Evidence for intracellular partitioning of serine and glycine metabolism in Chinese hamster ovary cells

Michael R. NARKEWICZ^{*,§}, S. David SAULS^{*}, Susan S. TJOA[†], Cecilia TENG[‡] and Paul V. FENNESSEY[†]

Department of Pediatrics, Sections of ^{*} Pediatric Gastroenterology, Hepatology and Nutrition, [†] Genetics and [‡] Neonatology, and the Hepatobiliary Center, University of Colorado School of Medicine, The Children's Hospital, Denver, CO 80218, U.S.A.

Serine hydroxymethyltransferase (SHMT) is the primary enzyme in the interconversion of serine and glycine. The roles of mitochondrial and cytosolic SHMT in the interconversion of serine and glycine were determined in two Chinese hamster ovary (CHO) cell lines that both contain cytosolic SHMT but either have (CHOm⁺) or lack (CHOm⁻) mitochondrial SHMT. Mitochondrial SHMT activity was significantly reduced in CHOm⁻ (0.24 ± 0.11 nmol/min per mg of mitochondrial protein) compared with CHOm⁺ (3.21 ± 0.66 nmol/min per mg of mitochondrial protein; $P = 0.02$) cells, whereas cytosolic SHMT activity was similar in CHOm⁻ and CHOm⁺ cells (1.09 ± 0.31 and 1.53 ± 0.12 nmol/min per mg of cytosolic protein respectively; $P = 0.57$). In CHOm⁺ and CHOm⁻ cells, the relative flux of glycine to serine measured with either [¹⁻¹³C]- or [²⁻¹³C]-glycine was similar (CHOm⁻: 538 ± 82 nmol/24 h per mg of DNA; CHOm⁺: 616 ± 88 nmol/24 h per mg of DNA; $P = 0.42$). In contrast, the relative flux of serine to glycine measured with [¹⁻¹³C]serine was

low in CHOm⁻ cells (80 ± 28 nmol/24 h per mg of DNA) compared with CHOm⁺ cells (3080 ± 320 nmol/24 h per mg of DNA; $P = 0.0001$). The rate of glycine production determined by [¹⁻¹³C]glycine dilution was lower in CHOm⁻ (1200 ± 200 nmol/24 h per mg of DNA) than CHOm⁺ (10200 ± 1800 nmol/24 h per mg of DNA; $P = 0.03$) cells, whereas glycine utilization was similar in the two cell lines. Serine production was similar in the two cell lines but serine utilization was lower in CHOm⁻ (3800 ± 1200 μmol/24 h per mg of DNA) than CHOm⁺ (6600 ± 1000 nmol/24 h per mg of DNA; $P = 0.0002$) cells. Increasing the serine concentration in the medium resulted in an increase in glycine production in CHOm⁺ but not in CHOm⁻ cells. Intracellular studies with [¹⁻¹³C]serine confirm the findings of decreased glycine production from serine. In CHO cells there is partitioning of intracellular serine and glycine metabolism. Our data support the hypothesis that mitochondrial SHMT is the primary pathway for serine into glycine interconversion.

INTRODUCTION

Serine and glycine are important amino acids involved in the provision of precursors and cofactors for nucleotide biosynthesis. Serine hydroxymethyltransferase (SHMT; EC 2.1.2.1) catalyses the reversible transfer of a methylene group from serine to tetrahydrofolate (THF) with the formation of glycine and 5,10-methyleneTHF (Figure 1) [1]. 5,10-MethyleneTHF is a carbon donor in thymidine synthesis and a precursor for the formation of other folate cofactors involved in purine biosynthesis.

SHMT exists as both mitochondrial (mSHMT) and cytosolic (cSHMT) isoforms encoded by separate genes [2]. The exact role

of the two isoforms of SHMT in serine and glycine metabolism is not certain. It has been suggested that the role of cSHMT is to provide methyleneTHF for nucleotide synthesis [1]. The role of the mSHMT isoform is still controversial. Some investigators have proposed that it is involved in the shuttling of one-carbon units across the mitochondrial membrane [3]. Other authors have proposed that mSHMT and the glycine-cleavage system (GCS) are involved in the coupled synthesis of serine from glycine within the mitochondria [4,5]. Others have proposed that mSHMT is responsible for glycine synthesis from serine [6] or for the provision of formate for purine biosynthesis [7].

We have previously reported stable-isotope analysis of serine and glycine metabolism in fetal hepatocytes [8]. However, as fetal hepatocytes contain both cSHMT and mSHMT activity, we could not determine whether they have similar or separate functions in serine and glycine metabolism. CHO glyA cells are a mutant CHO cell line that lacks mSHMT activity (referred to here as CHOm⁻). CHOm⁻ cells are derived from CHO K1 cells (referred to here as CHOm⁺) that have detectable mSHMT activity [9]. Previous studies have shown that CHOm⁻ cells require glycine for growth and have low rates of glycine synthesis [6,9]. In this study, we sought to clarify the role of mSHMT in the metabolic interconversion of serine and glycine using stable-isotope tracers in CHOm⁻ compared with CHOm⁺ cells. These cell lines allow clarification of the respective roles of the mitochondrial and cytosolic isoforms of SHMT in the metabolic interconversion and biosynthesis of serine and glycine. We hypothesized that there is intracellular partitioning of the interconversion of serine and glycine involving the two isoforms of SHMT.

