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Physiological changes in glutathione metabolism in foetal and newborn rat liver

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Glutathione metabolism was studied in isolated hepatocytes from foetal, newborn and adult rats. The GSH/GSSG ratio decreased 15–20-fold through the foetal–neonatal–adult transition. This was mainly due to an increase in GSSG. All enzyme activities involved in the glutathione redox cycle tend to increase during that transition, but the relative increases in glutathione peroxidase and glutathione *S*-transferase were 3–5 times those of glutathione reductase or glucose-6-phosphate dehydrogenase. GSH synthesis from methionine as a sulphur source was 6 times lower in foetal than in adult hepatocytes. However, when *N*-acetylcysteine was used as a sulphur donor to by-pass the cystathionine pathway, the rates of GSH synthesis were similar in foetal and adult cells. This is due to the fact that cystathionase activity in foetal cells is very low. This low activity is reflected in the blood amino acid pattern, where the concentration of cysteine rises from 8 to 52 μM from foetuses to adult rats. This supports the idea that cysteine may be an essential amino acid for the premature animal.

INTRODUCTION

During the foetal–neonatal transition, dramatic changes in the O_2 partial pressure in tissues occur. These are due both to the cardiovascular changes and to the high O_2 pressure in the atmospheric air. These changes in O_2 availability may result in oxidative stress to cells (Sies, 1986). GSH, the major intracellular non-protein thiol compound in mammalian cells, protects them against oxidative stress (Meister & Anderson, 1983; Viña *et al.*, 1986).

In vertebrates, the cystathionine pathway is essential to provide an adequate intracellular supply of cysteine (Greenberg, 1975). A key enzyme in this trans-sulphuration pathway is cystathionase, which cleaves cystathionine to yield cysteine (Finkelstein & Mudd, 1967). Previous work by Sturman *et al.* (1970), Gaull *et al.* (1972) and Jackson (1989) showed that cystathionase activity is absent from brain and liver of foetuses and premature infants, suggesting that cysteine might be an essential amino acid for premature infants and, consequently, that the latter may require dietary cysteine because of a limited capacity for methionine trans-sulphuration. However, Zlotkin & Anderson (1982) questioned this view by measuring the development of cystathionase activity in liver and other tissues, and suggested that it might be sufficient to ensure an adequate supply of cysteine from methionine. Intracellular cysteine concentration is very low (Tateishi *et al.*, 1974) and is rate-limiting for hepatic GSH synthesis (Tateishi *et al.*, 1974; Viña *et al.*, 1978). Thus, physiological changes in glutathione metabolism might occur during the perinatal period. We studied these changes in rat liver by measuring not only cystathionase activity, but also its relationship to GSH and cysteine synthesis, the activity of the enzymes involved in the glutathione redox cycle, the GSH/GSSG ratio and the amino acid profile in foetal, newborn and adult rats.

MATERIALS AND METHODS

For experiments with foetuses, albino Wistar rats on day 22 of gestation (200–300 g body wt.), fed on a stock laboratory diet, were killed between 09:00 and 10:00 h. Newborn rats from the same strain were killed 24 h after birth. Isolated hepatocytes from foetal and newborn rats were obtained and incubated [$(10–12) \times 10^6$ cells/ml] as described by Roncero *et al.* (1986). Isolated hepatocytes from adult (3 month old) rats were obtained by the method of Berry & Friend (1969). Enzymic activities were measured in livers homogenized in 0.1 M-phosphate buffer (pH 7.2) at 4 °C, and metabolites were determined in freeze-clamped livers or in incubated hepatocytes treated as described by Viña *et al.* (1978). For amino acid analysis, whole arterial blood was collected and treated as described by Viña *et al.* (1981).

GSH and GSSG were determined as described by Akerboom & Sies (1981). Cysteine was measured as described by Gaitonde (1967) and cystathionase activity as described by Sturman *et al.* (1970). Glutathione peroxidase activity was measured as described by Flohé & Gunzler (1986), glutathione reductase as described by Akerboom & Sies (1981), glucose-6-phosphate dehydrogenase as described by Bergmeyer (1974), and glutathione *S*-transferase as described by Habig *et al.* (1974).

For calculations, we used a wet-weight/dry-weight ratio of 3.7 ± 0.5 ($n = 5$) obtained for foetal hepatocytes as described by Krebs *et al.* (1974) for adult hepatocytes. This value was practically identical for neonatal hepatocytes. All results are means ± S.D. for the numbers of observations in parentheses.

