Biochem. J. (1987) 241, 491–498 (Printed in Great Britain)

Key role of L-alanine in the control of hepatic protein synthesis

Dolores PÉREZ-SALA, Roberto PARRILLA* and Matilde S. AYUSO
Instituto de Endocrinología y Metabolismo 'G. Marañón', Centro de Investigaciones Biológicas, C.S.I.C., Velazquez 144, 28006 Madrid, Spain

We investigated the effects of administration of single amino acids to starved rats on the regulation of protein synthesis in the liver. Of all the amino acids tested, only alanine, ornithine and proline promoted statistically significant increases in the extent of hepatic polyribosome aggregation. The most effective of these was alanine, whose effect of promoting polyribosomal aggregation was accompanied by a decrease in the polypeptide-chain elongation time. The following observations indicate that alanine plays an important physiological role in the regulation of hepatic protein synthesis. (1) Alanine was the amino acid showing the largest decrease in hepatic content in the transition from high (fed) to low (starved) rates of protein synthesis. (2) The administration of glucose or pyruvate is also effective in increasing liver protein synthesis in starved rats, and their effects were accompanied by an increased hepatic alanine content. (3) An increase in hepatic ornithine content does not lead to an increased protein synthesis, unless it is accompanied by an increase of alanine. The effect of alanine is observed either *in vivo*, in rats pretreated with cycloserine to prevent its transamination, or in isolated liver cells under conditions in which its metabolic transformation is fully impeded.

INTRODUCTION

There is now a large body of information indicating that amino acids carry out important physiological regulatory functions in addition to their role as protein constituents or energy fuels. The role of amino acids as neurotransmitters in the central nervous system is now very well established [1]. In non-excitable tissues amino acids have also been reported to control a large variety of metabolic processes. Hormone secretion [2,3], glycogen synthesis [4], gluconeogenesis [5–7], ureogenesis [8], pancreatic secretion [9], protein synthesis [10,11] and degradation [12,13], among others, are known to be influenced by the presence of amino acids, in a manner that cannot be merely explained by their potential value as energy fuels. The regulatory significance of most of these observations remains to be established, since the information has been obtained under non-physiological conditions with high concentrations of amino acids and isolated organs, cells or enzymes as experimental models. However, in support of a possible regulatory role of amino acids *in vivo* are the observations that changes in the metabolic activity of the intact animal attained by food deprivation [14], pathological processes [15] or hormone administration [16,17] are always accompanied by distinct changes in the amino acid patterns of plasma and tissues.

Two lines of evidence suggest that hepatic protein synthesis can be controlled by the amino acid supply. Firstly, perfusion of isolated rat livers with mixtures of amino acids at 10 times their normal plasma concentrations maintains maximal rates of protein synthesis and prevents polyribosomal breakdown [18]. Secondly, protein synthesis in isolated rat cells is stimulated by certain individual amino acids at concentrations within their physiological range [19,20]. The significance of these observations remains unclear, largely owing to uncertainties about the methodology utilized for measuring protein synthesis. The problem is even more complicated if we take into account that the response of liver-cell protein synthesis to amino acids is highly influenced by the nutritional status [21,22].

The present work is an attempt to clarify the physiological role of amino acids in the control of hepatic protein synthesis *in vivo*. For this purpose we have studied parameters of protein synthesis *in vivo*, which are independent of variations in the specific radioactivity of the amino acid precursor [23,24]. It is concluded that a correlation seems to exist between hepatic content of L-alanine and rates of protein synthesis.

EXPERIMENTAL

Animals

Male Wistar albino rats weighing 200–220 g were used. The animals were either fed *ad libitum* or starved for 48 h before the experiment was started.

Chemicals

[U-^{3}H]Valine (10 Ci/mol) was purchased from Amersham International (Amersham, Bucks., U.K.). All the reagents were of the highest possible purity, and most of them were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Enzymes were purchased from Boehringer (Mannheim, Germany). L-Cycloserine was a gift from Dr. W. E. Scott of Hoffman–La Roche (Nutley, NJ, U.S.A.). Collagenase was purchased from Worthington (Freehold, NJ, U.S.A.).