Figure 1 Metabolic pathways for the interconversion of serine and glycine

GCS, glycine-cleavage enzyme system. *C is carbon-2 of serine.

Abbreviations used: SHMT, serine hydroxymethyltransferase; GCS, glycine-cleavage enzyme system; FCS, fetal calf serum; MPE, mole percent excess; GC/MS, gas chromatography/mass spectrometry; THF, tetrahydrofolate; PLP, pyridoxal 5'-phosphate; CHO, Chinese hamster ovary.

§ To whom correspondence should be addressed.

EXPERIMENTAL

Materials

CHOM⁺ cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). CHOM⁻ cells were kindly provided by Dr. Kao [9]. Cells were grown to 80% confluence in Ham's F12 medium supplemented with 7% fetal calf serum (FCS), 10 units/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml kanamycin and 2.5 µg/ml amphotericin B at 37 °C, 5% CO₂ and 95% humidity. Medium was changed every 48 h. Cells utilized for media stable-isotope experiments were grown in six-well plates (Corning Laboratory Sciences Company, Corning, NY, U.S.A.). Cells used for enzyme analysis and intracellular stable-isotope studies were grown in T75 flasks. In some studies, media were supplemented with either serine or glycine to achieve concentrations three to four times higher than in normal F12 medium (106 ± 6 and 149 ± 15 nmol/ml respectively).

Media were obtained from Gibco (Grand Island, NY, U.S.A.) and FCS was from Hyclone (Logan, UT, U.S.A.). THF and pyridoxal 5'-phosphate (PLP) were from Fluka (Ronkonkoma, NY, U.S.A.). L-[3-¹⁴C]serine and L-[1-¹⁴C]glycine were from ICN (Costa Mesa, CA, U.S.A.). Stable isotopes ([1-¹³C]glycine, [2-¹³C]glycine and [1-¹³C]serine, all 99% atom excess) were obtained from Cambridge Isotope Laboratories (Andover, MA, U.S.A.). Solutions of the stable isotope of interest were prepared in sterile distilled water and sterilized by filtration. Stock solutions were analysed by gas chromatography/mass spectrometry (GC/MS) and all were more than 99% pure. All other chemicals and reagents were from Sigma (St. Louis, MO, U.S.A.) or Fisher (Pittsburg, PA, U.S.A.) and were of the highest grade available.

Stable-isotope studies

After the cells had reached 80% confluence, medium was removed by aspiration and fresh medium was added that contained an approx. 30% enrichment of the stable isotope of interest ([1-¹³C]glycine, [2-¹³C]glycine or [1-¹³C]serine) such that the unenriched medium contained an additional 40 nmol/ml tracer for glycine or 30 nmol/ml for serine. Medium without added stable isotope was analysed in parallel as a blank for the determination of natural abundance and changes in medium amino acid concentration. Baseline samples of enriched and unenriched media were taken at time 0 (*t*₀). In a typical experiment, cells were cultured for 24 h in the presence of the stable isotope of interest. Preliminary experiments indicated that the change in medium enrichment was linear for over 24 h. At 24 h, the medium was aspirated, centrifuged for 2 min at 10 000 *g* (4 °C) and snap-frozen for subsequent amino acid and GC/MS analysis. Cellular DNA was determined on cell trichloroacetate precipitates by the method of Burton [10]. Duplicate or triplicate wells were used for each culture and each medium sample was analysed in triplicate.

As SHMT is a reversible enzyme, in some experiments, we determined the effect of increasing the medium serine or glycine concentration on serine and glycine metabolism. For these experiments, cells were cultured as above except that the medium was supplemented with a 3–5-fold excess of either serine (high serine) or glycine (high glycine).

For the studies of intracellular enrichment, cells in three–five T75 flasks were grown to 80% confluence. At *t*₀, medium was removed and fresh medium supplemented with a 20–30% enrichment of [1-¹³C]serine was added. An aliquot of the enriched medium (*t*₀) was taken for subsequent analysis of serine and glycine concentrations and enrichments. Cells were cultured for 24 h in the presence of the enriched medium and, at *t*₂₄, 1 ml

aliquots of medium were removed from each flask and pooled for subsequent analysis. The remainder of the medium was then removed and the cells were harvested with trypsin, washed once with PBS containing 5% FCS and twice with ice-cold PBS. Preliminary experiments with radiolabelled serine and glycine had demonstrated that there was no residual radioactivity in the wash by the third wash. The cells were pelleted and resuspended in an equal volume (w/v) of sterile distilled water. Cells were disrupted by sonication. An aliquot was removed for DNA and protein analysis, cellular debris was removed by centrifugation, and the supernatant was snap-frozen for subsequent GC/MS analysis.

