Pathways of Gluconeogenesis from L-Serine in the Neonatal Rat

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Gluconeogenesis from amino acid precursors was increased in the perfused liver of neonatal rats compared with that of adults. Quinolinate (5 mM) was less inhibitory to glucose formation from serine and hydroxyproline in the neonatal rat than in the adult, suggesting that the main route for glucose synthesis from these precursors in neonatal liver does not involve pyruvate as an intermediate.

Serine is an effective glucogenic precursor in adult rat liver (see review by Snell & Walker, 1973). The route by which serine is converted into glucose is nevertheless the matter of some controversy. The alternative pathways, (A) via pyruvate and initiated by L-serine dehydratase (EC 4.2.1.13) and (B) via hydroxypyruvate and initiated by L-serine-pyruvate aminotransferase (EC 2.6.1.51), are outlined in Scheme 1. Much evidence suggests that the activity of L-serine dehydratase is increased *in vivo* under conditions associated with enhanced gluconeogenesis (see Snell & Walker, 1973). More recently a similar relationship between L-serine-pyruvate aminotransferase and increased gluconeogenesis has been shown (Rowell *et al.*, 1969, 1972a, 1973). Further evidence for the involvement of this aminotransferase in gluconeogenesis has come from work with quinolinic acid, a potent inhibitor of phosphoenolpyruvate carboxykinase (EC 4.1.1.32), which can inhibit glucose formation from alanine by blocking the conversion of pyruvate into glucose. However, quinolinate was much less inhibitory to glucose formation from serine (Lardy *et al.*, 1969; Metz *et al.*, 1972), suggesting a potential glucogenic pathway for this precursor not involving pyruvate as an intermediate (see Scheme 1).

During neonatal development of the rat, hepatic gluconeogenesis from a number of substrates is increased over the adult capacity and this is accompanied by appropriate changes in the activity of the enzymes involved (Snell & Walker, 1973; Walker & Snell, 1973). The activity of L-serine-pyruvate aminotransferase is elevated throughout the suckling period and, more significantly, at a time when L-serine dehydratase activity is, for the most part, at a lower value than in the adult (Snell & Walker, 1973; Rowell *et al.*, 1973). In the present work the hepatic capacity for gluconeogenesis from serine (and some other amino acid precursors) has been measured in the perfused liver of neonatal and adult rats, and the effect of quinolinate on this process *in vitro* investigated.

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Methods

The rats were an albino Wistar strain maintained as previously described (Snell & Walker, 1972). Adults were males weighing 150–200 g. All rats were starved overnight for 16–20 h, and the neonatal animals were kept in an humidicrib at an ambient temperature of 35°C during this period. The perfusion technique used was that of Hems *et al.* (1966), except that the duodenum was cannulated immediately after the start of perfusion to ensure the free flow of bile. Perfusion was carried out with 50 ml of bicarbonate-saline (Krebs & Henseleit, 1932), containing 2.5% bovine serum albumin (fraction V), dialysed for 2 days against four changes of bicarbonate-saline, and erythrocytes from aged human blood (to give a final haematocrit of 20%). Perfusion was achieved with a hydrostatic-pressure head of 12–15 cm of perfusion medium and this gave flow rates through the liver ranging from 3 to 5 ml/min per g of tissue for all the animals.

Livers were perfused for 38 min with perfusion medium before the addition of substrates as 0.2 M neutral solutions to give an initial circulating concentration of about 10 mM. Quinolinic acid was freshly dissolved and neutralized, and added to the medium 30 min from the beginning of the perfusion to give an initial circulating concentration of about 5 mM. Sampling was begun at 40 min and continued at 15 min intervals for a further 60 min. Glucose was determined in HClO₄ extracts of perfusion medium by a glucose oxidase method (Krebs *et al.*, 1963). The rate of formation of glucose by the perfused liver was followed by measuring changes in the concentration of glucose in the perfusion medium with time.

Results and discussion

The rates of glucose formation from various amino acid precursors in the perfused liver of adult and 10-day-old neonatal rats are shown in Table 1. For each precursor the rate of gluconeogenesis was

Scheme 1. Alternative pathways for gluconeogenesis from L-serine (A) via L-serine dehydratase and (B) via L-serine-pyruvate aminotransferase

The pathways of potential amino acid precursors of L-serine are also shown. The dashed line encompasses reactions taking place predominantly intramitochondrially. For the sake of clarity possible mechanisms for the transfer of oxaloacetate out of the mitochondrion are not indicated.

Table 1. Inhibition by quinolate of gluconeogenesis from alanine in perfused neonatal and adult liver

The final perfusate of amino acid precursors was 10mM, and of quinolate, 5mM. Quinolate, when present, was added 30min after the beginning of perfusion and substrates were added at 38min. Results are shown $\pm$ S.E.M. with the numbers of observations in parentheses.