Injections and collection of liver biopsies and plasma samples

The animals were anaesthetized with Nembutal (40 mg/kg body wt.) about 10 min before they were surgically manipulated. Small liver biopsies were taken as

* To whom correspondence should be addressed.

previously described [24] and immediately frozen with aluminium clamps cooled in liquid N_2 [25]. Blood was taken with heparinized syringes from the aortic bifurcation, and plasma samples were collected.

Analytical techniques

Determination of the state of aggregation of hepatic polyribosomes. The polyribosomal profiles were obtained from homogenates of liver biopsies that were immediately cooled by immersion in ice-cold buffer A (50 mM-triethanolamine, pH 7.3, 5 mM-$MgCl_2$ and 25 mM-KCl). After the addition of sodium deoxycholate (final concn. 1.3%, w/v), the extracts were centrifuged at 10000 *g* for 10 min. The polyribosomes were purified by centrifugation through discontinuous sucrose gradients, made up as previously described [24], for 16 h at 55000 rev./min in a Beckman 65 fixed-angle rotor. The discontinuous sucrose gradients contained 10% liver cell sap, known to content a potent inhibitor of ribonucleases [26,27]. The pellets were resuspended in 0.2 ml of buffer A, and approx. 2 A_{260} units were layered on 5 ml linear 20–40% (w/v) sucrose gradient made up in the same buffer. The gradients were centrifuged in a Beckman SW 50.1 rotor for 35 min at 45000 rev./min, and then pumped from the bottom of the tube through the flow cell of an Isco u.v.-monitoring system, and the A_{254} was continuously recorded.

Determination of the rate of elongation of hepatic polypeptide chains. This was done by measuring the distribution of radioactivity incorporated into nascent peptides (*n*) on the polyribosomes and into total peptides (*t*). For these determinations, 20 μCi of [^{3}H]valine was injected into the portal vein of anaesthetized rats, and at the indicated times small biopsies (about 1 g) were taken in such a way that only two biopsies were taken from each liver (at 0.5 and 1.5 min or at 1 and 2 min, respectively). To prevent haemorrhage, liver lobules were ligated immediately before the biopsies were taken. The details of the procedure for the determination of polypeptide-chain completion times were as previously described [23,24]. The rate of elongation was calculated from the determined time of completion of peptide chains, that is the time needed for the ratio of radioactivity incorporated in nascent peptides into the polyribosomes to the radioactivity incorporated into total peptides (n/t) to be decreased by 50%.

Determination of the concentration of amino acids in plasma and liver. For the determination of the plasma amino acid concentration, plasma samples (0.8 ml) were deproteinized with 10% (w/v) sulphosalicylic acid. For the determination of the hepatic amino acid content, frozen liver biopsies were freeze-dried, and 200 mg dry wt. of tissue was then homogenized with 2 ml of 8% (w/v)