Mass isotope-enrichment analysis

Incorporation of ¹³C label into glycine (using [1-¹³C]serine as the tracer) or serine (using either [1-¹³C]glycine or [2-¹³C]glycine as the tracer) was determined at 0 and 24 h by GC/MS as previously described [11]. Briefly, a 250 µl aliquot of medium was acidified with acetic acid, passed through anion/cation-exchange (AGCE AG 50W-X8) resin and, after washing with water, amino acids were eluted with 6% (w/v) NH₄OH. After being dried under vacuum, the samples were derivatized at 100 °C for 30 min with 200 µl of *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide (Pierce, Rockford, IL, U.S.A.) and acetonitrile (1:5 v/v). After being cooled, the samples were analysed by GC/MS as previously reported [11]. Changes in mole percent excess (MPE) was determined by monitoring at mass = 247/246 for (*M*+1) glycine and at mass = 391/390 and 392/390 for (*M*+1) and (*M*+2) serine.

Amino acid analysis

Medium amino acid concentrations were determined by two separate methods. First, they were determined at 0 and 24 h on a JEOL amino acid analyser with norleucine as the internal standard as previously reported [12]. In addition, at the time of the GC/MS analysis, medium glycine and serine concentrations were determined using a standard curve of serine and glycine standard additions from 5 to 500 nmol/ml. In preliminary experiments, we determined the coefficient of variation, using the latter method, and found it to be ±1% for both serine and glycine. This method was linear up to a medium serine or glycine concentration of 1 µmol/ml.

Analysis of enzyme activity

Cells were cultured to 80% confluence in six to ten T75 flasks, harvested with trypsin, collected by low-speed centrifugation, washed once in PBS/5% FCS and then twice more with cold PBS. After resuspension in a small volume of homogenization buffer (0.25 M sucrose, 0.1 mM EGTA, 10 mM Hepes, pH 7.4) cells were lysed by 10–20 strokes in Dounce glass/glass homogenizer and then ten passes with a motor-driven glass/Teflon homogenizer. Cell debris was removed by low-speed centrifugation (100 *g* for 2 min at 4 °C). The resulting supernatant was centrifuged at 10 000 *g* for 30 min at 4 °C. The mitochondrial pellet produced was washed twice more with homogenization buffer and resuspended in a small volume of homogenization buffer. The 10 000 *g* supernatant was centrifuged at 100 000 *g* for 60 min at 4 °C yielding the cytosolic fraction.

GCS activity was assayed by measuring the release of ¹⁴CO₂ from [1-¹⁴C]glycine in the fresh mitochondrial suspension by the method of Jois et al. [13,14] with added Ca²⁺ for maximal stimulation. SHMT activity was assayed by measuring the

Table 1 Medium serine and glycine concentrations

Medium serine and glycine concentrations were determined before and after 24 h of culture. Values are means $\pm$ S.E.M. for seven separate determinations. +, Net production; —, net utilization. * $P < 0.01$ compared with similar amino acid concentration in the other two conditions.

Media conditions and cell type	[Glycine] at t_0 (nmol/ml)	Δ Glycine (nmol/24 h per ml)	[Serine] at t_0 (nmol/ml)	Δ Serine (nmol/24 h per ml)
CHOM ⁺ , normal	149 $\pm$ 15	+107 $\pm$ 29	106 $\pm$ 6	—51 $\pm$ 6
CHOM ⁺ , high serine	168 $\pm$ 23	+281 $\pm$ 84	591 $\pm$ 117*	—412 $\pm$ 101
CHOM ⁺ , high glycine	621 $\pm$ 132*	—48 $\pm$ 47	118 $\pm$ 8	—41 $\pm$ 10
CHOM [–] , normal	149 $\pm$ 15	—80 $\pm$ 15	106 $\pm$ 6	+40 $\pm$ 14
CHOM [–] , high serine	168 $\pm$ 23	—55 $\pm$ 28	591 $\pm$ 117*	—57 $\pm$ 73
CHOM [–] , high glycine	621 $\pm$ 132*	—125 $\pm$ 50	118 $\pm$ 8	+78 $\pm$ 18

production of [¹⁴C]methyleneTHF from [3-¹⁴C]serine by the method of Geller and Kotb [15]. For assay of mSHMT and citrate synthase activities, mitochondria were sonicated in 0.125 M sucrose/17.5 mM Hepes, pH 7.4/0.05 mM EGTA, and, after centrifugation at 100 000 *g* for 60 min at 4 °C, the supernatant was used. Citrate synthase activity was determined spectrophotometrically by the method of Shepherd and Garland [16]. Protein was determined by the method of Lowry as modified by Hartree [17].

