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Dietary Regulation of Ornithine Transcarbamylase mRNA in Liver and Small Intestine

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Abstract

In the rat, changes in dietary protein intake give rise to changes in the levels of ornithine transcarbamylase (OTC) in liver and small intestine—an increase in liver and decrease in small intestine. The changes in enzyme level are accompanied by similar changes in levels of specific mRNA. Thus in liver, there is an increase in the level of specific mRNA when protein intake is increased, whereas in small intestine there is a small decrease. Comparison of changes in specific mRNA with total poly-A-containing RNA showed that the change in OTC mRNA in liver paralleled the change in total RNA levels. In contrast, in small intestine the small decrease in OTC mRNA levels when protein intake was increased was in the face of an increase in the level of total mRNA. Whereas the level of OTC is 20-fold higher in liver than in small intestine, the mRNA level for the enzyme differs by only 2·5-fold.

Introduction

Ornithine transcarbamylase (OTC) is found exclusively in the mitochondria of hepatocytes in liver and enterocytes in the small intestine of ureotelic animals. It catalyses the conversion of ornithine and carbamyl phosphate to citrulline. In the liver, the citrulline is converted to urea by the action of the cytoplasmic urea cycle enzymes, but in the small intestine these enzymes are present at only trace levels. Consequently, the citrulline synthesized by this tissue is available for release into the bloodstream. Much of the citrulline is subsequently utilized in the synthesis of arginine in the kidney (Featherston *et al.* 1973; Windmeuller and Spaeth 1980, 1981).

The importance of intestinal citrulline production in the endogenous synthesis of arginine was demonstrated in this laboratory when selective blockage of small-intestinal OTC activity led to significant reductions in blood arginine level and impaired growth in young rats (Hoogenraad *et al.* 1985). The liver enzymes presumably could not contribute significantly to blood arginine levels because of high arginase activity. Thus, OTC appears to serve separate functions in liver and small intestine. In the former, it takes part in the detoxification of ammonia; in the small intestine, it is important for the arginine supply.

We have shown that small-intestinal OTC is identical to the hepatic form of the enzyme but that the enzymes in each tissue are regulated differently in response to the protein content of the diet (Wraight *et al.* 1985). Hepatic OTC levels were 1·7-fold higher in rats maintained on a 60% casein diet, compared with rats on a 15% casein diet. In con-

Abbreviations used: OTC, ornithine transcarbamylase (EC 2.1.3.3); BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; TE, 10 mM Tris–HCl pH 7·6/1 mM EDTA; SSC, 0·15 M NaCl/0·015 M Na citrate, pH 7·0.

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trast, small-intestinal OTC levels were slightly lower in the rats on the 60% casein diet. Concordant results were obtained in a study on the effect of glucagon on liver and small-intestinal OTC and carbamylphosphate synthetase; the liver enzymes were induced by glucagon, but the small-intestinal enzymes were unaffected (Ryall *et al.* 1986). These enzyme adaptations were accompanied by similar changes in the levels of specific mRNA.

In the present study, we demonstrate that the changes in OTC levels in each tissue in response to dietary transitions are accompanied by similar changes in the levels of OTC mRNA in each tissue. We show that the slight reduction in small intestinal OTC in response to an increase in dietary protein is accompanied by a similar reduction in the OTC mRNA in that tissue. Furthermore, we provide evidence that this effect in small intestine does not represent a generalized change in the levels of mRNA but is specific for OTC mRNA.

Materials and Methods

Animals

Female Wistar rats, aged 2 months and weighing between 180 and 220 g were maintained on a standard Purina laboratory chow. Rats were transferred to a pre-conditioning diet containing 30% protein (30%, w/v) casein, 53% (w/v) dextrin, 9% (w/v) vegetable oil, 2% (w/v) agar, 4% (w/v) salt mix and 2% (w/v) vitamin mix (Schimke 1962). After 6 days, ten rats each were transferred to either a 15% protein diet (15% casein, 68% dextrin, etc.) or a 60% protein diet (60% casein, 23% dextrin, etc.) and three rats were maintained on the 30% protein diet. After 8 days, total RNA was prepared from the liver and small intestine of each animal, as well as from the kidney of a rat fed on the 30% protein pre-conditioning diet.

