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ORIGINAL ARTICLE

The effects of a low protein diet on amino acids and enzymes in the serine synthesis pathway in mice

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Abstract L-Serine is required for cellular and tissue growth and is particularly important in the immature brain where it acts as a crucial neurotrophic factor. In this study, the levels of amino acids and enzymes in the L-serine biosynthetic pathway were examined in the forebrain, cerebellum, liver, and kidney after the exposure of mice to protein-restricted diets. The levels of L-serine, D-serine, and L-serine-*O*-phosphate were quantified by HPLC and quantitative Western blotting was used to measure changes in protein levels of five enzymes in the pathway. The L-serine biosynthetic enzyme phosphoserine phosphatase was strongly upregulated, while the serine degradative enzymes serine racemase and serine dehydratase were downregulated in the livers and kidneys of mice fed low (6%) or very low (2%) protein diets for 2 weeks compared with mice fed a normal diet (18% protein). No changes in these enzymes were seen in the brain. The levels of L-serine increased in the livers of mice fed 2% protein; in contrast, D-serine levels were reduced below the limit of detection in the livers of mice given either the 6 or 2% diets. D-Serine is a co-agonist at the NMDA class of glutamate receptors; no alterations in NMDA-R1 subunit expression were observed in liver or brain after protein restriction. These findings demonstrate that the expression of L-serine synthetic and degradative enzymes display reciprocal changes in the liver and kidney to increase L-serine and decrease D-serine levels under conditions of protein restriction, and that the brain is insulated from such changes.

Keywords L-Phosphoserine · L-Serine-*O*-phosphate · Protein restriction · *N*-methyl-D-aspartate · Phosphoserine phosphatase · Serine racemase · Taurine

Introduction

L-Serine is a dietary non-essential amino acid that is generated in the body, in part, by proteolysis. In mammals, the predominant intrinsic synthetic pathways vary in different tissues and during different stages of development. For instance, the majority of L-serine synthesized by the human fetal liver comes from glycine by the combined action of the glycine cleavage system and serine hydroxymethyltransferases (SHMT) (Narkewicz et al. 1996), whereas in the adult human kidney and in the central nervous system (CNS) most of the L-serine is synthesized via a route known as the phosphorylated L-serine pathway (Lowry et al. 1987; Fell and Snell 1988; Snell and Fell 1990). The phosphorylated pathway encompasses three enzymatic steps (Fig. 1). In the first step, 3-phosphoglycerate derived from glycolysis is metabolized into phosphohydroxypyruvate by 3-phosphoglycerate dehydrogenase (3-PGDH) (Yamasaki et al. 2001). In the second step, phosphohydroxypyruvate is converted into L-phosphoserine (also known as L-serine-*O*-phosphate or L-SOP) by the vitamin B6-dependent enzyme, phosphoserine aminotransferase (PSAT) (Hester et al.

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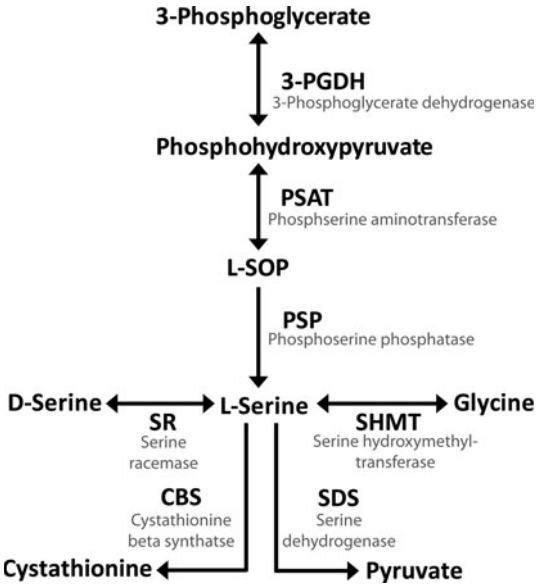

Fig. 1 Pathways for the biosynthesis and catabolism of L-serine

1999). L-SOP is then dephosphorylated by phosphoserine phosphatase (PSP) (Collet et al. 1999) to produce L-serine.

Several major routes of serine catabolism also exist. L-Serine can be isomerized to D-serine by the enzyme serine racemase (SR) (Wolosker et al. 1999), converted to glycine by the cytosolic (SHMT1) and mitochondrial (SHMT2) isoforms of SHMT (Stover et al. 1997; Macfarlane et al. 2008), metabolized into pyruvate by serine dehydratase (SDS) (Nakagawa et al. 1967), or converted into cystathionine by cystathionine beta-synthase (Kraus et al. 1993).