Calculations

The percentage of glycine utilized for serine production determined using [1-¹³C]glycine is given by: tracer carbon in serine at 24 h/tracer carbon in glycine utilized in 24 h expressed as a percentage of tracer glycine utilized. The percentage of serine utilized for glycine production determined using [1-¹³C]serine is given by: tracer carbon in glycine at 24 h/tracer carbon in serine utilized in 24 h expressed as a percentage of tracer serine utilized.

Since the enrichment (MPE) in the medium of the added stable isotope (serine or glycine) should not change unless there is cellular production or release of unlabelled amino acid, the production of the amino acid of interest (aa) can be determined by the stable-isotope dilution method using the following formula:

$$[(\text{MPE}_{\text{aa}} \text{ at } t_0 \times \text{AA concn.}_{\text{aa}} \text{ at } t_0) / (\text{MPE}_{\text{aa}} \text{ at } t_{24})] - \text{AA concn.}_{\text{aa}} \text{ at } t_0$$

Utilization is given by:

$$[(\text{MPE}_{\text{aa}} \text{ at } t_0 \times \text{AA concn.}_{\text{aa}} \text{ at } t_0) / (\text{MPE}_{\text{aa}} \text{ at } t_{24})] - \text{AA concn.}_{\text{aa}} \text{ at } t_{24}$$

These data were corrected for medium volume and for cellular DNA content to adjust for cell number.

Glycine production from serine is then given by:

$$\% \text{Ser utilized for Gly} \times \text{Ser utilization} \times 0.01$$

Serine production from glycine is given by:

$$\% \text{Gly utilized for Ser} \times \text{Gly utilization} \times 0.01$$

Although the calculation of absolute flux of tracer carbon to tracee carbon requires steady-state conditions that were not present in these experiments, the relative flux of tracer carbon to tracee carbon can be determined for serine to glycine and glycine to serine. Comparisons between cell types of the relative flux rates are valid if the initial concentrations and MPE of the tracer are equivalent between the two cell types and cultures as was true

for the studies performed in media without supplemental serine or glycine. The relative flux of serine to glycine in 24 h ([1-¹³C]serine as tracer) can be calculated from:

$$0.01 \times \text{MPE of Gly at } t_{24} \times \text{Concn. of Gly at } t_{24} / \text{mg of DNA}$$

The relative flux of glycine to serine in 24 h ([1-¹³C]glycine as tracer) can be calculated from:

$$0.01 \times \text{MPE of Ser at } t_{24} \times \text{concn. of Ser at } t_{24} / \text{mg of DNA}$$

Statistics

Values are presented as means $\pm$ S.E.M. Differences between the two cell types during simultaneous culture were compared by the paired *t* test with significance taken at $P = 0.05$. Differences between multiple culture conditions were determined by analysis of variance with significance taken at $P = 0.05$ by the Scheffe *F* test.

RESULTS

Enzyme activity

As expected, mSHMT specific activity was significantly reduced in CHOM[–] (0.24 $\pm$ 0.11 nmol/min per mg of mitochondrial protein) compared with CHOM⁺ (3.21 $\pm$ 0.66 nmol/min per mg of mitochondrial protein; $P = 0.02$; $n = 6$) cells. In addition, there was no significant difference in the activity of cSHMT (1.09 $\pm$ 0.31 nmol/min per mg of cytosolic protein for CHOM[–] cells and 1.53 $\pm$ 0.12 nmol/min per mg of cytosolic protein for CHOM⁺ cells; $P = 0.57$). Mitochondrial SHMT activity accounted for 40 % of the total SHMT activity in the CHOM⁺ cells ($n = 2$) and the percentage recovery of SHMT activity was 60–80 %. Higher recovery rates with more vigorous homogenization led to unacceptable mitochondrial contamination of the cytosolic fraction. GCS activity was very low in both cell types (< 0.1 nmol/min per mg of protein, the lower limit of detection of the assay; $n = 3$). Mitochondrial citrate synthase specific activity was not significantly different between CHOM[–] and CHOM⁺ cells (318 and 311 nmol/min per mg of mitochondrial protein respectively; $n = 3$). Citrate synthase activity was used as a marker for mitochondrial contamination of the cytosol; there was less than 5 % of this enzyme activity in the cytosolic fraction ($n = 3$). Thus the decrease in mSHMT activity appears to be due to a specific reduction in this enzyme and not a global defect in the mitochondria.

Changes in medium amino acid concentration

Medium serine and glycine concentrations at t_0 and the net change in their concentrations over 24 h are shown in Table 1. In CHOM⁺ cells, cultured in F12 medium without supplemental

serine or glycine (CHOm⁺, normal), there was an increase in medium glycine concentration and a reduction in medium serine concentration over 24 h. Compared with those in unsupplemented medium, CHOm⁺ cells in high-serine medium exhibited an eightfold greater decline in medium serine concentration over 24 h ($P = 0.01$) with a two-fold increase in medium glycine concentration ($P = 0.08$). CHOm⁺ cells in high-glycine medium exhibited a decrease in medium glycine concentration over 24 h ($P = 0.03$) with no effect on the change in medium serine concentration. In CHOm⁻ cells in unsupplemented medium, there was a reversal of the pattern observed in CHOm⁺ cells, with a decline in medium glycine concentration over 24 h ($P = 0.008$) and an increase in medium serine concentration ($P = 0.004$). In CHOm⁻ cells in high-serine or high-glycine medium, there were no significant effects on the changes in medium serine and glycine concentrations compared with those in normal medium.