Isolation of Total RNA

Animals from each dietary regimen were killed by exsanguination, then the liver, kidneys, and small intestine were processed by a method adapted from those of Harding and Rutter (1978) and Liu *et al.* (1979). Livers and kidneys were excised and frozen in liquid nitrogen. Small intestine was excised and rinsed in phosphate-buffered saline then frozen in liquid nitrogen. Pieces of tissue (1 g) were broken off while frozen and immediately homogenized in 20 ml of 4 M guanidine thiocyanate, 0·1 M Tris–HCl, pH 7·4, 10% (v/v) β-mercaptoethanol, using an Ultra-Turrax TP 18/10 disintegrator for 40 sec at top speed; the homogenate was centrifuged at 15 000 *g* for 20 min at 4°C. The supernatants were layered onto 0·5 ml 5·7 M CsCl, 0·1 M disodium EDTA (pH 7·5), and centrifuged at 100 000 r.p.m. for 1 h at 20°C in a Beckman TL-100 bench-top ultracentrifuge. The RNA pellet was resuspended in 200 μl 10 mM Tris–HCl, pH 7·6, 1 mM EDTA (TE), then precipitated in 20 μl 2 M sodium acetate, pH 5·0, and 400 μl absolute ethanol at −20°C overnight. After being centrifuged at 12 000 *g* for 10 min at 4°C, the pellet was washed once in 1 ml 70% (v/v) ethanol, dissolved in 200 μl TE and stored at −70°C.

Absorbance readings at 260 and 280 nm were taken in a Varian Cary Model 118 spectrophotometer and the amount of RNA was calculated, based on the assumption that A_{260}/A_{280} equals 2·0 for 40 μg ml^{-1} pure RNA (Maniatis *et al.* 1982). A_{260}/A_{280} ratios ranged from 1·7 to 2·1, indicating that protein contamination was not significant.

Northern Blot Analysis

Total RNA from liver, small intestine and kidney was analysed by the 'Northern' transfer or 'RNA-blot' technique after electrophoresis in a 1% (w/v) agarose gel containing formaldehyde. Sample preparation, gel electrophoresis and transfer onto nitrocellulose membrane were all performed as described in Maniatis *et al.* (1982). After transfer, the nitrocellulose was baked *in vacuo* at 80°C for 2 h then soaked in 0·1% (w/v) Ficoll, 0·1% (w/v) polyvinyl-pyrollidone, 0·1% (w/v) BSA, 0·9 M NaCl, 50 mM sodium phosphate, pH 7·7, 5 mM EDTA, 50% (v/v) formamide, 0·1% (w/v) SDS, 100 μg ml^{-1} denatured herring sperm DNA for 4 h at 42°C. The filter was probed for 16 h at 42°C with an OTC cDNA (McIntyre *et al.* 1985), which had been labelled with ^{32}P by nick-translation. The membrane was washed twice in 4 × SSC/0·1% (w/v) SDS for 10 min each, then twice in 0·1 × SSC/0·1% (w/v) SDS for 30 min each, all at 42°C (1 × SSC = 0·15 M NaCl, 0·015 M Na citrate, pH 7·0). The membrane was blotted dry and autoradiographed at −80°C.

Dot-blot Hybridization

Total RNA from rat liver, small intestine and kidney was denatured in formaldehyde as described by White and Bancroft (1982) to give 60 μg ml^{-1} final concentration of denatured RNA for liver and 270 μg ml^{-1} denatured RNA for small intestine and kidney.

Serial dilutions in 15 × SSC were made in 96-well microtitre plates and applied to a nitrocellulose membrane with a Schleicher and Schuell Minifold apparatus under vacuum. The membrane was air dried, baked *in vacuo* at 80°C for 2 h, then pre-hybridized, probed and washed as described for the Northern analysis. Radioactivity was determined by autoradiography or by cutting out the nitrocellulose spots and measuring the ^{32}P by liquid scintillation counting.