Biologically, L-serine is an important metabolic precursor in the synthesis of proteins, sphingolipids, other amino acids, and nucleotides (Furuya 2008). The importance of L-serine is underscored by reports of genetic mutations in 3-PGDH, PSAT, and PSP in humans. Although rare, mutations in all three enzymes produce a common series of clinical outcomes including abnormal brain development (e.g. microcephaly), seizures, and mental retardation (de Koning et al. 2003; de Koning and Klomp 2004; Veiga-da-Cunha et al. 2004; Hart et al. 2007). Furthermore, L-serine supplementation during development has been demonstrated to partially normalize some of the symptoms associated with these enzymatic abnormalities (de Koning 2006). From a neurochemical perspective, the L-serine precursor L-SOP has been proposed as a possible endogenous agonist or co-agonist at the Group III metabotropic glutamate receptors (Antflick et al. 2009), while the L-serine metabolite D-serine is a co-agonist at the NMDA subtype of ionotropic glutamate receptor (Shleper et al. 2005; Panatier et al. 2006).

The regulation of the enzymatic machinery responsible for serine production and metabolism has been linked to adaptations to dietary protein excess and protein restriction (Noguchi et al. 2008). It has been reported that the expression of 3-PGDH, the enzyme representing the "committed step" in L-serine biosynthesis, is upregulated in response to a low protein diet (Mauron et al. 1973; Achouri et al. 1999; Nagao et al. 2009). In addition, the activities of the other enzymes in this pathway, PSAT and PSP, have also been shown to be upregulated in response to a low protein diet (Lund et al. 1985; Guynn et al. 1986). However, it remains unknown if the increased enzymatic activity is a consequence of elevated expression of PSAT and PSP. A downregulation of SDS induced by amino acid-deficient diets has also been reported (Mauron et al. 1973; Xue et al. 1999; Nagao et al. 2009), but less is known regarding the other enzymes responsible for serine metabolism.

The objective of the present study was to more clearly define the effects of ingesting a low protein diet on the enzymes in the serine pathway. To this end, we conducted a comprehensive simultaneous analysis of the levels of five enzymes in the L-serine metabolic pathway, and also measured serine and related amino acids after the administration of a low protein diet. Mice were fed diets containing very low (2%), low (6%), or normal (18%) protein for 2 weeks and changes in the expression of the enzymes responsible for L-serine synthesis (PSAT and PSP) and L-serine metabolism (SR, SDS, SHMT) were quantitatively analyzed by Western blot analysis of forebrain, cerebellum, liver and kidney tissue. In addition, amino acid analysis of forebrain and liver was conducted to ascertain the effect of protein restriction on tissue amino acid levels. Our results demonstrate that PSP is selectively upregulated and that SR and SDS are selectively downregulated in the livers of mice fed a low protein diet.

Materials and methods

Animals and diets

For dietary studies, 8-week-old male C57BL/6 mice were used. The mice were fed ad libitum standard rodent chow (normal, 18% protein), TD.92203 (2% protein, 79.6% carbohydrate, 5.5% fat, Harlan Laboratories, Madison, WI) or TD.90016 (6% protein, 75.6% carbohydrate, 5.5% fat Harlan Laboratories, Madison, WI) for 2 weeks. The treatment and analysis groups ranged from four mice in each of the three diet groups in the amino acids analyses, to 4–8 mice in each group for the Western blot quantitation, to 12 mice in each group for the body and organ weight determinations. Mice were rapidly killed by cervical dislocation at 10 weeks of age and the brains, livers, and kidneys were removed and immediately weighed. For the

mouse brain dissections, whole brains were quickly removed and the forebrain was separated from the cerebellum and brain stem using a scalpel. The brain stem was then separated from the cerebellum. The forebrain and cerebellum were taken for analysis as described below.

