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Decreased Expression of Transporters Reduces Folate Uptake across Renal Absorptive Surfaces in Experimental Alcoholism

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Abstract In this study, we examined the mechanistic insights of folate reabsorption during alcoholism, considering enhanced renal excretion as one of the major contributing factors to alcohol-induced folate deficiency. Male Wistar rats were fed 1g/kg body weight/day ethanol (20% solution) orally for 3 months. The results on characterization of the folate transport system in renal basolateral membrane (BLM) suggested it to be a carrier-mediated, acidic pH-dependent and saturable one. Chronic ethanol feeding decreased the uptake mainly by increasing the K_m and decreasing the V_{max} of the transport process at the BLM surface. At the molecular level, reduced folate transport activity in renal tissue during chronic ethanol ingestion was attributable to decreased expression of reduced folate carrier (RFC) and folate binding protein (FBP). Antibodies against RFC protein revealed a parallel change in RFC expression in both brush border and BLM surfaces during chronic alcoholism. Such findings highlight the role of downregulation of RFC and FBP expression and provide mechanistic insight into the observed reduced folate transport efficiency at renal absorptive surfaces in alcoholism, which may result in low blood folate levels commonly observed in alcoholics.

Keywords Folate binding protein · Reduced folate carrier · Alcoholism · Basolateral · Brush border · Transport

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Introduction

Folate-mediated one-carbon metabolism is of fundamental importance for various cellular processes, including DNA synthesis and methylation. Due to the exogenous requirement of folate in mammals, there exists a well-developed epithelial folate transport system for regulation of normal folate homeostasis including intestinal uptake, renal tubular reabsorption and tissue distribution. The renal uptake of folate involves glomerular filtration followed by tubular reabsorption of filtered folate, which is essential for the conservation and normal homeostasis of this important vitamin (Sabharanjak & Mayor, 2004). Failure to reabsorb filtered folate would result in urinary losses of approximately 1 mg/day and the subsequent rapid depletion of body stores. A high-affinity folate binding protein (FBP) or folate receptor and low-affinity transporter, the reduced folate carrier (RFC), concentrated respectively in the brush border membrane (BBM) and basolateral membrane (BLM) of proximal tubule cells, are the candidate proteins involved in folate conservation (Elwood, Deutsch & Kolhouse, 1991; Wang et al., 2001). Moreover, the exact mechanism by which folates are transported, processed and released by the proximal tubule cells remains largely unresolved.

It is a well-established fact that folic acid deficiency is the hallmark of alcoholism worldwide. Excessive alcohol intake exerts a multifaceted impact on the bioavailability and subsequent metabolism of folate and, more broadly, on one-carbon metabolism as a whole (Halsted et al., 2002). Earlier studies had suggested that both acute and chronic ethanol ingestion were associated with high loss of urinary folate (McMartin et al., 1985, 1989) and, hence, contribute to the observed folate deficiency during alcoholism (Halsted, 2001). In an attempt to gain mechanistic insight into

this phenomenon, we have earlier shown that in renal BBM decreased transport of folate takes place, which is associated with altered uptake and binding kinetics in chronic ethanol-fed rats (Hamid & Kaur, 2005, 2006). However, the kinetic behavior of the folate transport system in renal BLM and molecular expression of transporters under physiological and alcoholic conditions have not been evaluated as yet. Studying this system holds great potential in understanding epithelial folate transport in the kidney under the conditions of derangement of folate homeostasis, not only in alcoholism but in renal diseases too. Therefore, the regulation of the expression of FBP and RFC in renal tissue has been sought concomitantly with the folate transport across the absorptive surface under alcoholic conditions. This study bears significance in view of the fact that these transporters are important determinants for the chemotherapeutic potential of various antifolates also.

Materials and Methods

Animals

Young adult male albino rats (Wistar strain) weighing 100–150 g were obtained from our institute's Central Animal House. The animals were housed in clean wire mesh cages with controlled temperature (23 ± 1°C) and humidity (45–55%) and a 12 h dark-light cycle throughout the study. The rats were randomized into two groups of six animals each, such that the mean body weights and the range of body weights for each group were similar. The rats in group I were given 1 g ethanol (20% solution)/kg body weight/day, and those in group II received an isocaloric amount of sucrose (36% solution) orally by Ryle's tube daily for 3 months. The rats were fed a commercially available pellet diet (Ashirwad Industries, Ropar, India) and water ad libitum. The body weights of rats were recorded twice weekly. Animals from both groups were killed under anesthesia using sodium pentothal, and blood was drawn for alcohol estimation 24 h after the last dose of ethanol (Burtis, Ashwood & Tietz, 1994).

Approval was sought from the Institutional Animal Ethical Committee and the Institutional Biosafety Committee before the commencement of the study.

