The Effects of Amino Acids on Albumin Synthesis by the Isolated Perfused Rat Liver

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Albumin synthesis was measured in the isolated perfused rat liver by using the livers of both well-fed and starved rats. Starvation markedly decreased albumin synthesis. The livers from starved rats were unable to increase synthesis rates after the addition to the perfusates of single amino acids or the addition of both glucagon and tryptophan. Arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine, added together to ten times their normal peripheral blood concentrations, restored synthesis rates to normal. The plasma aminogram (i.e. the relative concentrations, of amino acids) was altered by depriving rats of protein for 48 h. The use of blood from the deprived rats as perfusate, instead of normal blood, decreased albumin synthesis rates significantly by livers obtained from well-fed rats. The addition of single amino acids, including the non-metabolizable amino acid, α -amino-isobutyric acid, to the above mixture increased albumin synthesis rates to normal values. It is concluded that amino acids play an important role in the control of albumin synthesis and that more than one mechanism is probably involved.

Whereas recent studies have confirmed that protein restriction decreases albumin synthesis both in man (Coles *et al.*, 1970; L. Kelman, S. J. Saunders, L. Frith, S. Wicht & A. Corrigan, unpublished work) and in the rat (Kirsch *et al.*, 1968), the mechanisms involved remain to be established. The changes in the plasma aminogram (i.e. the relative concentrations of amino acids) in protein-calorie malnutrition (Holt *et al.*, 1963; Saunders *et al.*, 1967) suggested that certain amino acids may influence protein synthesis selectively. Indeed, Pronczuk *et al.* (1968) have shown that tryptophan deficiency results in diminished incorporation of amino acid into rat liver protein, with associated ribosomal disaggregation. Similar results have been obtained in mice (Sidransky *et al.*, 1968). Whereas tryptophan appears to have this specific function *in vivo*, the work of Hanking & Roberts (1964, 1965) suggests that individual amino acids can stimulate protein synthesis and increase ribosomal activity *in vitro*, irrespective of the size of the amino acid pool. In contrast, Baliga *et al.* (1968) have shown that the absence of any amino acid (with the exception of isoleucine) results in ribosomal disaggregation.

Therefore, we have studied the influence of amino acids on albumin synthesis in the isolated perfused rat liver.

While this work was in progress, Rothschild *et al.* (1969) reported that tryptophan and, to a lesser

extent, isoleucine were able to stimulate albumin synthesis in the liver of starved rabbits. Important differences between the present study and that of Rothschild *et al.* (1969) suggest that more than one mechanism is operative.

Experimental

Perfusion apparatus and technique

Livers were taken from male Wistar rats (320–350 g) housed under controlled conditions of temperature, humidity and lighting.

Two groups of rats were used: (1) rats fed on a 20% protein diet *ad lib.* and (2) rats starved for 24 h. Both groups had free access to water.

The perfusate consisted of 60 ml of blood, 2 ml of 4.2% NaHCO_3 , 40 mg of heparin (heparin injection British Pharmacopoeia; Boots Pure Drug Co. Ltd., Nottingham, U.K.) and 25 ml of Plasmalyte B (Baxter-Saphar Laboratories Ltd., Johannesburg, South Africa) containing, in mmol/l: Na^+ 130, Cl^- 109, K^+ 4, Mg^{2+} 1.5, NaHCO_3 28. The final packed cell volume of the perfusate was 24–28%. Blood was obtained by cardiac puncture under diethyl ether anaesthesia from two groups of rats: (1) rats fed *ad lib.* (perfusate 1) and (2) rats on a zero-protein diet for 48 h (perfusate 2). This feed consisted of maize starch with mineral and vitamin supplements

(2.2% vitamin diet fortification mixture, Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.).