Western blot analysis

Tissue samples were homogenized in 50 mM Tris–HCl and 1% SDS, pH 7.4 and the protein concentrations were determined in the solubilized tissue samples by the BCA assay (Sigma) and diluted to 1 mg/ml in 2% SDS (w/v), 62.5 mM Tris, 10% glycerol, and 0.1 M dithiothreitol for electrophoresis. Equal amounts of protein were loaded in each lane (8–12 µg of protein were loaded per lane, depending on the abundance of the protein target). After protein separation on 10% polyacrylamide gels, the proteins were transferred onto a nitrocellulose membrane (Pall) and probed with the following primary antibodies: chicken anti-PSAT 1:4,000 (Genway Inc.), rabbit anti-PSP 1:1,000 (Antflick et al. 2009), mouse anti-SR 1:1,000 (BD Biosciences), mouse anti-SDS 1:800 (AbCam), sheep anti-SHMT1 1:10,000 (Liu et al. 2001), generously provided by Dr. Patrick Stover, Cornell University), and mouse anti-GAPDH 1:40,000 (Sigma). After a series of washes, the blots were incubated with secondary goat anti-rabbit horseradish peroxidase (HRP) 1:2,000 (Sigma), donkey anti-mouse HRP 1:2,500 (Jackson Immunoresearch), goat anti-chicken HRP 1:10,000 (Jackson Immunoresearch), and donkey anti-sheep HRP 1:20,000 (Jackson Immunoresearch) followed by chemiluminescent detection with West Pico ECL (Pierce). The blots were imaged with the FluorChem multi-image light cabinet (Alpha Innotech) and densitometry was analyzed with AlphaEaseFC software (Alpha Innotech). To account for small errors in sample preparation and loading, the band intensity of the target enzymes in each sample was normalized to the band intensity for GAPDH. The normalized expression values were averaged from 4 to 8 mice from each diet.

Amino acid analysis

Amino acids were analyzed in mouse forebrain and liver samples by HPLC using a procedure described by (Grant et al. 2006) as modified by Rauw et al. (2009). Briefly, the analytical method involved reaction of the supernatants from forebrain and liver homogenates in methanol with a derivatizing reagent consisting of a mixture of *N*-isobutyryl-L-cysteine and *o*-phthaldialdehyde, and separation of the derivatized L-SOP and amino acids on a Waters Alliance HPLC system equipped with a fluorescence detector. Values from four mice per group were averaged.

Statistical analyses

All statistical analyses were carried out by first conducting a one-way ANOVA followed by Tukey's post hoc analysis where appropriate (using GraphPad Prism). The values for the F statistics and the overall P values are listed in "Results" section while the results of the post hoc analyses are shown in the figure legends.

Results

Body and tissue weights

The initial weights of the mice at the start of the experiment, before the introduction of the modified diets, were virtually identical at day 0. After 7 days on the 2% diet, weight loss was evident in these mice; significant differences were observed in the body weights of the mice fed with the 2% diet when compared with the controls fed with 6 and 18% diets (21.7 ± 0.3 g vs. 23.4 ± 0.3 g and 24.1 ± 0.5 g, mean ± SEM, $p < 0.05$; Fig. 2a) ($P = 0.0005$, $F = 9.705$). The differences in weight were even more pronounced at the conclusion of the experiment on day 14 (2%, 21.4 ± 0.4 g; 6%, 23.2 ± 0.3 g; 18%, 25.0 ± 0.5 g). Significant differences were found between each pair of groups analyzed ($P < 0.0001$, $F = 15.68$). Overall, the mice fed the very low protein (2%) diet lost weight, the mice fed the low protein (6%) neither gained nor lost weight and the mice fed the normal protein (18%) diet gained weight (Fig. 2a). The weight loss in the mice fed the very low protein diet was expected in light of the phenomenon that in rodents, dietary aversion and anorectic behavior appears when insufficient levels of indispensible amino acids are present in the diet (Rogers and Leung 1973; also see Gietzen et al. 2007).

At the end of the 14-day feeding period, the wet organ weights were determined for the brain (forebrain and cerebellum, collected separately), liver, and kidney. No differences were detected in the weights of the forebrain and cerebella between the three dietary groups, however, differences were observed in the peripheral organ weights (Fig. 2b). Significant decreases in the liver weights were observed in the groups fed the very low and low protein diets compared with the group fed with the normal protein diet (2%, 760.3 ± 43.8 mg; 6%, 1094.2 ± 32.5 mg; 18%, 1262.6 ± 43.8 mg) ($P < 0.0001$, $F = 38.51$). In addition, a significant difference in liver weight was observed between the groups fed the low and very low protein diets. Kidney weight was also sensitive to protein restriction ($P < 0.0001$, $F = 21.36$); a trend towards decreased kidney weight with reduced protein intake was observed (2%,