Chemicals

Radiolabeled [3′,5′,7,9-^{3}H]-folic acid, potassium salt with specific activity 24.0 Ci/mmol, was purchased from Amersham Pharmacia Biotech (Hong Kong, PR China). D-[U-^{14}C]-glucose with specific activity 140 mCi/mmol was provided by Radioisotope Division, Bhabha Atomic Research Centre (Mumbai, India). DL-Dithiothreitol (DTT) or Cleland's reagent were purchased from Sigma Aldrich (St. Louis, MO). Cellulose nitrate membrane filters (0.45 μm) were obtained from Millipore (Bedford, MA). Diaminobenzidine (DAB), horseradish peroxidase (HRP)-labeled anti-rabbit immunoglobulin G (IgG) secondary antibodies were obtained from Bangalore Genei (Bangalore, India).

Isolation of Membrane Vesicles from Renal Tissue

Rat renal cortical brush border membrane vesicles (BBMVs) were prepared by differential centrifugation as described earlier (Hamid & Kaur, 2006). Basolateral membrane vesicles (BLMVs) from renal cortices were prepared by the self-generating Percoll gradient method of Scalera et al. (1980) with some modifications. Renal tissue was flushed with ice-cold normal saline and homogenized in ice-cold buffer containing 250 mM mannitol and 12 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/Tris, pH 7.4, using a Waring blender (Waring Products Division, New Hartford, USA) blender for 3 min and then centrifuged at 2,500 × *g* for 20 min. The supernatant was then centrifuged at 22,000 × *g* for 25 min and the resulting fluffy layer of the pellet resuspended in the same buffer followed by homogenization in a glass Teflon homogenizer. The resulting homogenate was mixed with Percoll at a concentration of 15.4% and centrifuged at 48,000 × *g* for 2 h. A distinct band of BLM was seen at the upper one-third of the Percoll gradient. The band was aspirated by a syringe and suspended in buffer composed of 100 mM mannitol, 100 mM KCl and 12 mM HEPES/Tris (pH 7.4) and centrifuged at 48,000 × *g* for 20 min. The pellet obtained was resuspended in loading buffer containing 280 mM mannitol and 20 mM HEPES/Tris (pH 7.4) and centrifuged at 48,000 × *g* for 20 min twice in order to wash out the residual Percoll in the purified membrane preparations. The final pellet representing BLMV was suspended in a small amount of loading buffer to obtain an approximately 5 mg/ml final protein concentration.

The specific activity of alkaline phosphatase and sodium-potassium-adenosine triphosphatase (Na^+,K^+-ATPase) were studied to check the purity of both renal BBM and BLM preparations. A 12- to 15-fold increase in alkaline phosphatase activity was observed generally in the BBM preparations with minimal activity of Na^+,K^+-ATPase. Moreover, BLM preparations showed 8- to 10-fold higher specific activity of Na^+,K^+-ATPase. The functional integrity of the vesicles was checked by the [^{14}C]-D-glucose uptake (Kessler et al., 1978), which revealed a transient overshoot of the intravesicular glucose concentration over its equilibrium uptake in the presence of

sodium gradient in BBMVs, whereas BLMVs did not show the overshoot phenomenon.

Transport of [^{3}H]-Folic Acid

Uptake studies were performed at 37°C using the incubation buffer of 100 mM NaCl, 80 mM mannitol, 10 mM HEPES, 10 mM 3-*N*-morpholinoethanesulfonic acid (MES, pH 5.5) and 0.5 μM [^{3}H]-folic acid unless otherwise mentioned. Ten microliters of vesicles (50 µg protein) were added to incubation buffer containing [^{3}H]-folic acid for fixed time intervals. The reaction was stopped by adding ice-cold stop solution, followed by rapid vacuum filtration. Nonspecific binding to the filters was determined by residual filter counts after filtration of the incubation buffer and labeled substrate without vesicles. Also, in the preliminary experiments, vesicular transport of [^{3}H]-folic acid in the presence of excess unlabeled folic acid was measured and amounted to a negligible value, suggesting the specificity of the transport process. The radioactivity remaining on the filters was determined by liquid scintillation counting (LS 6500; Beckman Coulter, Fullerton, CA). To determine the binding component of folate uptake, vesicles were equilibrated for 90 min in incubation buffers containing increasing concentrations of mannitol. Optimal conditions for maximum transport were chosen for various experiments performed to determine the kinetic characteristics of folate transport as described (Hamid & Kaur, 2006). For determination of the inhibition constants, [^{3}H]-folic acid at 0.5 and 1.0 μM concentrations were used in the presence of a range of concentrations of either methotrexate or unlabeled folic acid, viz., 1, 2, or 3 μM. In order to determine the role of ATP as a driving force in folic acid uptake in BLMV preparations, 1 mM Mg^{2+}-ATP was included in the incubation buffer.

