Renal Tubular Reabsorption of Folate Mediated by Folate Binding Protein 1

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Renal tubular reabsorption of filtered folate is essential for the conservation and normal homeostasis of this important vitamin. Different molecular mechanisms have been implicated in epithelial folate transport, including folate receptors. Defective expression or antibody inactivation of these is associated with embryonic defects also correlated with low folate intake; however, their contribution to renal tubular folate reabsorption has not been established. With the use of targeted inactivation of the folate binding protein 1 (folbp1) and folate binding protein 2 (folbp2) genes in mice, the role of folate receptors in renal epithelial folate reabsorption was evaluated during low and normal folate intake. Inactivation of folbp1 was associated with (1) loss of 3H-folic acid binding to crude kidney membranes, (2) increase in renal folate clearance, and (3) increase in urinary excretion and decrease in renal uptake of injected 3H-methyltetrahydrofolate. No changes in renal folate handling were observed as a result of folbp2 inactivation. Thus, folbp1 is essential for normal renal tubular folate reabsorption, preventing excessive urinary folate loss. Folbp1 is heavily expressed in choroid plexus, yolk sac, and placenta, supporting a role of folbp1 in folate transport in other tissues. The greatest significance of folbp1 for renal folate uptake was observed at conditions of low folate intake, providing a possible explanation for the ability of folate supplementation to prevent developmental defects associated with folbp1 inactivation.


Epithelial folate transport is essential for normal folate homeostasis, regulating intestinal uptake, renal tubular reabsorption, and tissue distribution of folate. Defects in folate homeostasis have been implicated in a number of conditions, including embryonic abnormalities, cancer, and cardiovascular disease. Several different mechanisms have been suggested to regulate epithelial folate uptake, including folate carriers and glycosylphosphatidylinositol–linked folate receptors (FR) (1–5). Thus far, four FR isoforms have been characterized in humans. FR-α is present predominantly in epithelial cells (6–8), FR-β is expressed at low to moderate levels in several different tissues (8,9) and differs with respect to affinities for different forms of folate (10). FR-γ1/γ2 are specific for hematopoietic cells (11,12). The human FR gene (FR-δ) predicts a 27.7-kD protein with a unique expression pattern in both adult and embryonic tissues (13). Both FR-α and FR-β and the reduced folate carrier (RFC) are expressed in the kidney (8,14–17), and in vitro studies have suggested a role for each of these transport systems in cellular folate uptake; however, their individual contribution varies between cell lines (1,2,5). FR have been located in the proximal tubule luminal membranes and endocytic apparatus (18,19), and kinetic studies indicate a role in renal tubular folate transport (20). Knockout of the folate binding protein 1 (folbp1) and folate binding protein 2 (folbp2) genes, the mouse equivalents of FR-α and FR-β, has shown that deletion of folbp1 is embryolethal but can be rescued by supplementing the dams with folate (21,22). Surviving mice have low plasma folate levels and may present with congenital defects involving the craniofacies, heart, eyes, and abdominal wall (22–24). Furthermore, the injection of anti-FR into pregnant rats was shown to cause embryonic damage (25), and autoantibodies against FR were recently identified in women who previously gave birth to an infant with neural tube defects (26). These data point to an important role of FR in folate homeostasis, although whether this is linked to epithelial folate transport or some other function of FR is currently unknown.

To establish whether FR are involved in renal folate transport, we examined tubular reabsorption of folate in mice with targeted gene knockout of folbp1 and folbp2. The data suggest an important role of folbp1 in renal tubular reabsorption of filtered folate, establishing for the first time a significant role for folbp1 in epithelia folate transport in vivo.