RESULTS AND DISCUSSION

Glutathione status and oxidative stress in foetal, newborn and adult rat liver

Table 1 shows that GSSG levels change from 0.001 μmol/g in the foetus to 0.012 μmol/g in the newborn. They further increase to 0.033 μmol/g in adult rats. In contrast with the dramatic changes found in GSSG, GSH levels did not change significantly in the foetal–neonatal transition (3.1 versus 3.4 μmol/g; see Table 1). Adult-rat hepatocytes had a higher GSH level (5.7 μmol/g), a value similar to previous findings (Viña *et al.*, 1978; Estrela *et al.*, 1988). The changes in GSH and GSSG levels

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Table 1. Glutathione status, glutathione-related enzymes and cystathionase activities in foetal, neonatal and adult rat livers

Livers from foetuses, newborn and adult rats were used. Abbreviations used: GR, glutathione reductase; G6PDH, glucose-6-phosphate dehydrogenase; GPx, Se-dependent glutathione peroxidase; GSH-S-t, glutathione *S*-transferase. Statistical significances are : $*P < 0.05$ versus foetal group; $\dagger P < 0.05$ versus neonatal group.

	Foetus	Newborn	Adult
GSH (μmol/g)	3.1±0.4 (6)	3.4±7 (10)	5.7±0.9 (9)*†
GSSG (μmol/g)	0.001±0.0002 (5)	0.012±0.005 (11)*	0.03±0.01 (4)*†
GSH/GSSG	3100	283	172
GR (units/g)	3.3±0.9 (7)	6.2±1.3 (4)*	9.1±3.0 (10)*†
G6PDH (units/g)	1.2±0.2 (10)	1.8±0.5 (7)*	2.2±1.0 (10)*
GPx (units/g)	3.7±1.6 (8)	9.1±2.4 (7)*	48.2±12.1 (9)*†
GSH-S-t (units/g)	13.3±1.2 (5)	14.7±1.9 (5)	90.5±30.7 (9)*†
Cystathionase (units/g)	0.9±0.7 (4)	1.8±0.3 (4)	3.2±0.1 (4)*†

resulted in marked changes in the GHS/GSSG ratio. Indeed, this ratio was much lower in neonatal than in foetal hepatocytes, and also in adult than in neonatal cells. In fact, the GHS/GSSG ratio in foetal hepatocytes was almost 20 times that in adult cells.

In an attempt to determine the possible reasons for the changes in glutathione status, we measured the activities of glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione peroxidase and glutathione *S*-transferase, i.e. the main enzymes involved in the glutathione redox cycle. We found that all the enzyme activities measured tended to increase from foetal to adult cells (Table 1). However, the activities of glutathione peroxidase and glutathione *S*-transferase increased much more than those of glutathione reductase and glucose-6-phosphate dehydrogenase (Table 1). Thus the activities of the enzymes using GSH, i.e. glutathione peroxidase and glutathione *S*-transferase, were about 10 times higher in adult than in foetal cells, whereas glutathione reductase and glucose 6-phosphate dehydrogenase, i.e. enzyme activities that tend to regenerate GSH, were only about twice as much in adult as in foetal cells. These enzymic changes may explain, at least in part, the marked shift towards oxidation of the GSH/GSSG pair in the foetal–neonatal–adult transition. Moreover, the increases in GSSG and in glutathione peroxidase activity may reflect the cellular adaptation to the increase in O_2 partial pressure that occurs during the foetal–neonatal transition.

Characteristics of glutathione synthesis in foetal, neonatal and adult hepatocytes

Hepatocytes are a good model to study GSH synthesis (Viña *et al.*, 1978). In previous studies we found that GSH synthesis from methionine is very active in hepatocytes (Viña *et al.*, 1978) and proceeds via the cystathionase pathway (Reed & Orrenius, 1977). We measured cystathionase activity in foetal livers, and found that it is very low, but rises during the foetal–neonatal transition (Table 1).

To determine if changes in cystathionase activity are reflected by changes in the characteristics of GSH synthesis, we compared the rate of GSH synthesis, using either methionine or *N*-acetylcysteine as cysteine sources. We used *N*-acetylcysteine instead of free cysteine because the latter is rapidly oxidized in the cell suspension medium, giving rise to free radicals (Sáez *et al.*, 1982), and is toxic (Viña *et al.*, 1983). *N*-Acetylcysteine is rapidly deacylated by liver cells, yielding free cysteine, but trans-sulphuration via the cystathionase pathway is required to obtain cysteine from methionine (Greenberg, 1975). Table 2 shows that the rate of GSH synthesis from *N*-acetylcysteine was similar in hepatocytes from foetal, neonatal or adult rats. However, the rate of GSH synthesis from methionine was significantly lower in foetal than in neonatal, and in neonatal than in adult, rat hepatocytes (Table 2). The lower rate of GSH synthesis in hepatocytes from foetal rats might be due to a lower rate of cysteine synthesis from methionine. To prove this, we compared the rate of cysteine synthesis from methionine in foetal, neonatal and adult hepatocytes, and found it to be 15±12 in foetal hepatocytes, 137±89 in hepatocytes from newborns and 248±138 (nmol/min per g wet wt.; all $n = 4$) in adult rats. Thus the trans-sulphuration pathway involving methionine indeed modulates the availability of free cysteine for GSH synthesis. Cystathionase activity, a key enzyme in the pathway, which also increases from foetal to adult cells (Table 1), may be acting as a rate-limiting step.