Precursor	Rate of glucose formation (μ mol/min per g of liver)		Percentage inhibition by quinolate	
	Adult	10-day-old rat	Adult	10-day-old rat
None	0.079 ± 0.010 (6)	0.088 ± 0.027 (6)	82 ± 9 (3)	68 ± 9 (3)
L-Alanine	0.327 ± 0.020 (10)	0.423 ± 0.035 (17)*	86 ± 8 (3)	74 ± 9 (3)
L-Serine	0.206 ± 0.023 (5)	0.539 ± 0.044 (5)†	60 ± 6 (4)	38 ± 6 (4)*
L-Hydroxyproline	0.181 ± 0.020 (8)	0.287 ± 0.014 (6)†	62 ± 5 (4)	35 ± 7 (4)*

* Statistically significant difference by Student's *t* test, $P < 0.05$.
† Statistically significant difference by Student's *t* test, $P < 0.01$.
‡ Statistically significant difference by Student's *t* test, $P < 0.001$.

significantly greater in neonatal than in adult liver. The enhanced gluconeogenic capacity of the neonatal rat observed in the present study agrees well with previous findings in the whole animal *in vivo* (Walker & Snell, 1973) and with liver slices *in vitro* (Ballard & Oliver, 1963; Yeung & Oliver, 1967; Vernon *et al.*, 1968). A greatly increased capacity for the hepatic synthesis of glucose from serine in the neonatal rat is noteworthy. This coincides with an elevated activity of L-serine-pyruvate aminotransferase but a diminished activity of L-serine dehydratase compared with adult values (Snell & Walker, 1973; Rowsell *et al.*, 1973), suggesting that the potential for glucose synthesis via hydroxypyruvate (Scheme 1, route B) may be preferentially enhanced in the neonatal rat.

Glucose formation from alanine was inhibited by 75–85% in the presence of 5mM-quinolinate in both adult and neonatal perfused liver (Table 1). However, quinolinate was significantly less inhibitory for serine gluconeogenesis compared with alanine gluconeogenesis in both the adult ($P < 0.05$) and the neonatal animal ($P < 0.02$). This again suggests that for serine the potential exists for a pathway of carbon to glucose that does not involve pyruvate as an intermediate. Further, the inhibition of gluconeogenesis from serine by quinolinate in the neonatal rat was significantly less ($P < 0.05$) than that observed in the adult (Table 1). This is taken as further evidence that the capacity for gluconeogenesis from serine via the aminotransferase pathway is increased in the neonatal animal.

In the case of the adult rat, other workers have also shown a less inhibitory effect of quinolinate on glucose formation from serine compared with alanine and have suggested that the gluconeogenic pathway from serine may not involve the intermediary formation of pyruvate (Lardy *et al.*, 1969; Metz *et al.*, 1972). Chan & Freedland (1971), comparing glucose formation from lactate and serine, found complete inhibition from both precursors in the presence of quinolinate, but in their experiments animals were starved for 4 days before perfusion and this resulted in a 4-fold elevation of L-serine dehydratase activity. Prolonged starvation causes a much greater increase of L-serine dehydratase activity than of L-serine-pyruvate aminotransferase activity (K. Snell, unpublished work) and so the enzymic potential for gluconeogenesis via pyruvate is greatly increased relative to that via hydroxypyruvate, and this probably accounts for the greater sensitivity to inhibition by quinolinate in these conditions.

In considering the physiological significance of the alternative routes of glucose synthesis from serine (Scheme 1), it may be relevant that in the rat the pathway via pyruvate is predominantly cytosolic in subcellular location whereas the aminotransferase

pathway is located intramitochondrially (Rowsell *et al.*, 1972b). This raises the possibility that the aminotransferase pathway may be involved in gluconeogenesis from a mitochondrial serine pool, and possible intramitochondrial sources of serine were considered. One of these may be hydroxyproline, which is oxidized in the mitochondria of liver to give pyruvate and glyoxylate (Adams, 1970). Pyruvate, of course, is a potent glucogenic precursor, and glyoxylate, after transamination to glycine, is potentially glucogenic via serine (Scheme 1). Gluconeogenesis from hydroxyproline was significantly increased in perfused neonatal rat liver compared with the adult (Table 1), which is in agreement with the relevant enzymic data (Snell & Walker, 1973). Experiments with quinolinate (Table 1) show that gluconeogenesis from hydroxyproline was not inhibited to the same extent as that from alanine, either in the adult ($P < 0.05$) or in the neonatal rat ($P < 0.02$). Moreover, inhibition by quinolinate was significantly less in the neonatal rat than in the adult. These findings are very similar to those obtained with serine as the glucogenic precursor, and suggest that hydroxyproline has a similar potential for gluconeogenesis not involving pyruvate as an intermediate, and that this potential is increased in the neonatal rat.

The extent to which the enhanced capacity for gluconeogenesis via hydroxypyruvate is realized in the neonatal rat and the actual precursors involved *in vivo* are not known. Arteriovenous differences have shown that serine is taken up by the adult liver and released into the circulation, as an 'end product' of amino acid metabolism, by the kidney (Aikawa *et al.*, 1973). The plasma serine concentration in the 10-day-old rat is higher than in the adult (Snell & Walker, 1973) and this may reflect an increased flow of serine to the liver at this time. Glycine, a direct precursor of serine in the liver, appears to be released into the circulation from extrahepatic tissues other than the kidney (Aikawa *et al.*, 1973), but its plasma concentration is considerably lower than the adult value throughout the neonatal period (K. Snell, unpublished work). Most of the hydroxyproline (and also glycine) in the body is found in collagen, which normally has a low turnover. Nevertheless, once released by collagen breakdown, hydroxyproline cannot be reincorporated and is either excreted or catabolized in the liver. There is a greatly increased urinary excretion of free hydroxyproline in young mammals (Neuberger & Richards, 1964) and it has been suggested that young adults catabolize collagen as a metabolic reserve for gluconeogenesis during starvation to a much greater extent than do older adults (Ball *et al.*, 1972). The major contribution to this collagen fraction is made by the skin, which in young rats is about 22% of the total body weight. Although body protein losses as

a whole do not contribute greatly to overall weight loss after 24h of starvation until after about 20 days *post partum*, skin protein losses show a peak contribution at 10 days *post partum* (Hahn & Koldovský, 1966). Increased collagen turnover in the neonatal animal may contribute hydroxyproline, and also glycine, as hepatic gluconeogenic precursors at this time.

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