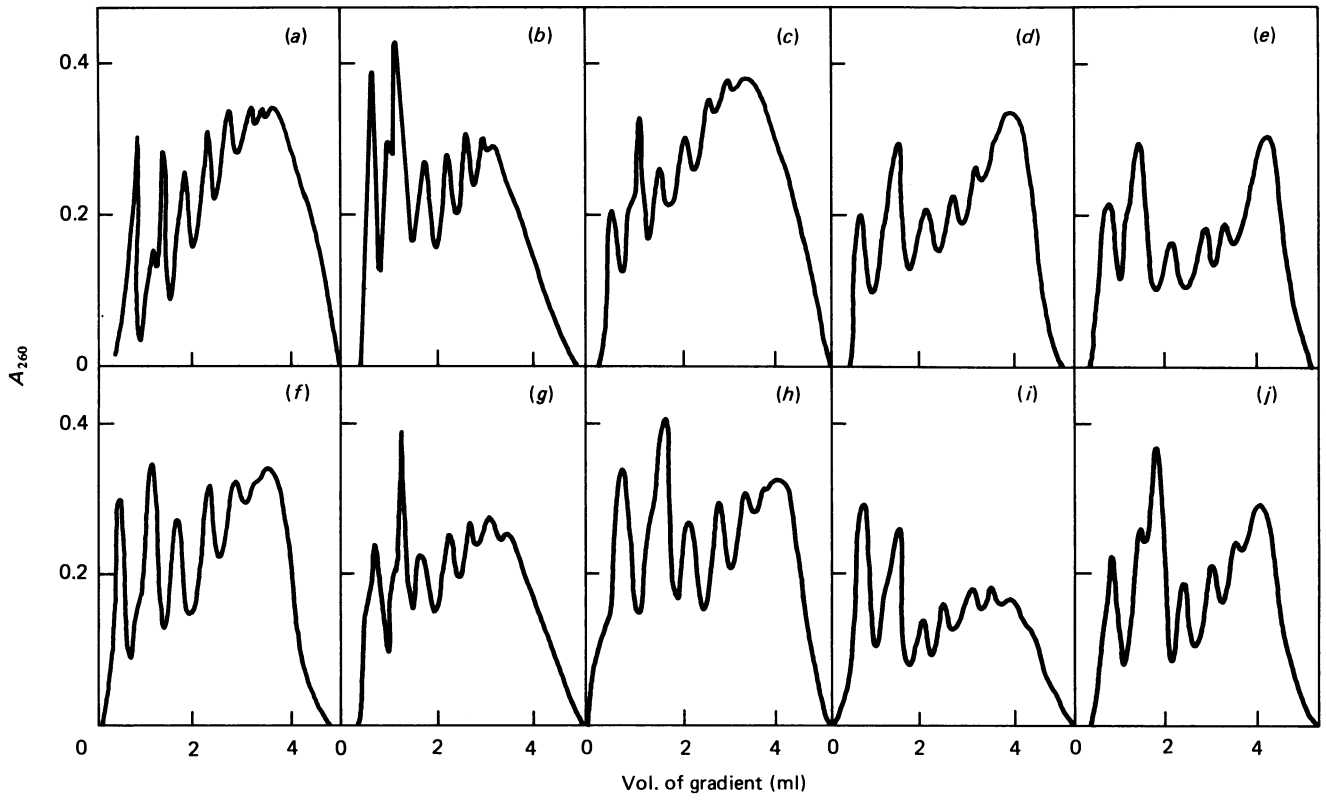

Fig. 1. Effect of starvation and of administration of amino acids to starved rats on the hepatic polyribosomal profiles

The polyribosomal profiles of livers of fed (*a*) or 48 h-starved animals (*b–j*) were determined as described in the Experimental section. The starved animals were treated intraperitoneally with either saline (*b*) or 1 mmol of the following amino acids dissolved in saline: alanine (*c*), proline (*d*), ornithine (*e*), lysine (*f*), arginine (*g*), methionine (*h*), valine (*i*) or glutamine (*j*). At 10 min thereafter liver biopsies were taken as described in the Experimental section.

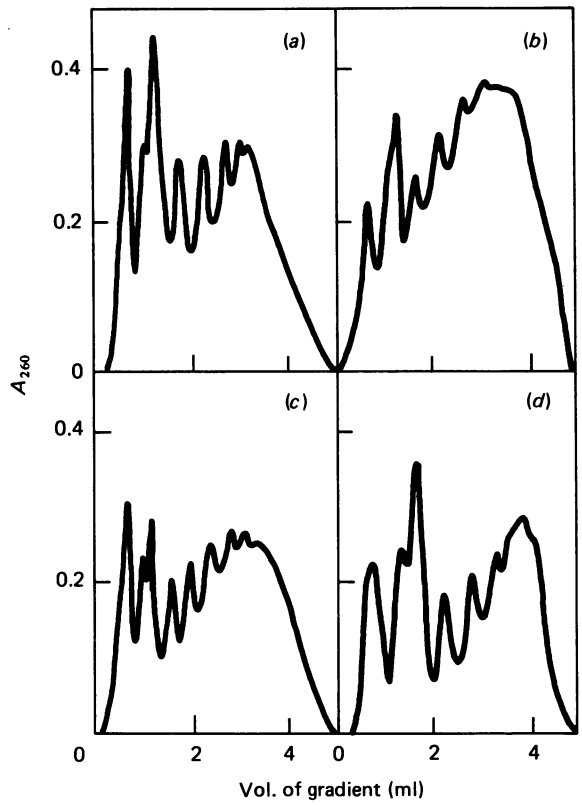

Fig. 2. Effect of the administration of pyruvate, glucose or insulin to starved rats on the hepatic polyribosomal profiles