Perfusions were performed according to the method of Miller *et al.* (1951) as modified by Fisher & Kerly (1964). Under diethyl ether anaesthesia the bile duct and portal vein were cannulated, then the liver was flushed with plasmalyte B and transferred to the perfusion cabinet. Outflow from the hepatic veins drained directly into a reservoir. O₂ + CO₂ (95:5) was passed into a glass 'thin-film' oxygenator at a flow rate of 750 ml/min. The cabinet was humidified, the temperature controlled at 38°C and the portal pressure kept at 18 cm of water. pH was kept at 7.35–7.42 and *p*O₂ and *p*CO₂ at 20.0–23.3 kN/m² (150–175 mmHg) and 4.7–5.3 kN/m² (35–40 mmHg) respectively. Perfusate urea and glucose concentrations were measured on a Technicon AutoAnalyser with appropriate standards.

Albumin synthesis rate

Sodium [¹⁴C] carbonate (250 μCi) (N.V. Phillips-Duphar, Amsterdam, The Netherlands; specific radioactivity 55 mCi/mm) was added to the reservoir after 30 min of perfusion. The perfusion was continued for a further 120 min to allow for complete release of labelled albumin into the perfusate.

Albumin synthesis rate was measured by the method of McFarlane (1963) and Reeve *et al.* (1963). Urea synthesis rate was determined from the difference between the perfusate urea concentrations before and after perfusion. The radioactivity incorporated into the guanidine carbon of arginine in albumin and into the carbon of urea was obtained from the end-perfusion sample. Urea specific radioactivity was measured on deproteinized samples, incubated with urease to produce ¹⁴CO₂ which was then released by the addition of acid. The volume of gas produced was measured manometrically on a high-vacuum gas train, collected in phenethylamine-methanol (1:1, v/v) and counted for radioactivity in a toluene liquid scintillator [2,5-diphenyloxazole and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] in a Beckman automatic scintillation spectrometer. Samples were counted to an error of less than 3%. The specific radioactivity of the guanidine carbon of arginine in albumin was obtained by extraction of albumin from the plasma by the acid-ethanol method (Korner & Debro, 1956). After acid hydrolysis at 110°C, the sample was passed through a bicarbonate-resin column to separate arginine, which was then incubated with arginase to produce urea. The sample was then treated as for urea. The rate of albumin synthesis was calculated as follows:

The factor 100/5.95 is obtained from the amount of arginine present in albumin, i.e. 100 g of albumin contains 5.95 g of arginine.

Other determinations

Plasma albumin was measured by the method of Fernandez *et al.* (1966). Only L isomers of amino acids (British Drug Houses, Poole, Dorset, U.K.) were added to the perfusate, each to a concentration of 10 μmol/ml, except in some experiments in which tryptophan and the branched-chain amino acids were added to a concentration of 0.05 μmol/ml. Arginine, asparagine, isoleucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine were added together to a concentration of ten times their normal peripheral blood concentration. Glucagon (United States Pharmacopoeia; Eli Lilly and Co., Indianapolis, Ind., U.S.A.), when used, was added initially at doses of 1 mg followed by 0.5 mg/h. Amino acids were measured by the method of Hamilton (1963) with a Technicon amino acid AutoAnalyser. Tryptophan was measured by the method of Hess & Udenfriend (1959).

Results

The amino acid concentrations of the perfusates used are shown in Table 1. Protein restriction for 48 h decreased the concentrations of isoleucine, leucine, valine, tyrosine, phenylalanine, histidine, ornithine, methionine and glycine.