Glycine metabolism

A comparison of serine and glycine metabolism in CHOm⁺ and CHOm⁻ cells in normal medium is shown in Table 2. In CHOm⁺ cells glycine production exceeded glycine utilization, with the resultant increase in medium glycine concentration over 24 h shown in Table 1. Compared with CHOm⁺ cells, glycine production was significantly less in CHOm⁻ cells ($P = 0.003$) with no significant difference in glycine utilization ($P = 0.61$), with the resultant decrease in medium glycine concentration over 24 h shown in Table 1. Compared with CHOm⁺ cells, there was a significant reduction in the relative glycine production from serine in CHOm⁻ cells ($P = 0.0001$) which paralleled the reduction in glycine production. Similarly, there was a reduction in the percentage of serine utilized for glycine synthesis ($P = 0.005$) and in serine utilization ($P = 0.0002$) compared with that in CHOm⁺ cells (Table 2). Thus cells without mSHMT activity (CHOm⁻) demonstrated only 11% of the glycine production found in CHOm⁺ cells. These data suggest a reduction in the capacity for relative glycine production from serine in CHOm⁻ cells to 3% of that in CHOm⁺ cells.

Effect of medium serine and glycine concentrations on glycine metabolism

In CHOm⁺ cells, there were no significant effects of high-serine or -glycine media on glycine production or utilization (Table 3).

Table 2 Comparison of serine and glycine metabolism in CHOm⁺ and CHOm⁻ cells in normal medium

Values are means $\pm$ S.E.M. for seven separate determinations.

	CHOm ⁺	CHOm ⁻
Serine utilized to synthesize glycine (%)	72.8 $\pm$ 6.7	3.2 $\pm$ 2.5
Relative glycine production from serine (nmol/24 h per mg of DNA)	3080 $\pm$ 320	80 $\pm$ 28
Glycine utilized to synthesize serine (%)	34.5 $\pm$ 5.7	19.4 $\pm$ 3.3
Relative serine production from glycine (nmol/24 h per mg of DNA)	616 $\pm$ 88	538 $\pm$ 82
Glycine production (nmol/24 h per mg of DNA)	10 200 $\pm$ 1800	1200 $\pm$ 200
Glycine utilization (nmol/24 h per mg of DNA)	5600 $\pm$ 1600	4600 $\pm$ 600
Serine production (nmol/24 h per mg of DNA)	5000 $\pm$ 800	7400 $\pm$ 1400
Serine utilization (nmol/24 h per mg of DNA)	6600 $\pm$ 1000	3800 $\pm$ 1200

Table 3 Comparison of the effects of medium serine and glycine concentrations on glycine metabolism in CHOm⁺ and CHOm⁻ cells

Cells were cultured for 24 h in the presence of [1-¹³C]glycine. The determination of utilization and production rates is described in the text. Values are means $\pm$ S.E.M. for seven experiments.

Media conditions and cell type	Glycine production (nmol/24 h per mg of DNA)	Glycine utilization (nmol/24 h per mg of DNA)
CHOm ⁺ , normal	10 200 $\pm$ 1800	5600 $\pm$ 1600
CHOm ⁺ , high serine	17 800 $\pm$ 3200	3400 $\pm$ 2400
CHOm ⁺ , high glycine	10 800 $\pm$ 2800	9600 $\pm$ 2400
CHOm ⁻ , normal	1200 $\pm$ 200	4600 $\pm$ 600
CHOm ⁻ , high serine	2400 $\pm$ 600	5600 $\pm$ 3200
CHOm ⁻ , high glycine	6600 $\pm$ 1800	18 600 $\pm$ 4800

In CHOm⁻ cells, increasing medium serine did not affect glycine production or utilization (Table 3). However, high medium glycine concentrations did result in an increase in both glycine production ($P < 0.02$) and utilization ($P < 0.01$) compared with that in CHOm⁻ cells in both normal and high-serine media.

Serine metabolism

As shown in Table 2, there was a significant reduction in serine utilization ($P = 0.0002$) with no change in serine production ($P = 0.11$) in CHOm⁻ cells compared with that in CHOm⁺ cells. Thus in CHOm⁺ cells, serine utilization exceeded production with a resultant decline in medium serine concentration over 24 h (Table 1). In CHOm⁻ cells, serine production exceeded serine utilization with a resultant increase in medium serine concentration over 24 h. There was an increase in the percentage of glycine utilized to synthesize serine in CHOm⁻ cells compared with that in CHOm⁺ cells ($P = 0.0001$), but the relative flux of glycine to serine was similar in the two cell types. The decline in serine utilization in CHOm⁻ cells was consistent with the reduction in glycine production, suggesting that a significant proportion of glycine is produced from serine.