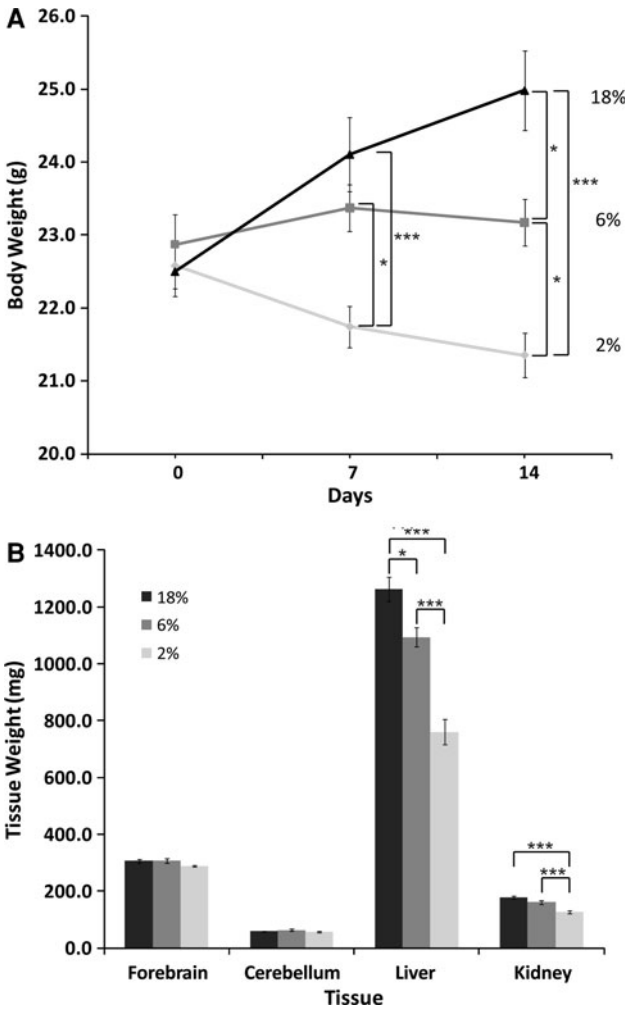

Fig. 2 Changes in body and organ weights in mice fed low protein or normal diets for 2 weeks. Values represent mean ± SEM. **a** Changes in body weight of mice fed diets composed of 2, 6 or 18% protein. **b** Changes in tissue weights. One-way ANOVA followed by Tukey's post hoc analysis was performed ($N = 12$ for each group). Significance is indicated by $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$

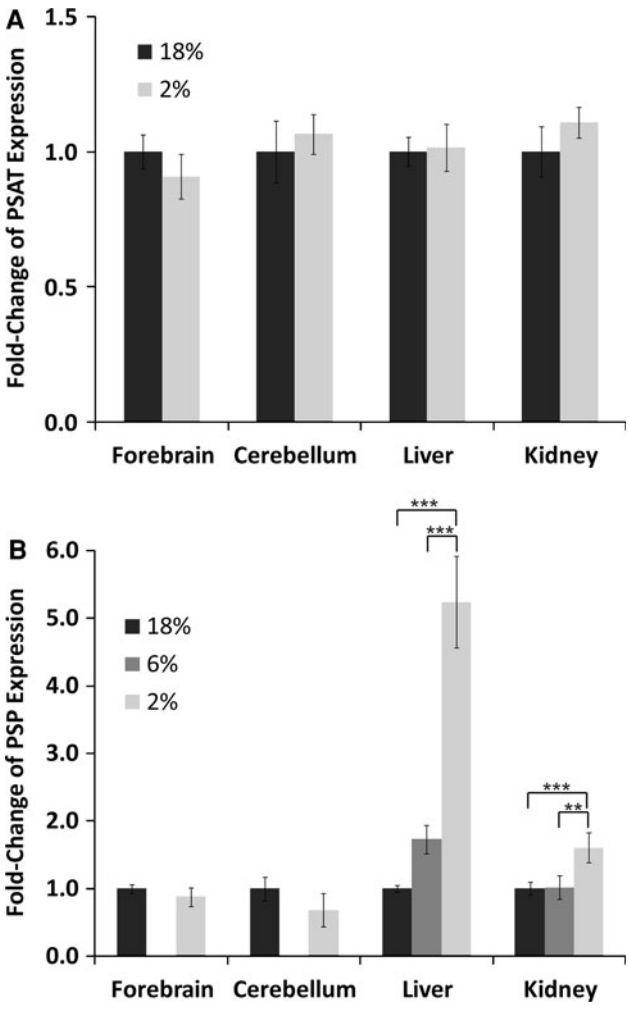

Fig. 3 Changes in the expression of the enzymes associated with L-serine synthesis in mice fed low protein or normal diets for 2 weeks. **a** Summary of PSAT expression based on quantitative Western blotting; **b** Summary of PSP expression based on quantitative Western blotting. Values represent mean ± SEM ($N = 4$–8 for each group). Significant differences are indicated as $**P < 0.01$, $***P < 0.001$

127.3 ± 5.0 mg; 6%, 160.4 ± 6.4 mg; 18%, 177.6 ± 5.6 mg, Fig. 2b).