Reverse Transcriptase-Polymerase Chain Reaction Analysis

Total RNA was isolated from the renal tissues following the method of Chomczynski & Sacchi (1987). cDNA synthesis was carried out from the purified and intact total RNA according to the manufacturer's instructions (Fermentas Inc., Maryland, USA) using Moloney murine leukemia virus reverse transcriptase (RT). Expression of RFC, FBP and β-actin was evaluated using sequence-specific primers corresponding to the sequence in the open reading frame: RFC forward 5′-GAACGTCCGGCA ACCACAG-3′, reverse 5′-GATGGACTTGGAGGCCC AG-3′; FBP forward 5′-ATGAGTGTTCCCCGAACTTG-3′, reverse 5′-GCATAGAACCTCGCCACTTC-3′; β-actin forward 5′-CACTGTGCCCATCTATGAGGG-3′, reverse 5′-TCCACATCTGCTGGAAGGTGG-3′. A 20-µl polymerase chain reaction (PCR) mixture was prepared in 1x PCR buffer consisting of 0.6 U of *Taq* polymerase; 2 μM of each primer for RFC, FBP and β-actin; along with 200 μM of each deoxynucleotide triphosphate. In optimized PCR, the initial denaturation step was carried out for 2 min at 95°C. The denaturation, annealing and elongation steps were carried out, respectively, for 1 min at 94°C, 1 min at 68°C and 1 min at 72°C for 35 cycles. The final extension step was carried out for 10 min at 72°C.

Western Blot Analysis

The BBM and BLM protein preparations were essentially the BBMV and BLMV preparations described earlier. For expression studies, BBM and BLM proteins (100–150 µg) isolated from renal tissues were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli (1970) and transferred to nitrocellulose membranes for 4–5 h at 4°C, and the transfer was carried out at 25 V or 300 mA. Western blotting was performed according to the procedure described by Towbin, Staehelin & Gordon (1979) using polyclonal primary antibodies as rabbit anti-rat RFC (1:500 dilutions; kindly provided by Dr. Hamid M. Said, Department of Physiology and Biophysics, School of Medicine, University of California, Irvine). The antibodies were raised against a specific region of rat RFC synthetic peptide corresponding to amino acids 495–512 of the rat RFC. Secondary antibodies used were goat anti-rabbit IgG, HRP-labeled (1:2,000 dilution).

Statistical Analysis

The data were computed as means ± standard deviation (SD). Group means were compared using Student's *t*-test, and analysis of variance was used wherever necessary. The acceptable level of significance was $P < 0.05$ for each analysis. All semiquantitative RT-PCR and Western blot analyses were performed on at least five separate occasions with comparable results.

Results

Estimation of Blood Alcohol Levels

In order to reestablish the suitability of the rat model for studies on experimental alcoholism under our experimental setup, the blood alcohol level was a required parameter.

The alcohol level was 91% higher ($P < 0.001$) in the chronic ethanol-fed group compared to the control group. Mean blood alcohol levels were 15.04 ± 1.96 and 1.77 ± 0.34 mg/dl, respectively, in ethanol-fed and control rats.

Kinetic Characterization of Transport of Folic Acid in BLMV

The time course of folate uptake at various intervals revealed that uptake in renal BLMV in the control group was maximal up to 30–45 s and then remained at this plateau level (Fig. 1). However, in the ethanol-fed group, maximal uptake was observed at 20 s and decreased thereafter. Moreover, there was a 56–76% decrease ($P < 0.001$) in folate uptake in the ethanol-fed group compared to the control group at different time courses. In order to determine the driving force for folate transport across the renal BLMV, the pH of the incubation buffer was varied from 5 to 8, keeping the intravesicular pH constant at 7.5. As shown in Figure 2, when we decreased the incubation buffer pH from 8 to 5, an increase in folic acid uptake was observed in both groups. This was evident when the uptake changed from neutral pH 7 to mildly acidic pH 5. Moreover, at the different pH points studied, folate uptake was 24–56% less ($P < 0.001$) in the ethanol-fed group compared to the control group. Notably, no such uniform decrease was observed in the alkaline pH range. The effect of varying medium osmolarities on folic acid uptake was examined to determine folic acid transport into the intravesicular space and to determine the binding component. There was a 75% decrease ($P < 0.001$) in uptake values when osmolarity was increased from 300 to 600 mosm in the control group, whereas a 59% ($P < 0.001$) decrease was observed in the ethanol-fed group under these conditions. The relationship between the uptake and the reciprocal of osmolarity indicated that uptake of folate was predominant in the intravesicular space and no significant binding was associated with the membrane surface (Fig. 3). Further, since the saturation kinetics is a notable characteristic feature of carrier-

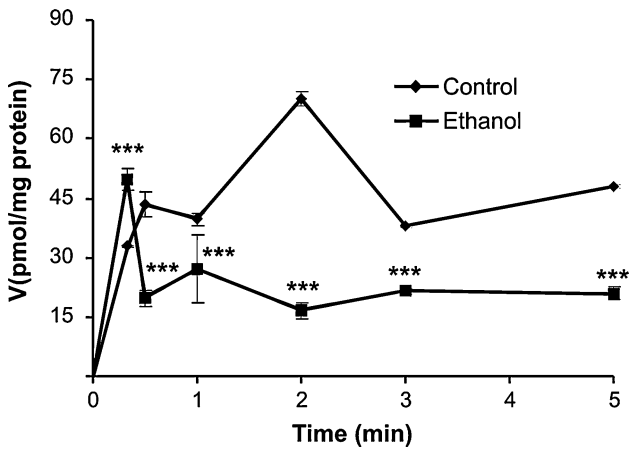

Fig. 1 [^{3}H]-Folic acid uptake in renal BLMVs as a function of time (minutes). The incubation buffer of pH 5.5 and [^{3}H]-folic acid concentration of 0.5 μM were used for uptake measurements. Each data point is the mean ± SD of three separate uptake determinations carried out in duplicate. ***$P < 0.001$ vs. control