Effect of medium serine and glycine concentrations on serine metabolism

Increasing the medium glycine concentration resulted in an increase in serine production in both CHOm⁺ ($P = < 0.02$) and CHOm⁻ ($P < 0.02$) cells, whereas increasing medium serine concentration had no effect on serine production. In contrast, high-serine medium produced an increase in serine utilization with no change in production in both CHOm⁺ and CHOm⁻ cells (Table 4; $P < 0.02$). These data suggest that serine production from glycine can be increased by increasing medium glycine concentration in both cells.

Coupling of SHMT and GCS

To investigate the possible sequential actions of SHMT and GCS, cells were cultured with either [1-¹³C]- or [2-¹³C]glycine and the enrichment in serine and glycine and glycine at 24 h was determined. If the actions of SHMT and GCS were sequential, the C-2 from [2-¹³C]glycine could be incorporated into serine in

Table 4 Comparison of the effects of medium serine and glycine concentrations on serine metabolism in CHOm⁺ and CHOm⁻ cells

Cells were cultured for 24 h in the presence of [1-¹³C]serine. The determination of utilization and production rates is described in the text. Values are means $\pm$ S.E.M. for seven experiments.

Media conditions and cell type	Serine production (nmol/24 h per mg of DNA)	Serine utilization (nmol/24 h per mg of DNA)
CHOm ⁺ , normal	5000 $\pm$ 800	6600 $\pm$ 1000
CHOm ⁺ , high serine	4400 $\pm$ 1400	23 800 $\pm$ 6400
CHOm ⁺ , high glycine	12 400 $\pm$ 3200	14 600 $\pm$ 3400
CHOm ⁻ , normal	7400 $\pm$ 1400	3800 $\pm$ 1200
CHOm ⁻ , high serine	10 800 $\pm$ 2800	17 400 $\pm$ 4400
CHOm ⁻ , high glycine	16 800 $\pm$ 3600	12 200 $\pm$ 2800

one of two ways. First, labelled glycine could be used for the synthesis of serine via SHMT (Figure 1). Secondly the C-2 of glycine could label methyleneTHF via GCS and then be used by SHMT for the synthesis of serine with resulting label at the C-3 of serine. If the pools of methyleneTHF were sufficiently enriched from the C-2 of glycine, a ¹³C-labelled methyleneTHF from the action of GCS could combine with a ¹³C-labelled glycine producing *M* + 2 serine. Using [1-¹³C]glycine, serine can be labelled only by the action of SHMT, as with GCS the C-1 of glycine is lost as CO₂. Therefore the ratio of enrichment in serine from [2-¹³C]glycine to that from [1-¹³C]glycine will be 1.0 if there is no sequential action of GCS and SHMT as both [2-¹³C]glycine and [1-¹³C]glycine will only be metabolized by SHMT. If the ratio is greater than 1.0, this would imply the sequential action of GCS and SHMT. As would be expected by the low level of GCS in CHO cells, the ratio of enrichment in serine at 24 h from [2-¹³C]glycine to that from [1-¹³C]glycine was 1.0. This was true for both CHOm⁺ and CHOm⁻ cells. This suggests that there is no significant sequential action of GCS and SHMT in CHO cells.

Intracellular serine and glycine enrichments

It is possible that analysis of media enrichment may not directly reflect intracellular processes. This might happen if there are intracellular pools of amino acids that are not in equilibrium with the medium. Thus we determined the enrichments and concentrations of intracellular free serine and glycine in CHOm⁺ and CHOm⁻ cells cultured for 24 h in the presence of an approx. 20% enrichment of [1-¹³C]serine. This isotope was chosen as it allows us to trace the production of glycine from serine, which is lower in CHOm⁻ cells than in CHOm⁺ cells.

The pattern of enrichment in intracellular free glycine from serine compared with that found in the medium is shown in Table 5. In CHOm⁺ cells cultured in the presence of [1-¹³C]serine, there was a significant increase in the intracellular enrichment of *M* + 1 glycine at 24 h similar to that in the medium. In contrast, in CHOm⁻ cells, there was very little increase in the intracellular or medium enrichment of *M* + 1 glycine (0.5 $\pm$ 0.1% in CHOm⁻ cells and 3.6 $\pm$ 0.1% in CHOm⁺ cells) at 24 h. Thus the medium enrichment (MPE) data parallel the intracellular data. There was, however, a proportionately lower intracellular concentration of serine than glycine in the CHOm⁻ cells compared with the relative concentrations of serine and glycine in the medium, suggesting either decreased transport of serine into the cell or glycine out of the cell in CHOm⁻ cells.

Table 5 Intracellular amino acid concentrations and enrichments

Cells were cultured for 24 h in medium enriched with [1-¹³C]serine. Medium and cells were then analysed for serine and glycine concentrations and enrichments as described in the text. Values are means $\pm$ S.E.M. for four separate determinations. * Significantly different from the similar medium ratio *P* = 0.0007; † significantly different from the similar medium ratio *P* < 0.05 by the paired *t* test.