L-Serine synthesizing enzymes

Quantitative Western blotting was performed on tissue homogenates with antibodies to PSAT and PSP. The first sample analyses were conducted comparing mice fed the very low 2% diet with mice fed the normal 18% protein diet. In most cases, in experiments where no changes were observed in the 2% mice that particular parameter was not measured in mice fed the 6% diet. No change in the expression of PSAT between the groups of mice fed the 2 and 18% diets was seen in any of the four tissues examined (Fig. 3a). In contrast, in response to the 2% diet, PSP was significantly upregulated (5.2-fold) in the liver when compared with the groups fed the 6% (1.7-fold) and 18% diets ($P < 0.0001$, $F = 47.67$). In the kidney, only the very low protein diet induced a significant upregulation in PSP expression (1.6-fold, $P = 0.0003$, $F = 10.60$); no change in PSP expression was detected in the kidneys of mice fed the low protein diet. These results show that, in the liver, PSP is upregulated in response to a low protein diet to augment de novo serine production.

L-Serine catabolizing enzymes

The expression levels of three enzymes responsible for serine catabolism were also examined. Serine racemase which interconverts L- and D-serine was significantly decreased in the livers of the mice fed both the very low and the low protein diets compared with the mice fed the

normal protein diet ($P < 0.0001$, $F = 13.49$). However, no changes in SR expression were detected in forebrain or kidney (Fig. 4a). Serine racemase protein was undetectable in the cerebellum in all samples, consistent with the results of Labrie et al. 2009 who did not detect SR activity in mouse cerebellum. Serine dehydratase, which converts L-serine into pyruvate for gluconeogenesis, was significantly decreased in liver ($P < 0.0001$, $F = 55.35$) and kidney ($P < 0.0001$, $F = 13.86$), but remained unchanged in the forebrains and cerebella of the mice fed the very low (2%) protein diets (Fig. 4b). The mice fed the low (6%) protein diet showed a similar significant reduction in serine SDS expression as the mice fed the 2% diet in the liver, but no reduction in SDS expression was observed in the kidneys of these mice. The expression of SHMT, which converts L-serine to glycine, was not altered in forebrain, cerebellum, liver or kidney (Fig. 4c). Taken together, these results showed that there was a reciprocal increase in L-serine synthesis (via increased expression of PSP), and a decrease in L-serine degradation (decreased expression of SR and SDS) in the liver in response to the low protein diet.

Amino acid levels

Amino acid levels were quantified in samples of forebrain and liver. The analytical assay (Rauw et al. 2009) was developed specifically for the simultaneous detection of L-SOP, L-serine and D-serine, but several additional amino acids including glycine and taurine that are related to the serine pathway could also be assayed simultaneously using this procedure. HPLC analysis of samples from mouse forebrain revealed no significant changes in the level of L-serine in mice fed the 2% diet compared with the control diet (Fig. 5a). In mice given the 6% diet, a significant reduction in the levels of L-SOP was observed compared with the very low and normal diet ($P = 0.0075$, $F = 8.952$). The drop in L-SOP levels in the forebrain of the mice fed the 6% diet was not observed in forebrains of the mice fed the more restricted 2% diet, or in the livers of mice fed either the 2% or the 6% diets. Thus, the decrease in L-SOP in the 6% animals needs to be confirmed, since no significant change in L-SOP was observed in the forebrain of the 2% mice.

In contrast to the forebrain, remarkable changes were observed in the hepatic amino acid levels (Fig. 5b). L-Serine displayed an inverse relationship with dietary protein intake, while D-serine and taurine decreased with a reduction in dietary protein intake. A modest increase in hepatic L-serine was observed in the group fed the 6% protein diet and further protein restriction with the 2% protein diet resulted in a significant increase in L-serine over the two other diets (2%, 106.27 ± 9.64 μg/g; 6%, 57.09 ± 1.75 μg/g; 18%, 50.88 ± 2.46 μg/g) ($P = 0.0002$, $F = 27.07$). Hepatic D-serine levels dropped below the limit of detection in both protein restricted diets