Materials and Methods

Animals

All animal experiments were carried out in accordance with the provisions for animal care license provided by the Danish National Animal Experiments Inspectorate. Male mice that were defective in folbp1 or folbp2 were produced by targeted gene knockout (21) and previously characterized (22,23,27). Beginning at 7 wk of age, folbp1−/− (n = 6), folbp1+/− (n = 6), folbp2−/− (n = 7), and...
wild-type (n = 6) mice were fed a low-folate diet (0.3 mg folate/kg with succinyl-sulfathiazole; Dyets #518841, Bethlehem, PA) for 38 d followed by a shift to a folate-replete diet (3 mg folate/kg; Dyets) for 25 d. At the end of both periods, mice were placed in metabolic cages with free access to water and received an intraperitoneal injection of 430,000 to 580,000 CPM (approximately 40 pmol) of \(^{3}H\)-(6S)-5-methyltetrahydrofolate (\(^{3}H\)-MTHF; 15 Ci/mmol; Moravek Biochemicals, Brea, CA) in 0.9% NaCl. Urine was collected into 2 ml of 10 mM sodium-ascorbate (Sigma, St. Louis, MO) for 24 h both before \(^{3}H\)-MTHF injection and 24 h after this. Blood was sampled from the retro-orbital sinus after urine collection on low-folate diet. After the mice had been fed the normal diet for 25 d and urine collection had been completed, the mice were anesthetized, blood was collected, and the kidneys were removed. All blood samples were hemiparinated and centrifugated for the preparation of plasma. Urine, plasma, and kidneys were stored at \(-80^\circ\)C. Kidneys from additional folbp1\(^/-/-\), folbp2\(^/-/-\), and wild-type mice were fixed by retrograde perfusion through the abdominal aorta with 2% paraformaldehyde or 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4).

**Crude Membrane Preparation**

Mouse kidneys were homogenized in 0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, 8.5 \(\mu\)M leupeptin, and 1 mM PMSF (pH 7.2), using an Ultraturrax T8 homogenizer (IKA Labortechnik, Staufen, Germany) at maximum speed for 30 s. After centrifugation at 4000 \(\times\) g for 15 min, crude membranes were isolated by centrifugation of the supernatant at 200,000 \(\times\) g for 1 h. Membrane protein concentration was determined using a Coomassie blue commercial assay (Bio-Rad Laboratories, Hercules, CA).

**Membrane Folate Binding**

\(^{3}H\)-folic acid binding was estimated using a method modified from Mason and Selhub (28). Approximately 13 to 28 \(\mu\)g of crude membrane preparations in 100 \(\mu\)l of 10 mM HEPES, 140 mM NaCl, 2 mM CaCl\(_2\), and 1 mM MgCl\(_2\) (pH 7.4) was incubated with 200 \(\mu\)l of 1 M acetate buffer (pH 3.5) to dissociate endogenous folate, followed by the addition of 7.2 kBq \(^{3}H\)-folic acid (approximately 6 pmol; Moravek Biochemicals) and normalization of pH 7.4 with 300 \(\mu\)l of 1 M K\(_2\)HPO\(_4\) (pH 8). The mixture was passed through equilibrated 0.45-\(\mu\)m cellulose filters (Millipore, Billerica, MA) and washed with 20 ml of 0.1 M K\(_2\)HPO\(_4\) (pH 7.4). Filters were dried, and retained radioactivity was counted in a LKB-Wallac 1211 Rackbeta scintillation counter with Ecoscint H scintillation fluid (National Diagnostics, Atlanta, GA). Additional kidney membrane binding experiments were performed in the presence of excess 0.5 nmol unlabeled folic acid. Counts from blanks representing buffer without kidney membranes were approximately 10% of total counts retrieved with crude wild-type membrane samples. Blanks were subtracted from total counts, and the amount of bound \(^{3}H\)-folic acid was determined by the parallel counting of appropriate \(^{3}H\)-folic acid standards.