Rats starved for 48 h received an intraperitoneal injection of a saline solution containing no addition (*a*), 1 mmol of pyruvate (*b*), 1 mmol of glucose (*c*) or 2 units of insulin (*d*); 10 min thereafter liver biopsies were taken and processed as described in the Experimental section for the determination of polyribosomal profiles.

$HClO_4$. After centrifugation at 13000 *g* for 5 min, 0.7 ml portions of the supernatant were adjusted to pH 2 with 1 M-lithium citrate, pH 1.3. The concentration of the different amino acids was determined by liquid chromatography.

Isolation and incubation of rat liver cells

For the preparation of isolated liver cells, the animals were not fed for 48 h before their experimental use. The procedure for isolation was described previously [28]. The cells were incubated in Krebs–Ringer bicarbonate buffer containing 2% Ficoll 70 (M_r 70000). The pH of the incubation mixture was 7.4 after equilibration with O_2/CO_2 (19:1). L-[^{3}H]Valine was utilized as the amino acid precursor, since this amino acid is poorly oxidized or transaminated by the liver cells [29]. Routinely, 1 ml portions of liver cells (40–60 mg wet wt./ml) were placed in plastic 25 ml Erlenmeyer flasks and incubated at 36.5 °C in a rotary shaker. The incubation medium contained 0.5 mM-L-[^{3}H]valine (sp. radioactivity 10 Ci/mol). This concentration seems to be near saturation [20]; thus minor changes in the size of the valine pool utilized for protein synthesis would not induce appreciable changes in the rate of protein labelling. At the end of the experiment, triplicate samples (0.1 ml) were immediately added to cold 10% trichloroacetic acid and processed as previously described [20].

The rate of utilization of alanine by liver cells was determined as follows: 2 ml portions of the cell suspension, made up in Krebs–Ringer/Ficoll 70 medium, were incubated at 36.5 °C in a rotary shaker. Samples (0.8 ml) were taken at zero time and after 1 h of incubation and immediately acidified with 7% $HClO_4$. After removal of the acid-insoluble material by centrifugation, the samples were adjusted to pH 7 with 3 M-KOH. The precipitate formed was removed by centrifugation, and a 0.8 ml sample of the supernatant was adjusted to pH 2 by addition of 1 M-lithium citrate, pH 1.3. Alanine concentration was determined by ion-exchange liquid chromatography. The gluconeogenic rate was determined as previously described [20].

Table 1. Effect of the intraperitoneal administration of individual amino acids, pyruvate, glucose or insulin to starved rats on the hepatic polyribosomal fraction

The experiment was performed as described in the legends of Figs. 1 and 2. The polyribosomal fraction was calculated as the ratio of the area under polyribosomal peaks to the area under monomer plus dimer peaks. The results are means ± S.E.M. for 17 experiments for control fed and 48 h-starved rats, and for 5 experiments for starved rats treated with the different compounds. The *P* value for the difference relative to the saline-treated starved rats was calculated by Student's *t* test; n.s., not significant.

Rats	Administered compound	Polyribosomal fraction	*P*
Fed	Saline	4.85 ± 0.6	0.001
Starved	Saline	1.68 ± 0.2	—
	Glucose	2.71 ± 0.5	0.05
	Pyruvate	2.58 ± 0.2	0.05
	Alanine	4.59 ± 1.4	0.01
	Proline	2.77 ± 0.4	0.05
	Ornithine	2.49 ± 0.2	0.05
	Lysine	2.36 ± 0.3	n.s.
	Arginine	2.18 ± 0.4	n.s.
	Methionine	2.14 ± 0.2	n.s.
	Valine	1.50 ± 0.3	n.s.
	Glutamine	1.94 ± 0.3	n.s.
	Insulin	1.81 ± 0.1	n.s.