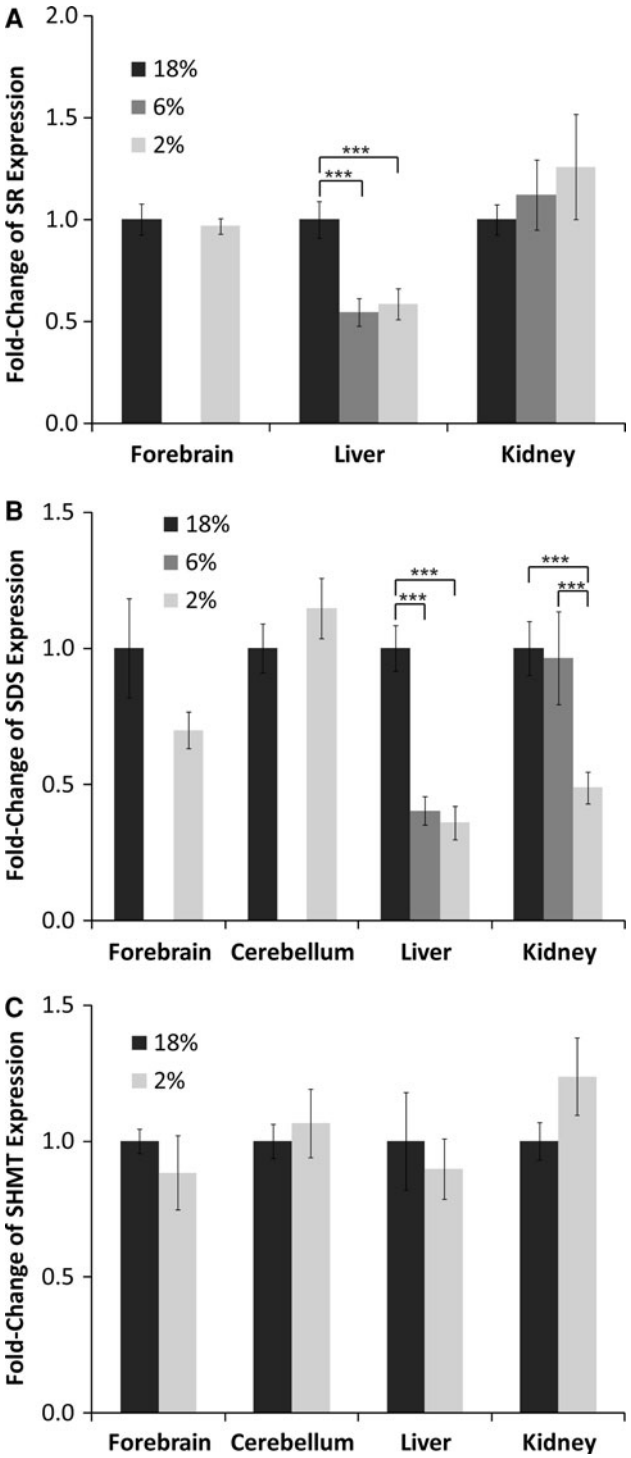

Fig. 4 Changes in the expression of the enzymes associated with serine metabolism. **a** SR expression. **b** SDS expression. **c** SHMT expression. Serine racemase was undetectable in the cerebellum in all samples. Values represent mean ± SEM; $N = 4$–8 for each group. Significant differences are indicated as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