**Folate Analyses**

Folate in plasma and urine was determined using a Lactobacillus casei assay essentially as described by Molloy and Scott (29). In short, 0.25 M sodium ascorbate was added to 25 \(\mu\)l of sample, making a total volume of 1 ml. Duplicate volumes of 50 and 100 \(\mu\)l of diluted sample or 0 to 100 \(\mu\)l of a folate standard, 500 pg/ml, prepared from folic acid (Sigma, St. Louis, MO), were added to 96-well microtiter plates. Additional 0.25 M sodium ascorbate was added to some wells to make a total volume of 100 \(\mu\)l in all. To each well was added 200 \(\mu\)l of folic acid medium prepared from a 57-mg/ml folic acid medium (Becton Dickinson, Sparks, MD) solution that contained 30 \(\mu\)g/ml chloramphenicol (Sigma), 0.75 mg/ml ascorbic acid (Sigma), 30 \(\mu\)l of Tween 80 (Sigma), and 200 \(\mu\)l of cryopreserved L. casei (donated by Dr. J.M. Scott, Trinity College, Dublin, Ireland). The plates were incubated for 48 h at 37°C, gently shaken, and read at 590 nm using an InerMed ImmunoReader NJ-2000. The amount of folate in each sample was determined from a standard curve based on the folate standards.

\(^{3}H\) activity in urine and kidney tissue was determined by liquid scintillation counting. Because the mice received an injection of \(^{3}H\)-MTHF on low-folate diet 25 d before repeated injection on normal diet, urine was collected 1 d before the second injection to examine whether labeled folate retained from the first injection would interfere with the results of the second injection. The activity in the urine before the second injection was \(<2\%\) of the activity collected after the second \(^{3}H\)-MTHF injection in all animals and, thus, considered insignificant.

Folate Analyses of tissues.

**Other Serum and Urine Analyses**

Creatinine and urea in mouse serum and urine were measured using an automatic equipment (Cobas-Integra, Hoffmann-La Roche, Switzerland).

**Calculations**

Renal folate, creatinine, and urea clearances were estimated by dividing 24-h folate, creatinine, or urea excretion by plasma folate, creatinine, or urea concentrations, respectively. The latter were determined from blood collected at the end of the 24-h urine sampling period. Thus, because calculations are based on single, end point serum concentrations, the calculated clearances should be considered estimates.

**Antibodies**

A polyclonal antibody raised against purified rat placental FR was donated by Dr. S.P. Rothenberg (State University of New York). Polyclonal sheep anti-rat-megalin antibodies have been described previously (30).

**SDS-PAGE and Immunoblotting**

Membrane samples were subjected to nonreducing SDS-PAGE using polyacrylamide minigels (Bio-Rad Mini Protein II) and transferred to nitrocellulose membranes. Blots were blocked with 5% skim milk in PBS with 0.1% Tween 20 (PBS-T; pH 7.5) for 1 h. After overnight incubation with primary antibody diluted in PBS-T with 1% BSA, the blots were incubated with horseradish peroxidase–conjugated goat anti-rabbit IgG (1:3000; Dako, Glostrup, Denmark) and visualized using ECL enhanced chemiluminescence system (Amersham Biosciences, Little Chalfont, UK). Controls involving incubation without primary antibody or incubation with nonspecific serum revealed no significant labeling. The expression of immunoreactive FR was evaluated by densitometry of immunoblots.

Equal amounts of membrane protein samples from different genotypes were blotted for the FR as described. Identical samples were run on similar gels and Coomassie blue stained. Both the ECL films and the Coomassie blue–stained gels were scanned using an AGFA Duoscan F40 scanner and Corel Photo Paint software. The labeling density of the specific bands on the immunoblots was quantified using custom-made software (31). Values were adjusted for...
the loaded amount of protein by dividing the density of the specific bands obtained by immunoblotting with the density of all bands in the corresponding Coomassie-stained gel. Values thus represent relative expression levels in arbitrary units given as means ± SEM and adjusting wild-type to 100%.