RESULTS

Effect of the administration of glucose, pyruvate or individual amino acids to starved rats on the state of aggregation of hepatic polyribosomes

Starvation for 48 h in the rat was accompanied by a pronounced breakdown of hepatic polyribosomes (Figs. 1*a* and 1*b*). This observation agrees with others made in liver [31,32] and other tissues [31,33,34], which indicate that in starved animals the rate of initiation of protein synthesis is decreased. Fig. 1 also shows representative tracings of the effect of the administration of several individual amino acids *in vivo* to starved rats on the state of polyribosome aggregation, and in Fig. 2 the effects of glucose, pyruvate and insulin are shown. A quantification of these experiments is presented in Table 1, in which the polyribosomal fraction has been calculated. As can be appreciated, glucose and pyruvate had a clear effect in

Table 2. Effect of starvation on plasma and liver contents of amino acids

The experiment was performed as described in the legends of Figs. 1 and 2. Each value is the mean ± S.E.M. of determinations on five animals.

	Plasma (nmol/ml)		Liver (μmol/g dry wt.)		Fed/starved ratio
	Fed	Starved	Fed	Starved	
Aspartate	9±4.5	19±7.6	11.2±1.3	5.7±1.7	1.9
Threonine	121±16	141±14	0.5±0.1	0.4±0.06	1.2
Serine	143±22	165±11	1.7±0.3	1.5±0.2	1.1
Glutamate	31±1.5	34±9	10.6±0.3	7.7±1.2	1.3
Glutamine	325±58	498±55	5.5±0.6	7.6±0.5	0.6
Proline	183±14	129±11	0.2±0.01	0.2±0.01	1.3
Glycine	174±15	241±8	4.8±0.5	4.8±0.6	1.0
Alanine	283±41	229±51	3.4±0.6	0.8±0.14	3.9
Citrulline	48±4	47±8	0.1±0.03	0.1±0.02	1.6
Valine	115±25	164±28	0.5±0.1	0.3±0.05	1.6
Methionine	30±6	38±4	0.05±0.02	0.1±0.002	0.5
Isoleucine	23±4	39±7	0.3±0.04	0.2±0.01	1.6
Leucine	86±14	124±16	0.2±0.04	0.1±0.03	1.6
Tyrosine	36±5	52±5	0.2±0.02	0.1±0.01	1.3
Phenylalanine	36±7	58±5	0.2±0.03	0.1±0.01	1.5
Ornithine	39±8	32±5	0.6±0.2	0.3±0.06	2.0
Lysine	355±49	303±32	1.6±0.2	0.9±0.2	1.7
Hystidine	45±5	60±7	1.8±0.2	1.0±0.6	1.6
Tryptophan	185±27	48±4	0.1±0.02	0.2±0.02	0.8
Arginine	57±9	88±7	0.80±0.05	0.08±0.002	1.0

increasing the polyribosomal fraction. It is unlikely that the action of these compounds was mediated by increased insulin secretion, since administration of this hormone by itself had no detectable effects (Fig. 2*d*). Of all the amino acids tested, only alanine, proline and ornithine displayed significant effects in increasing the proportion of ribosomes in polyribosomes. Alanine had a particularly striking effect, restoring the state of polyribosomal aggregation to the normal 'fed' values.