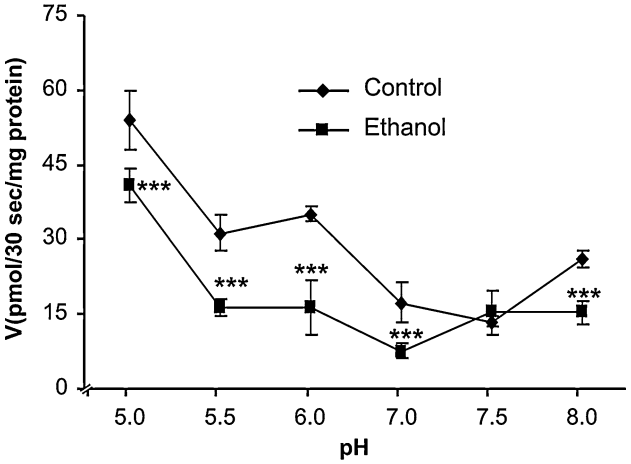

Fig. 2 Determination of [^{3}H]-folic acid uptake in renal BLMV with respect to pH optimum. BLMVs were incubated in buffers of different pH and 0.5 μM [^{3}H]-folic acid concentration for 30 s. Each data point is the mean ± SD of three separate uptake determinations carried out in duplicate. ***$P < 0.001$ vs. control

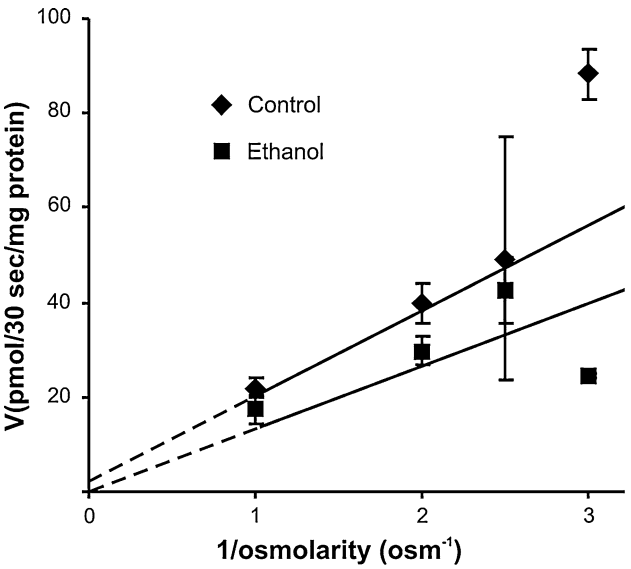

Fig. 3 [^{3}H]-Folic acid uptake in renal BLMVs as a function of 1/osmolarity. BLMVs were incubated in buffers of varying osmolarity with pH 5.5 for 90 min before transport measurements. Uptake was carried out after adding 0.5 μM [^{3}H]-folic acid for 30 s. Each data point is the mean ± SD of three separate uptake determinations carried out in duplicate

mediated transport, kinetic studies were performed in the presence of increasing concentrations of the substrate from 0.125 to 2.0 μM. The initial velocity determined at 30 s and at pH 5.5 showed that in both groups the saturation phenomenon with a plateau at 1.5 μM of substrate concentration was indicative of Michaelis-Menten kinetics (Fig. 4). At different concentrations of folic acid, uptake was found to be 35–79% less in the ethanol-fed group. The apparent K_m values were 0.07 ± 0.001 and 0.14 ± 0.002 μM in the control and chronic ethanol-fed groups, respectively ($P < 0.001$), as calculated by Lineweaver-Burk plot (Fig. 4, inset). In addition, the V_{max} values were 50.0 ± 5.3 and 20.0 ± 2.6 pmol/30 s/mg protein in the control and ethanol-fed groups, respectively ($P < 0.001$).

Furthermore, in order to determine the specificity of the transport system in renal BLM and the fact that folate transport is inhibited by structural analogues like methotrexate in a variety of cell types, the effect of various concentrations of methotrexate or unlabeled folic acid on 0.5 and 1.0 μM concentrations of [^{3}H]-folic acid uptake was determined. The results showed that both the analogues inhibited the uptake of folic acid in a dose-dependent manner. The inhibitory (Dixon) constants for methotrexate (Fig. 5a) were 3.10 ± 0.28 and 3.50 ± 0.32 μM, respectively, in the control and ethanol-fed group, whereas the inhibitory constants for unlabeled folic acid (Fig. 5b) were 2.60 ± 0.14 and 4.10 ± 0.32 μM, respectively. Interestingly, folate transport was found to be Na^+-, S-S group- and ATP-independent as incubation buffers containing, respectively, either K^+ or DTT or ATP did not influence the folate uptake process (*data not shown*). In addition, by decreasing the temperature from 37°C to 27°C, the rate of uptake was found to remain constant (temperature coefficients of 1.10 vs. 1.04 in the control and ethanol-fed groups, respectively).