Ultrastructure and Immunocytochemistry
Small blocks of perfusion-fixed kidney cortex were postfixed in the same fixative. For ultrastructural analysis, glutaraldehyde-fixed kidney cortex was further postfixed in 1% OsO4 en bloc stained with uranyl acetate, dehydrated in ethanol, embedded in Epon 812 (TAAB; Aldermaston, Berks, UK), sectioned on a Reichert Ultracut S microtome (Reichert, Vienna, Austria), stained with lead and uranyl acetate, and observed in a Philips CM 100 electron microscope. For immunocytochemistry, fixed kidney cortex was infiltrated with 2.3 M sucrose and frozen. Semithin cryosections were incubated with the primary antibody, followed by incubation with horseradish peroxidase–conjugated secondary antibody and visualization by incubation with diaminobenzidine and 0.03% H2O2. Sections were counterstained with Mayers hematoxylin before examination under a Leica DMR microscope equipped with a Sony S CCD color video camera. Controls involving sections that were incubated without the primary antibody or incubated with nonspecific rabbit or sheep serum revealed no significant labeling.

Statistical Analyses
Initial Bartlett testing of the data suggested that the data do not represent samples of similar variance. Thus, the equivalent nonparametric test (the Kruskall-Wallis test) was applied followed by Dunn’s multiple comparisons test as post hoc testing comparing folbp1–/–, folbp1+/−, and wild-type, or folbp2−/− and wild-type. Wilcoxon matched pairs signed rank test was used for paired comparisons. P < 0.05 was considered significant. Data represent mean ± SEM.

Results
Kidney Function and Structure
One folbp1−/− mouse had to be excluded during the initial experimental phase, as it seemed ill. All other mice thrived and survived throughout the study. Except for a higher mean weight of folbp2−/− mice compared with wild-type mice that were maintained on the low-folate diet, no differences were identified between genotypes when comparing animal and kidney weight, urine output, urinary creatinine and urea excretion, or creatinine and urea clearance (Table 1). A small yet significant increase in urinary creatinine and urea excretion was observed in wild-type mice when going from the low-folate to normal diet. No other significant changes were observed in the basic parameters as a result of the different diets, when comparing mice of the same genotype (Table 1). The proximal tubule ultrastructure of folbp1−/− mice appeared normal (Figure 1), and immunocytochemistry revealed no apparent difference in the immunolocalization of other proximal tubule membrane proteins, including megalin (data not shown).

Expression of Folate-Binding Proteins
Immunoblotting on crude kidney membranes revealed an approximately 40-kD protein band in wild-type, folbp1+/−, and folbp2−/− representing immunoreactive FR (Figure 2a). No band was observed in folbp1−/−, and no other bands were identified in kidney membranes from any of the genotypes. When similar amounts of total membrane protein were blotted, no other bands were identified in kidney membranes from any of the genotypes. When similar amounts of total membrane protein were blotted, the intensity of the band seemed greater in wild-type compared with both folbp1+/− and folbp2−/− specimens, although this was not evaluated statistically.

Immunocytochemistry confirmed the total absence of immu-

Table 1. Animal data and parameters of renal functiona

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<th>Diet</th>
<th>Low Folate</th>
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<td>Wild-Type</td>
<td>folbp1−/−</td>
</tr>
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<td>No. of animals</td>
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<td>6</td>
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<td>Animal weight (g)</td>
<td>20.7 ± 0.4</td>
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<tr>
<td>Total kidney weight (g)</td>
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<td>ND</td>
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<tr>
<td>Urine output (ml/24 h)</td>
<td>2.1 ± 0.6</td>
<td>1.8 ± 0.3</td>
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<tr>
<td>Urine creatinine (µmol/kg per 24 h)</td>
<td>130 ± 20c</td>
<td>150 ± 30</td>
</tr>
<tr>
<td>Urine urea (mmol/kg per 24 h)</td>
<td>34 ± 6c</td>
<td>37 ± 6</td>
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<tr>
<td>Estimated creatinine clearance (ml/kg per min)</td>
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<td>Estimated urea clearance (ml/kg per min)</td>
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aData are mean ± SEM. ND, not determined.
bSignificantly different compared with wild-type mice on low-folate diet (Kruskal-Wallis test followed by Dunn’s multiple comparisons test as post hoc testing).
cSignificantly different compared with wild-type mice on normal diet (Wilcoxon matched pairs signed rank test).
noreactive protein in the brush border of folbp1−/− in contrast to the normal labeling of proximal tubule apical membranes in wild-type and folbp2−/− mice (Figure 3). Additional labeling was observed in the parietal cells of Bowman’s capsule in these mice, demonstrating for the first time expression of FR within the renal corpuscle.