	CHOm ⁺	CHOm ⁻
Medium serine concn. at <i>t</i> ₂₄ (nmol/ml)	31 $\pm$ 2.6	227 $\pm$ 3
Medium glycine concn. at <i>t</i> ₂₄ (nmol/ml)	305 $\pm$ 8	34 $\pm$ 2
Medium serine/glycine ratio	0.097 $\pm$ 0.010	6.76 $\pm$ 0.45
Intracellular serine concn. at <i>t</i> ₂₄ (nmol/mg of DNA)	19 $\pm$ 8	55 $\pm$ 18
Intracellular glycine concn. at <i>t</i> ₂₄ (nmol/mg of DNA)	104 $\pm$ 35	27 $\pm$ 11
Intracellular serine/glycine ratio	0.16 $\pm$ 0.03	2.33 $\pm$ 0.80†
Medium MPE for <i>M</i> + 1 serine at <i>t</i> ₂₄ (%)	10.3 $\pm$ 0.3	6.4 $\pm$ 0.4
Medium MPE for <i>M</i> + 1 glycine at <i>t</i> ₂₄ (%)	3.6 $\pm$ 0.1	0.5 $\pm$ 0.1
Medium serine/glycine MPE ratio	2.88 $\pm$ 0.10	11.75 $\pm$ 0.83
Intracellular MPE for <i>M</i> + 1 serine at <i>t</i> ₂₄ (%)	4.9 $\pm$ 0.4	2.8 $\pm$ 0.3
Intracellular MPE for <i>M</i> + 1 glycine at <i>t</i> ₂₄ (%)	2.4 $\pm$ 0.4	0.9 $\pm$ 0.2
Intracellular serine/glycine MPE ratio	2.14 $\pm$ 0.18*	3.01 $\pm$ 0.26*

DISCUSSION

In this study, we have presented data that suggest that, in CHO cells, there is intracellular partitioning of the interconversion of serine and glycine by the respective isoforms of SHMT. We have documented this in three ways. First, in CHOm⁻ cells, which lack mSHMT activity, compared with CHOm⁺ cells, which have mSHMT activity, we have found, using [1-¹³C]serine as the tracer, a significant reduction in the relative flux of serine to glycine (Table 2). Secondly, we have shown using the stable-isotope-dilution method with [1-¹³C]glycine that, in CHOm⁻ cells, glycine production is significantly reduced compared with that in CHOm⁺ cells (Table 2). In addition, using the stable-isotope-dilution method with [1-¹³C]serine, serine utilization is significantly lower in CHOm⁻ than in CHOm⁺ cells, paralleling the decrease in glycine production. Thirdly, we have shown that, at 24 h, the intracellular enrichment of free glycine derived from [1-¹³C]serine is likewise lower in CHOm⁻ than CHOm⁺ cells (Table 5). All of these data support the concept that, in CHO cells, mSHMT is responsible for the majority of the synthesis of glycine from serine. Physiologically, these data are consistent with the glycine auxotrophy of CHOm⁻ cells [9].

Despite the lack of mSHMT activity, CHOm⁻ cells maintain a relative flux of glycine to serine and serine production and glycine utilization rates comparable with those found in CHOm⁺ cells (Table 2). These data are consistent with the concept that the role of cSHMT is in part to convert glycine into serine. Indeed, the similar rates in CHOm⁻ and CHOm⁺ cells of the relative flux of glycine to serine and serine production suggest that nearly 100% of serine production is due to the action of SHMT or other enzymes present in both cell types. In addition, since increasing the glycine concentration in the medium increases serine production in both CHOm⁺ and CHOm⁻ cells, this supports a role for cSHMT in serine biosynthesis from glycine.

Thus, there appears to be intracellular partitioning of serine and glycine biosynthesis between cSHMT (glycine to serine) and

mSHMT (serine to glycine). Although the exact mechanism for this partitioning is not unknown, there are several possibilities. One might be regulation of the local serine and glycine concentrations of the cytosol and mitochondria or of multiple pools of serine and glycine within the cell. If serine is the primary source for glycine biosynthesis, there is a discrepancy between the lower glycine production in CHO⁻ compared with that in CHO⁺ cells (1200 compared with 10200 nmol/24 h per mg of DNA) and the less dramatic decrease in serine utilization (3800 compared with 6600 nmol/24 h per mg of DNA). One possible explanation for our inability to account fully for the change in glycine production between CHO⁺ and CHO⁻ cells by changes in serine utilization is that exogenously supplied serine (i.e. medium serine) may not completely equilibrate with the intracellular serine pool utilized for glycine biosynthesis. This is further supported by the lack of equilibration of exogenously supplied [1-¹³C]serine with the intracellular pools in CHO⁻ cells (Table 5). This may be due to differential transport of serine and glycine into the mitochondria, but this hypothesis awaits further investigation. Thus intracellular partitioning of the serine pool (perhaps in the mitochondria) might be one mechanism by which biosynthetic partitioning is achieved. Although the exact measurement of serine utilization and production might be affected by this phenomenon, it would not affect the major conclusion of this study that there is partitioning of glycine biosynthesis to the mitochondria.