Expression of mRNA Corresponding to RFC and FBP

To determine, whether the decrease in renal transport activity observed earlier in the ethanol-fed group was due to decreased expression of the RFC and/or FBP genes regulating folate transport in the kidney, RT-PCR analysis was performed. For this, sequence-specific primers in the coding regions of the RFC and FBP genes were used in order to obtain fragments of sizes 489 and 370 bp, respectively, during the amplification reaction using β-actin (588 bp) as an internal control. Densitometric analysis of RT-PCR products on 1.2% agarose gel revealed that the mean RFC (Fig. 6a, b) and FBP (Fig. 7a, b) mRNA level in the ethanol-fed group was 1.5- and 2.5-fold lower, respectively, compared to the control group.

Expression of RFC Protein Levels

Specific polyclonal antibodies raised against the synthetic peptide of the rat RFC protein were reacted to purified renal BBMV and BLMV preparations separated on SDS-PAGE and transferred onto nitrocellulose membrane, and immunoreactivity was obtained at ~65 kDa. Interestingly,

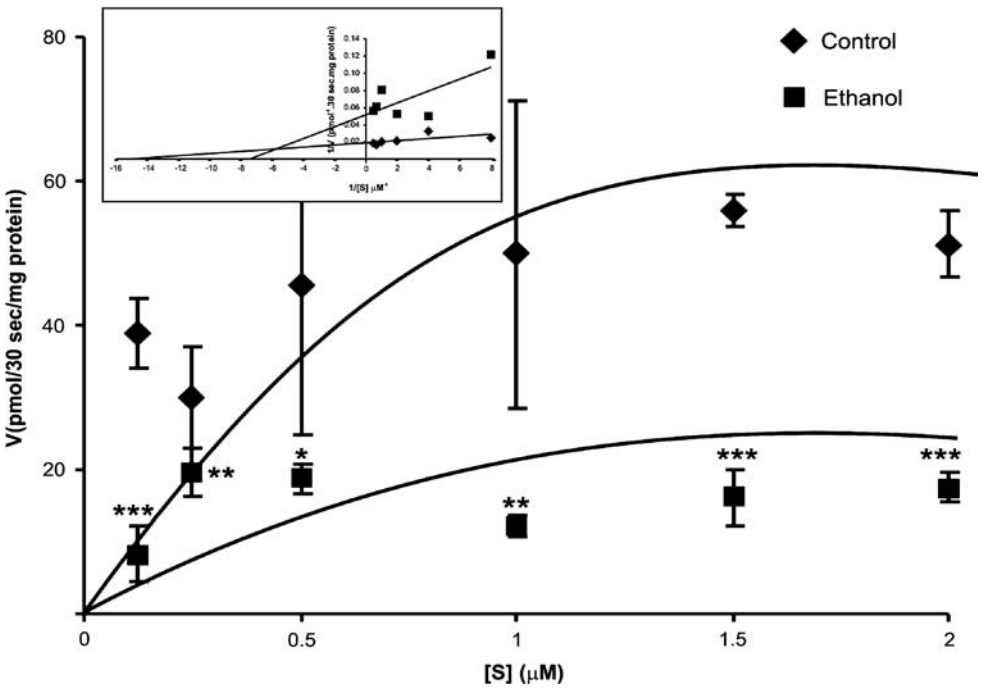

Fig. 4 [^{3}H]-Folic acid uptake in renal BLMVs as a function of substrate concentration (*inset*, Lineweaver-Burk plot). Uptake was measured by varying [^{3}H]-folic acid concentration 0.125–2.0 μM in incubation medium of pH 5.5 after incubating BLMVs for 30 s. Each data point is the mean ± SD of three separate uptake determinations carried out in duplicate. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control

