunted in apcMin/+sgk1−/− mice. Thus, SGK1-dependent Na+ retention could have led to hypervolemia. Increased SGK1 expression following treatment with a PPARγ agonist was similarly shown to enhance plasma volume (2), an effect, in part, dependent on SGK1.

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In conclusion, plasma aldosterone and corticosterone levels, plasma volume, and blood pressure were higher in mice carrying the defective APC gene. The effects are partially reversed by lack of SGK1 pointing to a role of this kinase in APC-dependent regulation of electrolyte homeostasis. However, SGK1 deficiency augments the effect of defective APC on plasma aldosterone levels, pointing to a SGK1-independent role of APC in the regulation of adrenal hormone release. Clearly, the signaling cascade of APC and SGK1 is more complicated than a simple serial chain, and the present data shed some light on but still do not fully clarify the final interactions.

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GRANTS

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