Table 2 shows the changes in albumin and urea synthesis rates, expressed as mg/h per 300 g of rat, for liver from well-fed rats. When perfusate (1) was used, an albumin synthesis rate of 1.75 ± 0.18 was obtained. The use of perfusate (2) decreased the synthesis rate to 1.11 ± 0.31 (0.01 < *P* < 0.02). Perfusate (2) with each amino acid added singly to a concentration of 10 μmol/ml significantly increased albumin synthesis rates (tryptophan, 0.01 < *P* < 0.02; isoleucine, 0.001 < *P* < 0.01; lysine, 0.001 < *P* < 0.01; glycine, 0.02 < *P* < 0.5; α-aminoisobutyric acid, 0.02 < *P* < 0.05; lysine, methionine and threonine together, 0.02 < *P* < 0.05). Neither the branched-chain amino acids nor tryptophan, added to a concentration of 0.05 μmol/ml to perfusate (2), had any effect on albumin synthesis (0.3 < *P* < 0.4 and 0.05 < *P* < 0.10 respectively). Arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine added together to perfusate (2), each to ten times their normal peripheral blood concentration, enhanced synthesis rates to a value of 2.38 (0.02 < *P* < 0.05). Urea synthesis

$$\text{Albumin synthesis rate} = \frac{\text{Total } ^{14}\text{C in the guanidine carbon of albumin in arginine}}{\text{Specific activity of newly formed urea}} \times \frac{100}{5.95}$$

Table 1. *Initial amino acid concentrations of the perfusates*

Perfusate (1) consisted of 60ml of blood from rats on a normal diet, 25ml of Plasmalyte B, 4ml of heparin (40mg) and 2ml of 4.2% NaHCO₃. Perfusate (2) was the same as perfusate (1), except that 60ml of blood taken from rats kept on a protein-free diet for 48h was used. Each result represents the mean $\pm$ s.d. with the number of observations in parentheses. *P* values indicate the significance of the change between perfusate (1) and perfusate (2). N.S. is not significant.

Amino acid	Perfusate (1) (μ mol/ml)	Perfusate (2) (μ mol/ml)	Significance
Isoleucine	0.056 $\pm$ 0.005 (7)	0.038 $\pm$ 0.009 (5)	0.001 < <i>P</i> < 0.01
Leucine	0.107 $\pm$ 0.012 (7)	0.062 $\pm$ 0.021 (5)	0.001 < <i>P</i> < 0.01
Valine	0.110 $\pm$ 0.011 (7)	0.051 $\pm$ 0.020 (5)	<i>P</i> < 0.001
Histidine	0.047 $\pm$ 0.014 (6)	0.028 $\pm$ 0.008 (5)	0.02 < <i>P</i> < 0.05
Phenylalanine	0.045 $\pm$ 0.005 (7)	0.030 $\pm$ 0.011 (5)	0.001 < <i>P</i> < 0.01
Tyrosine	0.058 $\pm$ 0.005 (7)	0.028 $\pm$ 0.008 (5)	<i>P</i> < 0.001
Lysine	0.160 $\pm$ 0.023 (7)	0.140 $\pm$ 0.011 (5)	N.S.
Alanine	0.407 $\pm$ 0.109 (6)	0.518 $\pm$ 0.039 (5)	N.S.
Citrulline	0.049 $\pm$ 0.013 (3)	0.040 $\pm$ 0.017 (2)	N.S.
Ornithine	0.123 $\pm$ 0.027 (7)	0.074 $\pm$ 0.020 (5)	0.001 < <i>P</i> < 0.01
Taurine	0.120 $\pm$ 0.005 (5)	0.120 $\pm$ 0.007 (3)	N.S.
Methionine	0.032 $\pm$ 0.005 (7)	0.020 $\pm$ 0.001 (5)	<i>P</i> < 0.001
Glycine	0.286 $\pm$ 0.049 (6)	0.133 $\pm$ 0.042 (4)	<i>P</i> < 0.001
Tryptophan	0.018 $\pm$ 0.004 (8)	0.014 $\pm$ 0.003 (8)	0.02 < <i>P</i> < 0.05

Table 2. *Albumin and urea synthesis rates in isolated rat liver perfusions for livers from well-fed rats*

For details see the text. Each result represents the means $\pm$ s.d. with the number of observations in parentheses.