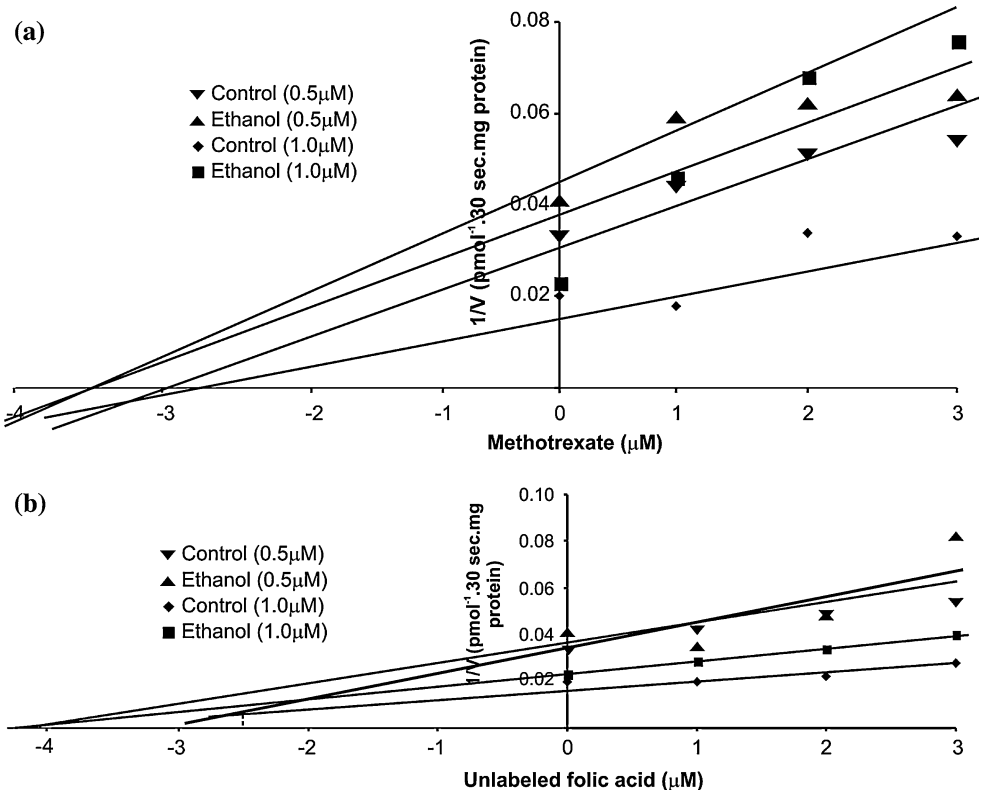

Fig. 5 Determination of the Dixon constants for methotrexate (**a**) and unlabeled folic acid (**b**) in renal BLMVs. [^{3}H]-Folic acid uptake was determined with its concentration at 0.5 and 1.0 μM in the presence of different concentrations of structural analogues (1–3 μM). Each data point is the mean ± SD of three separate uptake determinations carried out in duplicate

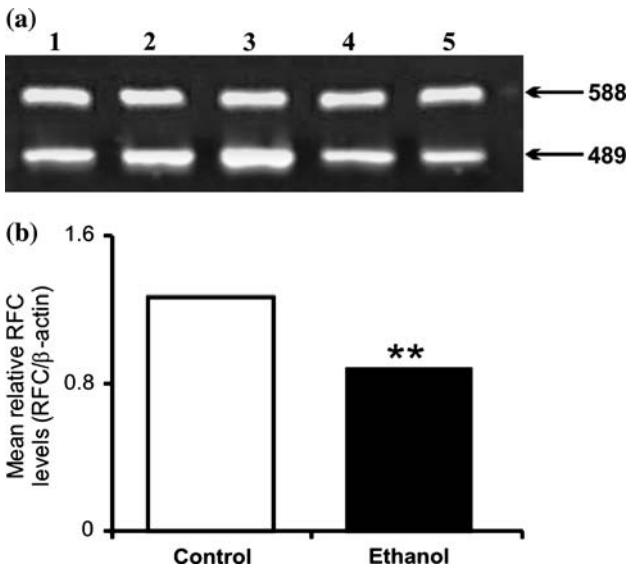

Fig. 6 RT-PCR analysis of RFC in renal tissue: (**a**) agarose gel electrophoresis and (**b**) densitometric analysis. Data are representative of 8–10 separate sets of experiments. $^{**}P < 0.01$ vs. control. Lanes 1–3, control; lanes 4 and 5, ethanol

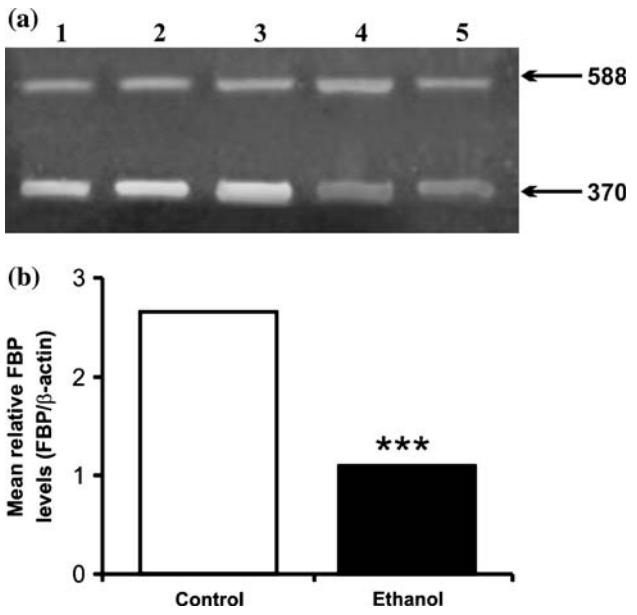

Fig. 7 RT-PCR analysis of FBP in renal tissue: (**a**) agarose gel electrophoresis and (**b**) densitometric analysis. Data are representative of 8–10 separate sets of experiments. $^{***}P < 0.001$ vs. control. Lanes 1–3, control; lanes 4 and 5, ethanol

in concordance with RFC mRNA, there was a significant decrease in RFC protein expression in both BBMV (Fig. 8a, b) as well as BLMV (Fig. 9a, b) preparations in the ethanol-fed group compared to the control group. The decreased expression in the ethanol-fed group was of the order of 1.5- and 3.0-fold in renal BBMV and BLMV, respectively. Importantly, RFC expression was higher in BLMV preparations compared to BBMV when an equal amount of protein was loaded on the gel.