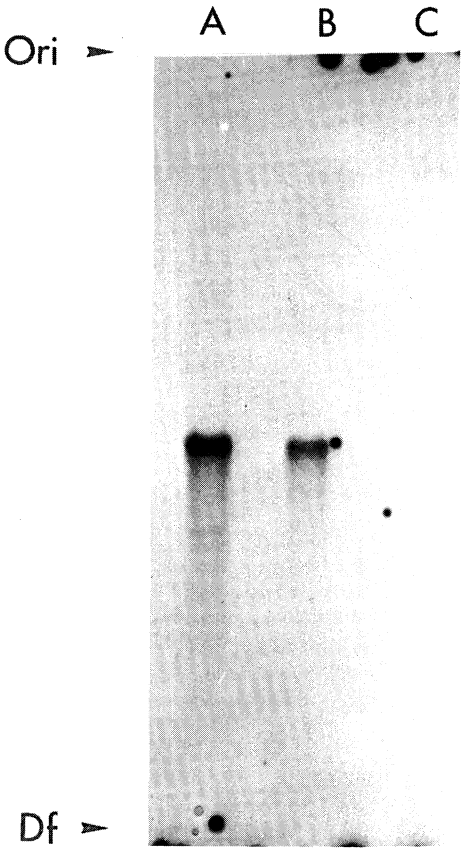

Fig. 1. Northern analysis of OTC mRNA from liver, small intestine and kidney. Total RNA from liver, small intestine and kidney was electrophoresed in a 1% agarose gel containing formaldehyde, then transferred to nitrocellulose membrane and probed with a ^{32}P-labelled OTC cDNA. Lane A, 5 μg total RNA from small intestine; B, 1 μg total RNA from liver; C, 5 μg total RNA from kidney; Ori, origin; Df, dye-front.

In order to estimate the relative levels of poly-A-containing RNA in each spot, separate dot-blots were probed with a synthetic oligo-dT, 30 bases in length. The oligonucleotide was end-labelled with γ-^{32}P-ATP (Maniatis *et al.* 1982). Total RNA from each tissue (1 μg) was spotted and baked as described above, then soaked in 0·1% SDS, 5 mM EDTA, 250 μg ml^{-1} herring sperm DNA, 0·2% (w/v) Ficoll, 0·2% (w/v) polyvinyl-pyrollidone, 0·2% (w/v) BSA, 5 × SSC for 4 h. The filter was then incubated in the same buffer containing the oligonucleotide probe for 60 h. The filter was washed in 4 × SSC for 30 min and in 2 × SSC/0·5% (w/v) SDS for 2 h. All steps were performed at ambient temperature. Radioactivity in each spot was determined by liquid scintillation counting.

Results and Discussion

Northern analysis (Fig. 1) showed that the mRNA for OTC from liver and small intestine is of the same apparent size. Size differences of less than 50 bases would not have been detected. No OTC mRNA was measurable in kidney and the labelled probe does not bind to any other material on the nitrocellulose filter.

From dot-blot hybridization analyses, small intestine contained 2·5-fold less OTC mRNA, per unit total RNA, than liver. This contrasts with the 20-fold lower activity of OTC in small intestine compared with liver (Wraight *et al.* 1985). Possible explanations for this difference could be that OTC mRNA is translated less efficiently in small intestine, the turnover rate of the enzyme is greater in small intestine, or that the efficiency of import of the cytoplasmic precursor for OTC into mitochondria is lower in small intestine.

The response in OTC mRNA to changes in dietary protein intake was different in liver and small intestine. As shown in Table 1, liver OTC mRNA showed a 2·5-fold increase, when expressed relative to total RNA, on a change from 15% to 60% protein. In small intestine, OTC mRNA levels decreased by 1·7-fold.