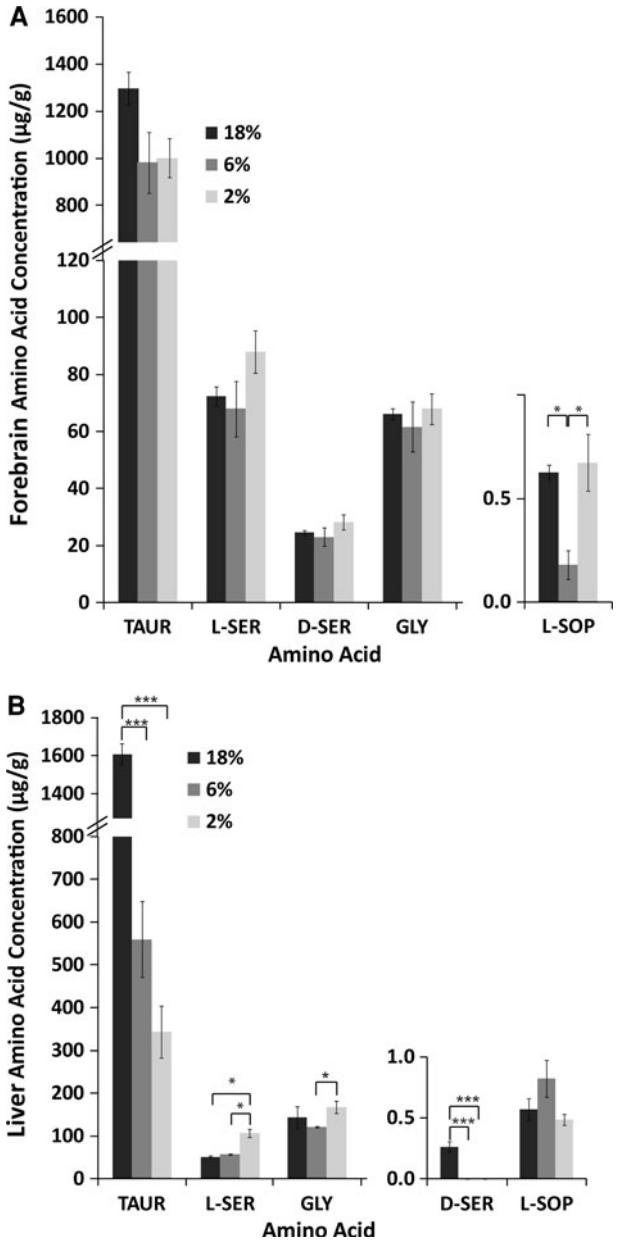

Fig. 5 Amino acid levels in the forebrains and livers of mice fed normal or low protein diets. **a** Forebrain amino acid levels and **b** liver amino acid levels. Values represent mean ± SEM in µg/g; $N = 4$ for each group. Significant differences are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

compared with the level in the mice fed the normal diet (0.26 ± 0.04 µg/g). Thus, the significant decrease in D-serine ($P < 0.0001$, $F = 36.15$) complemented the similar reduction in hepatic SR induced by protein restriction.

The analytical method used for quantitation of amino acids was compatible with the measurement of taurine concentrations in tissues. Hepatic taurine was also sensitive to protein restriction. Taurine concentrations decreased with the amount of dietary protein intake and were significantly reduced in both the low 6% and the very low 2% protein diets compared with the normal diet (2%, 342.69 ± 60.91 µg/g; 6%, 559.28 ± 88.52 µg/g; 18%, 1,608.23 ± 56.24 µg/g) ($P < 0.0001$, $F = 93.44$).

Discussion

We assessed the effects of protein-restricted diets on L-serine and related amino acids, and the enzymes responsible for L-serine synthesis and metabolism in the brain and in peripheral organs. We found that the amount of PSP, the enzyme responsible for the generation of L-serine from L-SOP, was strongly induced in a dose-dependent fashion with decreasing dietary protein in the liver, and to a lesser extent in the kidney. Although elevated PSP enzymatic activity after protein restriction has been demonstrated previously (Lund et al. 1985; Guynn et al. 1986), it was not determined whether this was due to induction of PSP activity per se, as neither mRNA nor protein were measured in those studies. Interestingly, our data show that in the liver, the large 5–6-fold induction of PSP after dietary protein reduction with the 2% diet produced a relatively small, albeit significant increase in L-serine. It is known that ambient levels of L-serine (approximately 500 µM) inhibit the activity of PSP, thereby reducing the conversion of L-SOP to L-serine (Fell and Snell 1988; Hawkinson et al. 1997). Thus, it appears that relatively large amounts of newly synthesized PSP protein are required for overwhelming this feedback inhibition and for producing adequate L-serine in the liver during dietary protein deprivation.

Surprisingly, unlike PSP, the levels of PSAT protein remained unchanged after protein restriction in all tissues tested. Of interest is a report that the mRNA coding for the enzyme responsible for the committed step in serine synthesis, 3-PGDH, is upregulated in response to protein restriction (Mauron et al. 1973; Nagao et al. 2009). Together, these findings indicate that the first enzyme (3-PGDH), and the last enzyme (PSP) in the L-serine biosynthetic pathway are both induced by low dietary protein, whereas the second enzyme in the pathway, PSAT, is not responsive to dietary protein intake. Snell and Fell 1990 suggested that PSAT may operate primarily in a feed-forward fashion. One explanation for the observation that PSAT is not induced after the ingestion of a low protein diet may be that this enzyme is constitutively active and that there may be no intrinsic need for regulatory control, since both the immediate upstream and downstream enzymes are induced when the plasma and hepatic levels of proteins and amino acids drop during dietary protein deprivation.