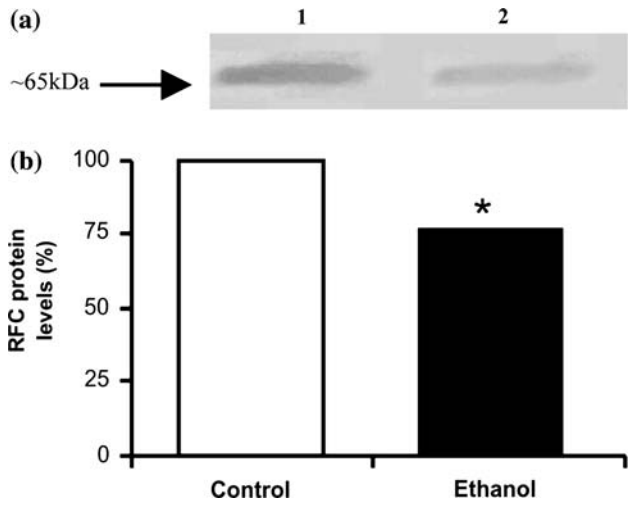

Fig. 8 (**a**) Immunoblot analysis of RFC in renal BBMV and (**b**) densitometric analysis. Data are representative of five separate sets of experiments. $^{*}P < 0.05$ vs. control. Lane 1, control; lane 2, ethanol

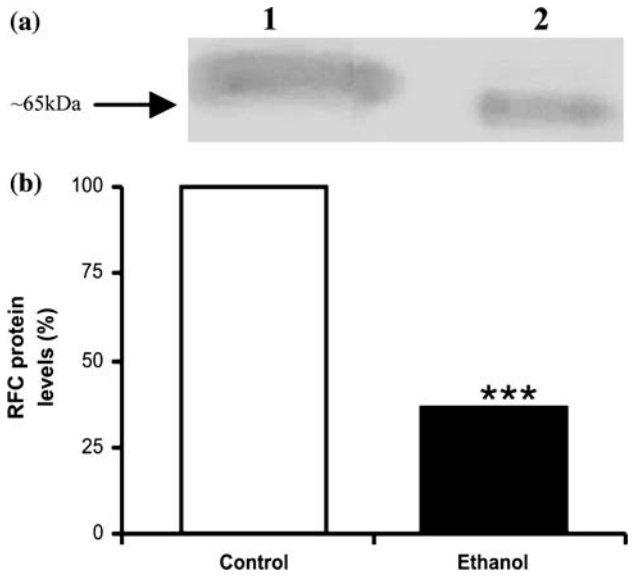

Fig. 9 (**a**) Immunoblot analysis of RFC in renal BLMV and (**b**) densitometric analysis. Data are representative of five separate sets of experiments. $^{***}P < 0.001$ vs. control. Lane 1, control; lane 2, ethanol

Discussion

The activities of folate transporters on kidney BBM play an important role in conserving folate excretion and reabsorption across the apical membrane of the proximal tubules (Sikka & McMartin, 1998). The BLM of the kidney mainly contains RFC as the major transport protein (Elwood et al., 1991; Wang et al., 2001), in contrast to FBP which is mainly responsible for folate transport in renal BBM (Birn, Nielsen & Christensen, 1997). Besides RFC and FBP, several other nonspecific transporters, like the renal organic anion transporter (OAT) family, are known to facilitate folate transport in the BLM of renal absorptive cells (Saito, Masuda & Inui, 1996). These transport system activities could become distinct in response to external stimuli such as alterations in folate availability and exposure to certain chemotherapeutic agents (Agnieszka, Patrycja & Malgorzata, 2000).

The higher blood alcohol concentrations observed in the chronic ethanol-fed group showed that a significant blood alcohol level is maintained during the course of ethanol administration. In contrast to chronic alcoholic studies, the blood alcohol levels observed in the present study were low. Such low blood alcohol levels were obtained because the measurements were carried out 24 h after the last dose of ethanol, and the peak blood alcohol levels in the present study would be much higher. Ethanol feeding at 1 g/kg body weight did not show any clinical signs of intoxication, similar to an earlier study (Muldoon & McMartin, 1994). After 3 months of chronic ethanol ingestion, reduced folate uptake across the BLM was observed at different time intervals in the ethanol-fed group.