Folate Binding to Kidney Membranes
To quantify membrane-associated folate binding, we measured high-affinity 3H-folic acid binding activity to crude kidney membranes including both plasma membrane and intracellular membranes (Figure 2b). No binding of 3H-folic acid to kidney membranes from folbp1−/− was identified while binding to kidney membranes in the presence of excess unlabeled folic acid was very low (Figure 2b).

Plasma and Urinary Folate
In accordance with previous observations (22), total plasma levels of folate were significantly lower in the folbp1−/− compared with wild-type mice on both low-folate and normal diets (Table 2). Mean plasma folate levels in folbp1+/− and folbp2−/− were between levels in folbp1−/− and wild-type mice, although not statistically significant from either. On normal diet, no significant differences in the urinary folate excretion rate were observed between genotypes despite differences in plasma folate levels. In contrast, on the low-folate diet, folbp1+/− mice revealed an approximately 10-fold increase in urinary folate excretion rate compared with wild-type and folbp1−/− mice. No significant differences in urinary folate excretion were observed between folbp1+/− or folbp2−/− and wild-type mice.

On normal diet, an approximately five-fold higher estimated renal folate clearance was observed in folbp1−/− compared with wild-type mice (Table 2). This was the result of unaltered urinary folate excretion despite lower plasma folate. The estimated renal folate clearance in folbp1−/− mice on normal diet is comparable to creatinine clearance (Table 2). On the low-folate diet, an approximately 34-fold higher estimated renal folate clearance was observed in the folbp1−/− mice when compared with wild-type mice. This elevated folate clearance is the combined result of an increase in urinary folate excretion.

Figure 1. Electron micrograph showing the apical part of a proximal tubule from perfusion-fixed folate binding protein 1-deficient (folbp1−/−) mouse kidney cortex. General ultrastructure appears normal with extensive microvilli (MV) and a well-preserved endocytic apparatus including invaginations, numerous endocytic vesicles (EV), dense apical tubules representing the membrane recycling compartment (arrows), and structurally normal mitochondria (MIT). Bar = 0.5 μm.

Figure 2. Expression of immunoreactive folate receptor (FR; a) and 3H-folic acid binding (b) in crude kidney membranes from folbp1−/− (lanes 1 to 3), folbp+/− (lanes 4 to 6), wild-type (lanes 7 to 9), and folbp2−/− (lanes 10 to 12) mice. Similar amounts (approximately 8 g) of total membrane protein as reflected by the similar stained lanes in the Coomassie blue-stained gel (a, insert, top left) were subjected to SDS-PAGE and immunoblotted using an antibody raised against rat placental FR. An approximately 40-kD protein band was identified in folbp1−/−, wild-type, and folbp2−/− mice, showing the expression of immunoreactive FR (a). No bands were observed in folbp1−/−, and no other bands were identified in any of the genotypes. The intensity of the bands was semiquantified as described (a, bottom bars). The positions of molecular weight markers are indicated to the right. The binding of 3H-folic acid to crude kidney membranes was determined as described previously (b, filled bars). No binding of 3H-folic acid to kidney membranes from folbp1−/− could be identified, whereas binding to kidney membranes from all genotypes in the presence of excess unlabeled folic acid was very low (b, open bars).
and decreased levels of plasma folate (Table 2). No significant differences in estimated folate clearance were observed between folbp1+/− or folbp2−/− and wild-type mice; however, a tendency toward higher urinary folate clearance was observed in folbp1+/− compared with wild-type mice on both low-folate and normal diet.