Table 2. GSH synthesis in foetal, neonatal and adult isolated hepatocytes

Hepatocytes from foetuses, newborn and adult rats were used in these experiments. Cells were preincubated with 0.5 mM-diethylmaleate as previously described (Sáez *et al.*, 1985). Abbreviations used: AA_1, 5 mM-Gln, 2 mM-Gly, 1 mM-Ser, 0.2 mM-Met; AA_2, 5 mM-Gln, 2 mM-Gly, 1 mM-Ser, 0.2 mM-*N*-acetylcysteine (NAC). All results are expressed as means±S.D., with the numbers of observations in parentheses. Statistical significances: $*P < 0.05$, versus foetal group; $\dagger P < 0.005$ versus neonatal group.

	GSH (nmol/min per g wet wt.)		
Additions	Foetus	Newborn	Adult
None	1.7±0.8 (4)	5.5±2.3 (4)*	15.1±0.7 (4)*†
AA_1 (Met)	20.0±4.4 (4)	61.7±7.7 (3)*	119±26 (4)*†
AA_2 (NAC)	106.5±7.4 (4)	87.7±9.1 (5)*	112.5±18.2 (6)†

We also measured the rate of cysteine and GSH synthesis from other amino acids involved in the trans-sulphuration pathway, such as *S*-adenosylmethionine or homocysteine. These are good precursors for GSH synthesis in hepatocytes from adult rats (Viña *et al.*, 1978; Stramentinoli *et al.*, 1979). However, we found that GSH synthesis in foetal hepatocytes is negligible (results not shown), in agreement with the idea that low cystathionase activity limits GSH synthesis in foetal hepatocytes. Thus we conclude that cystathionase activity can limit cysteine synthesis, and therefore GSH synthesis, in foetal cells, and that this limitation disappears progressively after birth.

Concentrations of amino acids in blood from foetuses, newborn and adult rats

Blood amino acid profiles reflect the activity of tissues and have been used as an index of metabolic and nutritional status

Table 3. Amino acid concentrations in whole blood from foetal, newborn and adult rats

Whole blood from foetuses, newborn and adult rats was used in these experiments. All results are expressed as means ± S.D. with the numbers of observations in parentheses. Statistical significances: *$P < 0.05$ versus foetal group; †$P < 0.05$ versus neonatal group.

Amino acid	Amino acid concn. (μM)		
	Foetus	Newborn	Adult
Gln	1978 ± 782 (14)	665 ± 184 (3)*	448 ± 147 (3)*
Glu	302 ± 97 (15)	165 ± 17 (3)*	102 ± 8 (3)*†
Gly	373 ± 96 (15)	564 ± 44 (3)*	113 ± 21 (3)*†
Ser	204 ± 72 (15)	181 ± 7 (3)	114 ± 10 (3)*†
Met	26 ± 29 (11)	34 ± 2 (3)	57 ± 29 (3)
Cyst(e)ine	8 ± 5 (14)	17 ± 3 (3)*	53 ± 14 (3)*†
Met/Cyst(e)ine	3.2	2.0	1.1
Met + Ser/ Cyst(e)ine	28.7	12.6	3.2

(Oberholzer & Briddon, 1990). Thus, we measured the possible changes in concentration of amino acids in blood of foetuses, newborn and adult rats.

Table 3 shows the blood concentrations of the amino acids involved in the cystathionase pathway. The ratio (Met + Ser)/ Cyst(e)ine may be taken as another indication of the activity of the pathway. If the low cystathionase activity in foetal and newborn liver is significantly reflected in the plasma levels of amino acids, we would expect this ratio to be lower in adult than in foetal or neonatal rats. Table 3 shows that the value for this ratio in foetal blood is 28.7, in neonatal blood is 12.6 and in adult blood is 3.2. The marked changes in this ratio during the first 24 h after birth are parallel to the changes in cystathionase activity. This shows that changes in the activity of this enzyme are relevant to changes in blood amino acid profiles and support the idea that cysteine may be essential in immature or premature infants (Sturman *et al.*, 1970), even though some cystathionase activity may be found in extrahepatic tissues. Since the metabolic situation of the foetus at day 22 of gestation is similar to that of premature infants (Cuezva *et al.*, 1980), these results may be important, as they suggest that it may be necessary to include cysteine sources other than methionine in solutions for parenteral nutrition for premature infants.

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