Table 1. Response of OTC mRNA to dietary transitions in liver and small intestine

Total RNA from three rats maintained on the 15% and 60% dietary regimens and three rats maintained on the 30% diet was spotted onto nitrocellulose and hybridized with a ^{32}P-labelled OTC cDNA. Radioactivity in the spots was determined by liquid scintillation counting. Values represent the mean ± s.d. *$P < 0·005$; **$P < 0·5$; versus rats on 15% casein diets (Student's *t*-test)

Protein content of diet	Amount of mRNA (c.p.m. ± s.d. per 100 μg total RNA) Liver	Small intestine
15%	931 ± 297	368 ± 118
30%	1140 ± 364**	341 ± 124
60%	2300 ± 352*	212 ± 50*

Table 2. Effect of dietary protein intake on the level of total poly-A-containing mRNA in liver and small intestine

Total RNA from ten rats maintained on the 15% and 60% dietary regimens and three rats maintained on the 30% diet was spotted onto nitrocellulose and hybridized with a ^{32}P-labelled oligo-dT probe as described in the text. Radioactivity in the spots was determined by liquid scintillation counting. Values represent the mean ± s.d. *$P < 0·005$; **$P < 0·25$; versus rats on 15% casein diets (Student's *t*-test)

Protein content of diet	Amount of poly-A-containing mRNA (c.p.m. ± s.d. per μg total RNA) Liver	Small intestine
15%	27300 ± 3130	25600 ± 3680
30%	30300 ± 2710**	28700 ± 2460**
60%	38600 ± 3040*	34400 ± 864*

To determine whether this difference represented generalized changes in the steady-state levels of mRNA in the two tissues, the amount of poly-A-containing RNA was measured in rats fed on the two diets. It was observed that, in the small intestine, the level of total poly-A-containing RNA increased with increasing protein intake (1·3 fold, $P < 0·005$, Table 2), which contrasts with the response to the OTC mRNA, which falls (1·7-fold, $P < 0·005$). In liver, poly-A-containing RNA levels increased (1·4-fold, $P < 0·005$, Table 1) in the same direction as OTC mRNA (increased 2·5-fold, $P < 0·005$). From these results, it appears that the response of OTC mRNA in the small intestine is a specific

change, but in liver, changes in OTC mRNA may, in part, reflect a more generalized adaptation in mRNA levels. Although OTC mRNA levels change in a different manner in the two tissues, OTC is encoded by one gene (Haan *et al.* 1982; Lindgren *et al.* 1984). These differences in response in liver and gut may reflect differences in the regulation of transcription of the OTC gene.

The results on OTC are in accord with results obtained for carbamylphosphate synthetase, the enzyme preceeding OTC in citrulline biosynthesis (Hurwitz and Kretchmer 1986). In that study, carbamylphosphate synthetase levels in liver increased by a factor of nearly 3-fold in response to an increase in the protein content of the diet from 8% to 50%, but the enzyme level in small intestine decreased by approximately 10%.

Another difference between liver and small intestine is that, despite a 20-fold difference in the level of OTC activity in liver and small intestine, there is only a 2·5-fold difference in the level of their specific mRNAs. A similar discrepancy between enzyme and mRNA levels was found by Ryall *et al.* (1985), who investigated carbamylphosphate synthetase in the two tissues. Since liver carbamylphosphate synthetase is known to turn over rather slowly (Nicoletti *et al.* 1977), the maintenance of the high hepatic levels of this enzyme does not require high rates of mRNA translation. Ryall *et al.* (1985) point out that the small-intestinal mucosa, which contains the mitochondrial urea cycle enzymes, has a short life span (approx. 5 days). The high turnover of all proteins in this cell type is therefore consistent with the idea that relatively high levels of mRNA are required to maintain the observed steady-state concentrations of the mitochondrial urea cycle enzymes.

The separate regulation of OTC in liver and small intestine is consistent with the idea that the intestinal mitochondrial enzymes serve a role different from the disposal of ammonia performed by their liver counterparts. While an increase in protein intake forces a need for increased rates of conversion of ammonia to citrulline in the liver, the need for *de novo* synthesis of arginine might be expected to decrease, or at least be unaltered.

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