Perfusate	Albumin synthesis rate (mg/h per 300g of rat)	Urea synthesis rate (mg/h per 300g of rat)
Perfusate (1)	1.75 $\pm$ 0.18 (8)	5.81 $\pm$ 1.93 (8)
Perfusate (2)	1.11 $\pm$ 0.31 (7)	6.03 $\pm$ 1.28 (7)
Perfusate (2) + tryptophan*	1.50 $\pm$ 0.23 (8)	6.93 $\pm$ 1.16 (8)
Perfusate (2) + isoleucine*	1.82 $\pm$ 0.34 (5)	6.18 $\pm$ 0.98 (5)
Perfusate (2) + lysine* + methionine* + threonine*	1.58 $\pm$ 0.36 (5)	15.58 $\pm$ 2.69 (5)
Perfusate (2) + lysine*	2.04 $\pm$ 0.61 (5)	12.90 $\pm$ 1.32 (5)
Perfusate (2) + glycine*	1.45 $\pm$ 0.24 (5)	11.02 $\pm$ 2.10 (5)
Perfusate (2) + α -amino- isobutyric acid*	1.47 $\pm$ 0.16 (5)	6.02 $\pm$ 0.81 (5)
Perfusate (2) + tryptophan†	1.45 $\pm$ 0.34 (8)	6.59 $\pm$ 0.55 (8)
Perfusate (2) + branched- chain amino acids†	1.23 $\pm$ 0.12 (5)	5.92 $\pm$ 0.66 (5)
Perfusate (1) + 11 amino acids‡	2.38 $\pm$ 0.43 (5)	14.30 $\pm$ 1.37 (5)

* Amino acid was added to a concentration of 10 μ mol/ml.

† Amino acid was added to a concentration of 0.05 μ mol/ml.

‡ Amino acids arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine were added together to a concentration of ten times their normal peripheral blood concentration.

rates were the same whether perfusate (1) alone or perfusate (2) alone was used. The addition of lysine, glycine, the 11 amino acids given together (see above) or a combination of methionine, lysine and threonine

significantly increased urea synthesis. However, the addition of tryptophan, isoleucine and α -aminoisobutyric acid had no effect on urea production despite an increase in albumin synthesis rates.

Table 3. *Albumin and urea synthesis rates in isolated rat liver perfusions for livers from 24h-starved rats*

For details see the text. Each result represents the mean $\pm$ S.D. with the number of observations in parentheses.

Perfusate	Albumin synthesis rate (mg/h per 300g of rat)	Urea synthesis rate (mg/h per 300g of rat)
Perfusate (1)	0.50 $\pm$ 0.24 (5)	6.28 $\pm$ 1.28 (5)
Perfusate (2)	0.65 $\pm$ 0.43 (5)	6.34 $\pm$ 0.97 (5)
Perfusate (2)+tryptophan*	0.46 $\pm$ 0.14 (5)	7.36 $\pm$ 1.83 (5)
Perfusate (2)+isoleucine*	0.59 $\pm$ 0.08 (3)	6.63 $\pm$ 0.50 (3)
Perfusate (2)+lysine*	0.48 $\pm$ 0.26 (5)	14.44 $\pm$ 1.31 (5)
Perfusate (2)+trypto- phan*+lysine*	0.70 $\pm$ 0.22 (5)	14.14 $\pm$ 2.09 (5)
Perfusate (1)+tryptophan*	0.61 $\pm$ 0.10 (3)	7.17 $\pm$ 1.42 (3)
Perfusate (1)+11 amino acids†	1.65 $\pm$ 0.33 (4)	15.90 $\pm$ 1.71 (4)
Perfusate (1)+glucagon + tryptophan‡	0.59 $\pm$ 0.03 (3)	10.53 $\pm$ 2.70 (3)

* Amino acid was added to a concentration of 10 μ mol/ml.

† Amino acids arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine were added together to a concentration of ten times their normal peripheral blood concentration.

‡ Tryptophan was added to a concentration of 10 μ mol/ml. A dose of 1 mg of glucagon was added initially, followed by 0.5 mg/h.