Urinary Excretion and Renal Accumulation of Injected ³H-MTHF

For examining handling of an acutely administered physiologic dose of MTHF and to study tissue uptake of folate, approximately 40 pmol of ³H-MTHF was injected intraperitoneally into mice on both the low-folate and the normal diet. Twenty-four-hour urinary ³H excretion confirmed the findings of total folate excretion, showing higher urinary excretion of label in folbp1−/− mice when compared with wild-type (Figure 4). Thus, the handling of an acutely injected physiologic MTHF dose is consistent with steady-state observations. After the normal diet period, the kidneys were removed and tissue accumulation of ³H was counted. Twenty-four-hour urinary ³H excretion was increased approximately 1.7-fold in folbp1−/− mice, which was associated with a similar, significant decrease in the accumulation of label within the kidney (Figure 4). This demonstrates that the increased excretion of folate associated with the folbp1−/− genotype is not due to changes in filtration as increased glomerular filtration along with unchanged tubular reabsorption would have resulted in unaltered or increased renal accumulation. Similar to the results of total folate handling, no significant changes in the urinary excretion and renal accumulation of ³H-MTHF were observed as a result of the folbp2 deficiency.

Discussion

The present study establishes an important physiologic role for the folbp1 protein in mouse renal, tubular reabsorption of folate. This conclusion is based on the finding of increased renal folate clearance in folbp1−/− mice both at low and normal folate intake showing impaired tubular uptake of filtered folate. The importance of the folbp1 protein is greater during folate deficiency than during normal folate intake. On the low-folate diet, renal folate clearance in wild-type mice is reduced to <1% of creatinine clearance, whereas folbp1−/− mice lose folate in the urine at a rate corresponding to approximately 23% of creatinine clearance despite an approximately 2.5-fold decrease in plasma folate. Plasma folate levels in folbp1−/− mice on normal diet are comparable to the levels in wild-type mice on the low-folate diet (26.1 ± 0.01 nmol/24 h). Therefore, the amount of filtered folate is assumed to be similar in these situations. Nevertheless, the amount of folate excreted in the urine of folbp1−/− mice is approximately 100 times higher than that detected in the wild-type mice (4.3 ± 0.4 vs. 0.04 ± 0.01 nmol/24 h), indicating that the tubular reabsorptive capacity related specifically to folbp1 in the low-folate situation is approximately 4 nmol/24 h in mice. The essential role of folbp1 in tubular folate transport is further supported by the total lack of kidney membrane ³H-folic acid binding associated with folbp1 deficiency. Injection of ³H-MTHF also confirms the important role of folbp1 for renal tubular reabsorption, showing increased urinary excretion and decreased renal accumulation in folbp1−/−. These findings, establishing for the first time in an in vivo model a direct role for folbp1 in epithelia folate uptake, confirms the hypotheses raised by the identification of FR in the kidney proximal tubule and by observations on folate transport in the kidney and in cultured kidney cells (14,20,32,33). In addition, folbp1 is heavily expressed in other absorptive epithelia, including the rodent yolk sac (34) and choroid plexus (15). These findings indicate a similarly important role for folbp1 in transepithelial folate transport in these tissues and provide a potential mechanism for the defective embryonic development observed in unsupplemented folbp1−/− mouse embryos (21). The estimated urinary loss of 4 nmol/24 h ascribed to folbp1 deficiency corresponds to the folate content in approximately 6 g of low-folate diet, which is more than the average daily intake. Thus, as a result of urinary loss, it is impossible to maintain adequate folate levels in folbp1−/− mice on the low-folate diet, contributing to the observed reduction in plasma folate levels.

The reduced folate clearance observed on low-folate diet in both folbp1−/− and wild-type animals is consistent with previous observations that urinary folate clearance decreases with decreasing plasma folate levels (35). Given a low-folate intake resulting in low plasma folate levels, reduced amounts of folate are filtered within the renal glomeruli. Under these conditions, folbp1 permits almost complete tubular reabsorption of folate, as shown by a wild-type folate clearance <1% of creatinine clearance. In contrast, folate clearance in folbp1-deficient mice
Table 2. Plasma folate, urinary folate excretion, and calculated renal folate clearance in wild-type, folbp1+/−, folbp+/-, and folbp2−/− mice on low-folate and normal diet*