The HPLC method that we developed for the analysis of L-SOP, L-serine, and D-serine was also amenable to measurement of taurine levels. A steep and highly significant drop in taurine concentrations in the livers of mice on the 6 and 2% diets, and smaller non-significant decline in the forebrain was observed. Previous studies have indicated that the majority of taurine is generated from cysteine metabolism (De La Rosa and Stipanuk 1985) and that taurine levels reflect the availability of cysteine which decreased with the amount of dietary protein intake in rat (Stipanuk et al. 2002). In the present study, the HPLC assay utilized was incompatible with the detection of cysteine. However, the fall in taurine levels with protein restriction observed here is suggestive of a commensurate decrease in the level of cysteine, most likely due to the decreased activity of cystathionine beta-synthase and reduced production of cystathionine from serine (Yamamoto et al. 1996). This is relevant to L-serine synthesis, as cysteine administration has been shown to induce a decrease in the level of 3-PGDH mRNA (Achouri et al. 1999) and to decrease the activity of PSP (Fallon et al. 1966), although in both cases it was not determined if the changes reflected the effects of cysteine directly, or a metabolite of cysteine such as taurine. In our experiments, a decrease in taurine levels after protein restriction was associated with increased L-serine concentrations via the increased expression of PSP and decreased expression of SR and SDS.

Of the three L-serine catabolic enzymes examined (SR, SHMT, and SDS), marked reductions in the levels of SDS were observed in both the liver and kidney, SR was reduced in the liver but not kidney, and SHMT was not significantly changed in any tissue. Interestingly, both SDS and SR were particularly sensitive to reductions in dietary protein. Unlike the induction of PSP, which was significantly elevated only after the 2% diet, SDS and SR levels were more sensitive to dietary protein restriction as they were significantly decreased after only modest protein restriction (6% protein diet). The SHMT1 isoform of SHMT analyzed here showed no change after treatment with a low protein diet. Although we cannot rule out the possibility that the protein restriction may affect the expression of the SHMT2 isoform, this seems unlikely in light of the functional overlap of these two isoforms (Anderson and Stover 2009), and the lack of significant elevation of glycine levels between the very low protein and normal diets in hepatic and forebrain tissue.

This is the first study to report on levels of SR after protein restriction. Complementing the decrease in the expression of SR in the liver was a decrease in the levels of D-serine to below the limit of detection after exposure to 6 and 2% protein diets. From the standpoint of L-serine production and catabolism, it appears that in the liver, a simultaneous induction of L-serine synthesis via the upregulation of 3-PGDH and PSP, and a reduction in L-serine catabolism via the downregulation of SDS and SR act in concert to maintain homeostatic levels of L-serine at the expense of pyruvate and D-serine.

Both D-serine and glycine can bind to the glycine site on the NMDA receptor R1 subunit and co-activate the receptor along with L-glutamate (binding to the NMDA-R2 subunits). However, D-serine has higher affinity for the NMDA receptor glycine binding site than does glycine itself and there is substantial evidence that D-serine is the preferred co-agonist in the brain (Mothet et al. 2000; Schell 2004; Balan et al. 2009; Labrie et al. 2009). Moreover, appropriate levels of D-serine may be required for human brain development (Fuchs et al. 2006). Although a dramatic decrease in D-serine in the liver subsequent to reduced protein was seen in our experiments, the brain levels of D-serine were not significantly affected by the low protein diets, suggesting that NMDA receptor function was likely not compromised. In fact, we measured levels of NMDA-R1 expression in the forebrain, liver, and kidney after exposure to 18 and 2% protein diets (see supplementary Fig. 1). The level of NMDA-R1 expression in the liver and kidney was variable from mouse to mouse as compared to the expression in forebrain which was more consistent, and no significant changes were seen in any of the three tissues after severe protein restriction on the 2% protein diet. We also note that no changes in NMDA-R1 expression were observed in the brains of SR knockout mice, despite the approximate 75–90% depletion of D-serine in whole brain, hippocampus, and cerebral cortex (Labrie et al. 2009), suggesting that if NMDA receptor protein expression in the CNS is affected by exposure to reduced levels of D-serine, it would appear to require a very severe depletion of this amino acid.

In contrast to profound changes in enzyme levels in the liver and kidney after dietary protein restriction, no alterations of any of the five enzymes studied was seen in the mouse forebrain or cerebellum. Moreover, amino acid analysis of the forebrain revealed that the levels of L- and D-serine were not significantly increased even after exposure to the very low 2% protein diet. These findings indicate that the CNS is relatively resistant to changes in the peripheral levels of L-serine biosynthetic and catabolic enzymes, and to fluctuations in the concentration of peripheral L-serine (and D-serine), even when access to dietary protein is severely restricted. The maintenance of brain levels of L-serine under such conditions is consistent with the role of this amino acid as an essential neurotrophic factor (Furuya et al. 2000).

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