Table 3 shows the change in albumin and urea synthesis rates expressed as mg/h per 300g of rat for liver from rats starved for 24h. Perfusion of the liver from starved rats resulted in a decrease in the albumin synthesis rates without affecting urea synthesis rates. The combination of the 11 amino acids mentioned above increased albumin synthesis rates markedly. Irrespective of the single amino acid added or the perfusate used, there was no change in the albumin synthesis rates. Glucagon with tryptophan failed to influence albumin synthesis. However, lysine, glucagon with tryptophan and the 11 amino acids added together increased the urea synthesis rates.

Glucose production was dependent on the nutritional state of the liver and was not influenced by the addition of amino acids or a change in the perfusate. Glucose production increased from 60mg/100ml in both groups to 110 and 215mg/100ml for liver of starved or fed rats respectively.

All livers were uniformly perfused at a rate of 1.5–2.0ml/min per g of liver. Bile production varied from 0.5–1.0ml/h.

Discussion

Eagle *et al.* (1961), Riggs & Walker (1963) and Schingoethe *et al.* (1967) have shown that, within limits, protein synthesis is augmented by increasing amino acid supply *in vitro*. Also, Amenta & Johnston (1963) increased their perfusate and liver free amino acid concentration by adding hydrazine and

thereby increased hepatic protein synthesis. The fact that the greater amino acid supply provided by perfusate (1) led to higher albumin synthesis rates tends to support this. John & Miller (1969), in an extensive study, demonstrated the major role of amino acids in accelerating albumin synthesis and the modifying influence of insulin, cortisone and growth hormone.

The mean value for albumin synthesis rates in the normal rat *in vivo*, as measured by the authors by using the [14 C]carbonate technique, was 9mg/h per 300g body wt. (L. Kelman, L. Frith & S. J. Saunders, unpublished work). In contrast, when perfusate (2) was used the value was 1.11 mg/h per 300g. However, perfusate (1) with its slightly higher concentrations of isoleucine, leucine, valine, histidine, phenylalanine, tyrosine, ornithine, methionine, glycine and tryptophan, was able to increase synthesis rates to 1.75 mg/h per 300g of rat. Clearly, the various synthesis rates obtained by different workers could reflect many factors including the perfusate amino acid concentration, dilution of blood and the nutritional and hormonal state of the blood or of the rats.

While the present study was in progress, Rothschild *et al.* (1969) reported that tryptophan and, to a lesser extent, isoleucine increased albumin synthesis when added to the blood perfusing an isolated liver taken from a starved rabbit.

Like those of Rothschild *et al.* (1969) the results reported in the present paper show marked slowing of albumin synthesis with the liver from starved rats.

However, unlike the findings of Rothschild *et al.* (1969), excess of single amino acids including tryptophan and isoleucine were ineffective in stimulating albumin synthesis. An attempt to increase protein catabolism, by glucagon infusion and then adding tryptophan, failed to increase albumin synthesis. The liver from starved rats responded to the addition of the previously mentioned 11 amino acids added to ten times their peripheral blood concentration. Probably the liver from starved rats, with its faster protein turnover than the rabbit liver, is too protein depleted by the time of perfusion to provide the other amino acids required.

Our remarkable and unexpected finding was the increased albumin synthesis resulting from the addition of single amino acids to the blood obtained from protein-depleted rats, perfusing livers derived from fed rats. This tends to support the findings of Hanking & Roberts (1964, 1965) that single amino acids are capable of stimulating protein synthesis *in vitro*. Further, the finding of Rothschild *et al.* (1969), that both isoleucine and tryptophan individually increased albumin synthesis, suggests that a mechanism other than that dependent on critical amino acid pool depletion is operative. It is of interest that α -aminoisobutyric acid, a non-metabolizable model amino acid, also has this property, suggesting that the mechanism is not one involving energy expenditure nor direct participation in amino acid metabolism. In addition, lysine is effective, supporting the concept that transamination is not involved.

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