An alternative mechanism for the regulation of intracellular partitioning of serine and glycine biosynthesis might be differential distribution of the cofactors necessary for the reactions involving SHMT, such as THF, methyleneTHF and PLP, among various intracellular pools, resulting in the partitioning of the reactions of SHMT. Pasternack et al. [7] have demonstrated that mitochondrial formate (synthesized from serine) is important for the provision of cytosolic one-carbon units for purine synthesis and have argued that there are separate pools of glycine within the mitochondria itself. Intracellular compartmentalization via regulation of the distribution of folate coenzymes by, for example, their polyglutamation status has been suggested [18,19]. In addition, transport of the various factors involved in the SHMT reaction may not be uniform [20]. These hypotheses also await further investigation.

In yeast, mutations in both cSHMT and mSHMT along with a third gene (GlyA) are required to yield glycine auxotrophy [21]. Mutants lacking only mSHMT did not demonstrate glycine auxotrophy [21]. In that study, only glycine auxotrophy was determined and no measurements of glycine synthesis or utilization were performed. In addition, there was no indication of

whether GCS activity was present in the yeast studied. The presence of GCS may impact on glycine and serine inter-conversion through the presence of cofactors such as methyleneTHF or by altering intramitochondrial glycine concentrations. Since the CHO⁻ cells were created by chemical mutagenesis, it is possible that CHO⁻ cells lack more than simply mSHMT [9]. Even if that is the case, our data would point to an as yet undetermined factor, absent from CHO⁻ cells, involved in the intracellular compartmentalization of serine and glycine interconversion that may also be present in yeast.

In summary, we have demonstrated that, in CHO cells, mSHMT is responsible for approx. 87% of glycine synthesis and virtually all of the synthesis of glycine from serine. In contrast, cSHMT appears to be primarily responsible for the biosynthesis of serine from glycine. The mechanism by which CHO cells regulate this intracellular partitioning of enzyme function remains to be determined.

This work was supported in part by a grant from the Children's Hospital Research Foundation, NIH grants DK34914 and HD31648.

REFERENCES

- Schirch, V. (1982) *Adv. Enzymol. Relat. Areas Mol. Biol.* **53**, 82–112
- Garrow, T. A., Brenner, A. A., Whitehead, V. M., Chen, X. N., Duncan, R. G., Korenberg, J. R. and Shane, B. (1993) *J. Biol. Chem.* **268**, 11910–11916
- Motokawa, Y. and Kikuchi, G. (1971) *Arch. Biochem. Biophys.* **146**, 461–464
- Lowry, M., Hall, D. E. and Brosnan, J. T. (1986) *Am. J. Physiol.* **250**, F649–F658
- Brosnan, J. T. (1987) *Can. J. Physiol. Pharmacol.* **65**, 2355–2362
- Pfendner, W. and Pizer, L. I. (1980) *Arch. Biochem. Biophys.* **200**, 503–512
- Pasternack, L., Laude, D. J. and Appling, D. (1994) *Biochemistry* **33**, 74–82
- Thureen, P. J., Fennessey, P. V., Battaglia, F. C. and Narkewicz, M. R. (1992) *Pediatr. Res.* **31**, 189A
- Kao, F., Chasin, L. and Puck, T. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1284–1291
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Loy, G. L., Quick, A. N. J., Teng, C. C., Hay, W. W. J. and Fennessey, P. V. (1990) *Anal. Biochem.* **185**, 1–9
- Townsend, S. F., Thureen, P. J., Hay, W. W. J. and Narkewicz, M. R. (1993) *In Vitro Cell. Dev. Biol.* **29A**, 592–596
- Jois, M., Hall, B. and Brosnan, J. T. (1990) *J. Biol. Chem.* **265**, 1246–1248
- Jois, M., Edwart, H. S. and Brosnan, J. T. (1992) *Biochem. J.* **283**, 435–439
- Geller, A. M. & Kotb, M. Y. (1989) *Anal. Biochem.* **180**, 120–125
- Shepherd, D. and Garland, P. B. (1969) *Biochem. J.* **114**, 597–610
- Hartree, E. F. (1972) *Anal. Biochem.* **48**, 122–127
- Appling, D. (1991) *FASEB J.* **5**, 2645–2651
- Garcia-Martinez, L. and Appling, D. (1993) *Biochemistry* **32**, 4671–4676
- Cybulski, R. and Fisher, R. (1976) *Biochemistry* **15**, 3183–3187
- McNeil, J., McIntosh, E., Taylor, B., Zhang, F., Tang, S. and Bogner, A. (1994) *J. Biol. Chem.* **269**, 9155–9165