It is important to mention here that the different components of the renal basolateral folate transport system can operate independently or synergistically with RFC, as suggested for FBP and RFC in renal BBM (Villanueva et al., 1998). It can be speculated that some members of the OAT family might also be involved in such uptake in renal BLM (Masuda et al., 1999). In this context, the medium osmolarity manipulations showed that the binding component is negligibly associated with the transport system of renal BLM. This finding was similar to earlier observations in intestinal (Said & Redha, 1987) and colonic (Dudeja et al., 2001) BLM preparations. Such findings indicate that the absence of a folate-binding component with the basolateral surface of actively folate-transporting tissue is general behaviour. The decreased uptake of folic acid was associated with a significant increase in K_m (0.07 ± 0.001 and 0.14 ± 0.002 μM) and a decrease in V_{max} (50.0 ± 5.3 and 20.0 ± 2.6 pmol/30 s/mg protein), and this suggests that the mechanism of folate transport across the renal basolateral surface does not operate efficiently in chronic alcoholism. Further, the lower values of K_m in BLM compared to BBM (Hamid & Kaur, 2006) propose that the basolateral surface is suited more for folate reabsorption in renal tissue. Importantly, reduced uptake of folate through cortical BBM and BLM after chronic ethanol feeding indicated less reabsorption, and this would contribute to the enhanced folate excretion in urine observed in alcoholism (McMartin et al., 1989).

The acidic pH was found to be a potential driving force of folate transport across the BLM surface as maximal uptake was obtained at pH 5.0. This was in contrast to that in intestinal BLM, where the optimal pH for folate

transport was found to be neutral (Hamid et al., unpublished data). The reduction in folate uptake in the ethanol-fed group was not uniformly observed in the alkaline pH range, which further suggested that acidic pH is important in the operation of the folate transport system in renal BLM. Transport under alkaline conditions may largely be mediated by the OATs in renal BLM as the latter are characterized by transporting organic anions efficiently in alkaline pH ranges. Furthermore, the analogues inhibited folate transport in a concentration-dependent manner, and the K_i values obtained were higher than the K_m values of folic acid transport in the absence of analogues. This suggested that structural analogues like methotrexate under such conditions are inefficiently transported or employ routes other than that of RFC.

Comparison of the transport characteristics of folic acid across the renal BBM (Hamid & Kaur, 2006) and BLM showed similarities only with regard to pH dependence. However, differences with respect to lower uptake capacity (lesser V_{max}) in renal BLM suggest that the two transport systems operate differently at BBM and BLM surfaces. Such findings are not surprising because the transport system comprises mainly FBP in BBM and RFC in BLM, which may share different chemistry on the membrane surfaces. Moreover, folate uptake in renal BLM, in contrast to renal BBM (Hamid & Kaur, 2006), was found to be temperature-, S-S group-, Na^+,K^+- and ATP-independent. Similar differences have been reported in the colonic apical and BLM surfaces (Dudeja et al., 2001; Dudeja, Torania & Said, 1997). The findings demonstrate that folate uptake involves a complex interplay between the transporter, substrate and some factors specific to the site. These observations are supported by earlier findings, which also suggested that the characteristics displayed by RFC depend on the cell context (Said, 2004). The diverse characteristics could be attributed to differences in membrane composition of BBM and BLM in renal tissue.

It is important to mention here that the physiological form of folic acid in the glomerular filtrate is 5-methyltetrahydrofolate (5-MTHF) and that the affinity of RFC is less toward folic acid compared to 5-MTHF. Earlier studies have shown that acute ethanol feeding increases the urinary excretion of 5-MTHF without simultaneously altering the excretion of folic acid (Eisenga, Collins & McMartin, 1989). The kinetic studies in the present report have limitations since they were carried out with [^{3}H]-folic acid in place of natural substrate 5-MTHF present at renal BLM. Nevertheless, the observed effect of chronic ethanol feeding on the expression of transporters, i.e., RFC and FBP, as discussed subsequently, is undeniable.

Furthermore, the decreased V_{max} in ethanol-fed rats might be due to reduced number of RFC and/or FBP molecules on renal plasma membrane. The decreased number of transporters in turn might reflect inhibition of some transcriptional, translational or posttranslational event or increased degradation of these transporters after chronic exposure to ethanol. Such findings demand a molecular mechanistic understanding, which could explain the role of expression of the transporter molecules in renal tissue during alcoholism. The observed downregulation of both RFC and FBP further stress that these transporters play roles in reduced folate transport efficiency, observed in the present study. The relatively greater decrease in mRNA expression of FBP in comparison to RFC during alcoholism is in agreement with the suggestion of Villanueva, Devlin & Halsted (2001). Importantly, the RFC protein profile studies further substantiated the mRNA expression data and showed decreased RFC protein levels in both BBM and BLM surfaces during alcoholism. In addition, high expression of RFC protein was observed in renal BLM compared to the corresponding BBM. Such an observation is in agreement with earlier studies (Wang et al., 2001) in which RFC was found to be concentrated in the BLM surface in the proximal tubules of renal tissue. Our studies suggested that transcriptional regulation operates in the renal folate transport system during alcoholism. Further studies addressing the molecular regulation of RFC and FBP synthesis and trafficking during alcoholism are required to evaluate the site of transcriptional downregulation.

In summary, chronic ethanol ingestion leads to decreased renal BBM and BLM folate uptake as a result of decreased mRNA expression of RFC and FBP, which was associated with reduced levels of RFC protein in BBM and BLM after alcohol ingestion.

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