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<td>Wild-Type</td>
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<td>Plasma folate (nM)</td>
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<td>11±2</td>
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<tr>
<td>Urinary folate excretion (nmol/24 h)</td>
<td>0.04±0.01</td>
<td>0.43±0.05bcd</td>
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<tr>
<td>Estimated folate clearance (ml/kg per min)</td>
<td>0.05±0.01</td>
<td>1.3±0.1b</td>
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<tr>
<td>Estimated folate clearance in % of creatinine clearance</td>
<td>0.9±0.2</td>
<td>23±2bcd</td>
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*aData are mean ± SEM.
*bSignificantly different from wild-type mice on similar diet (Kruskal-Wallis test followed by Dunn’s multiple comparisons test as *post hoc* testing).
*cSignificantly different from folbp1+/− mice on similar diet (Kruskal-Wallis test followed by Dunn’s multiple comparisons test as *post hoc* testing).

Figure 4. 24-hour urinary excretion of 3H-label after intraperitoneal injection of approximately 40 pmol of 3H-MTHF during low-folate and normal diet conditions (left axis scale), as well as renal accumulation of label after normal diet (right axis scale). Values are expressed in percentage of total injected label. Significantly less label is excreted on the low-folate diet compared with mice of the same genotype under normal diet.

100% of creatinine clearance that may be expected when folate is freely filtered and tubular reabsorption is defective. However, in addition to free folate, plasma contains both specific and nonspecific folate binders, the latter probably being albumin (36–38). Whereas total protein binding of folate in rat serum has been estimated to be approximately 20% over a wide range of concentrations (20), the concentration of the approximately 35 kD, high-affinity plasma folate binding protein secreted from hemopoietic cells (11,12), is assumed to be more constant and thus carrying a larger fraction of total plasma folate at low folate concentration. Filtration of this folate-protein complex is followed by tubular reabsorption, most likely by a megalin-mediated process (39), providing a potential mechanism of tubular folate uptake that is independent of membrane-associated folbp1 and may play a role at low plasma folate concentrations. Finally, it cannot be excluded that other, very low capacity reabsorptive mechanisms may operate under low-folate conditions.

The absence of 3H-folic acid binding to kidney membranes of folbp1−/− mice suggests that the folbp1 gene product is the major membrane folate binder in the kidney. This is further supported by the total lack of immunoreactive FR in folbp1−/− kidney cortex. Thus, although FR-β mRNA has been identified in human kidney (8), the role of folbp2 in mouse kidney remains unclear. The present data do not support a role for folbp2 in renal folate reabsorption. Immunoblotting revealed lower levels of FR in folbp1+/− and folbp2−/− kidneys, both showing a tendency of lower plasma folate concentrations, compared with the wild-type mice. Because it has been shown that low folate intake and low plasma folate levels are associated with decreased expression of FR in rat kidney (40), it is possible that the decreased expression of immunoreactive FR in folbp1+/− and folbp2−/− kidneys reflects this rather than a direct effect of gene knockout.

on the normal diet is approximately 100% of creatinine clearance, suggesting little or no net tubular reabsorption of folate. It is interesting that on the low-folate diet, the folate clearance in folbp1−/− mice is only approximately 20% rather than the
Although this study clearly shows an important role of folbp1 in renal epithelial uptake of folate, it does not exclude a significant function of other folate transport proteins. The kidney expresses both FR and RFC (17,41,42). Proximal tubule cell culture studies have implicated RFC in apical transport of MTHF (43,44), and RFC has been located by immunocytochemistry to basolateral membranes in kidney tubules (17). Thus, RFC may be involved in apical folate transport at high folate concentrations or in the cellular exit of reabsorbed folate.

In conclusion, this study has established the important role of folbp1 in renal tubular reabsorption of folate in vivo. The greatest relative importance of folbp1 is observed at low-folate intake. Although folbp2 has been identified in kidney, the present study does not support a role in folate reabsorption.

Acknowledgments

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References