Table 2 shows the plasma and hepatic contents of amino acids in fed and starved rats. The hepatic content, as well as the ratio of liver to plasma concentrations, of all amino acids was either decreased or not changed in the starved animals, with glutamine, methionine and tryptophan being the only exceptions. This observation probably reflects the utilization of amino acids to meet the increased gluconeogenic demand characteristic of the starved state [35,36]. The most pronounced changes were in the hepatic contents of ornithine and alanine, the very amino acids whose exogenous administration restored the polyribosomal state of aggregation (Fig. 1 and Table 1). It therefore seems plausible to conclude that their availability could somehow control the rate of hepatic protein synthesis. Although proline also increased polyribosomal aggregation in starved rats (Table 1), its hepatic content did not change significantly (Table 2). Thus the physiological significance of the proline effect is doubtful. Table 3 shows the influence of the administration *in vivo* of glucose, pyruvate or individual amino acids on the hepatic concentrations of alanine and ornithine. Only those agents capable of inducing polyribosomal aggregation, with the single exception of proline, induced significant increases in the hepatic content of alanine. In contrast, similar increases were observed in ornithine content regardless of the administered agent. Since ornithine administration increases alanine even above the value in fed liver, this observation suggests that this amino acid could act on protein synthesis through its ability to raise the hepatic alanine content. It has been previously reported that administration of tryptophan to starved animals [37,38] or administration of threonine to rats maintained on a threonine-free diet [39] also results in increased polyribosomal aggregation. However, under our experimental conditions, there is no correlation between the effect of glucose, pyruvate or several individual amino acids on the polyribosomal aggregation and the hepatic content of threonine or tryptophan (results not shown). Moreover, during starvation, where polyribosomal breakdown increases, the hepatic content of threonine does not change significantly, and the tryptophan content, in agreement with a previous report [40], increases (Table 2). The data presented above suggest that alanine could be playing an important regulatory role in hepatic protein synthesis.

Effect of alanine on the rate of hepatic polypeptide-chain elongation *in vivo*

The rate of polypeptide-chain elongation was measured after alanine administration by determination of the polypeptide-chain completion time. Fig. 3 shows how the administration of alanine increases the rate of decay of the n/t ratio. This change in slope implies a 30% decrease in the time taken to assemble average polypeptide chains. Assuming a ribosome content of 3 nmol/g wet wt. [24] and taking into account the proportion of ribosomes in polyribosomes (Fig. 1), these

Table 3. Effect of the administration of individual amino acids, glucose or pyruvate to starved rats on the concentrations of alanine and ornithine in plasma and liver

The experiment was performed as described in the legends of Figs. 1 and 2. The alanine and ornithine contents in plasma and liver were determined by liquid chromatography as described in the Experimental section. Values are means ± S.E.M. for five experiments.

Rats	Administered compound	Alanine content		Ornithine content	
		Plasma (nmol/ml)	Liver (nmol/g dry wt.)	Plasma (nmol/ml)	Liver (nmol/g dry wt.)
Fed	Saline	283±41	3360±667	39–8	645±218
Starved	Saline	229±51	845±141	31±5	237±35
	Glucose	188±25	1320±330	32±5	511±29
	Pyruvate	266±34	5053±1386	37±5	488±110
	Alanine	5090±1570	96260±14400	24±4	544±151
	Proline	283±51	875±128	36±4	368±51
	Ornithine	208±23	4040±720	4810±610	4810±5250
	Glutamine	21±35	47±60	65±22	366±18
	Lysine	218±47	1015±136	33±10	514±133

data indicate that protein-synthesis activity was increased from 1.02 to 1.92 nmol/min per g.

Relationship between the effect of alanine in stimulating protein synthesis and its metabolic fate

During starvation, alanine is the most important physiological substrate for hepatic gluconeogenesis [41]. It is therefore likely that in our experiments much of the alanine administered to starved rats is rapidly converted into glucose in the liver. For this reason, experiments were designed to ascertain whether alanine had to be metabolized in order to exert its effect on hepatic protein synthesis. L-Cycloserine, a potent inhibitor of alanine aminotransferase [42], was utilized to prevent pyruvate formation and its further conversion into glucose. In order to simplify and improve the accuracy, each animal was used as its own control. At 20 min after the intraperitoneal administration of 100 μmol of L-cycloserine, a liver biopsy was taken by ligating one of the small liver lobules. Immediately thereafter, 90 μmol of alanine dissolved in 0.2 ml of 0.9% NaCl was injected intraportally, and a second biopsy was taken 10 min later. Under these conditions alanine raised the polyribosomal fraction from 2.7±0.45 to 3.65±0.8 (mean± S.E.M. of nine experiments), strongly suggesting that its metabolic conversion may not be required to exert its action on protein synthesis. However, administration of L-cycloserine induced a significant hyperglycaemia, raising the possibility that glucose and not alanine is the effector molecule.

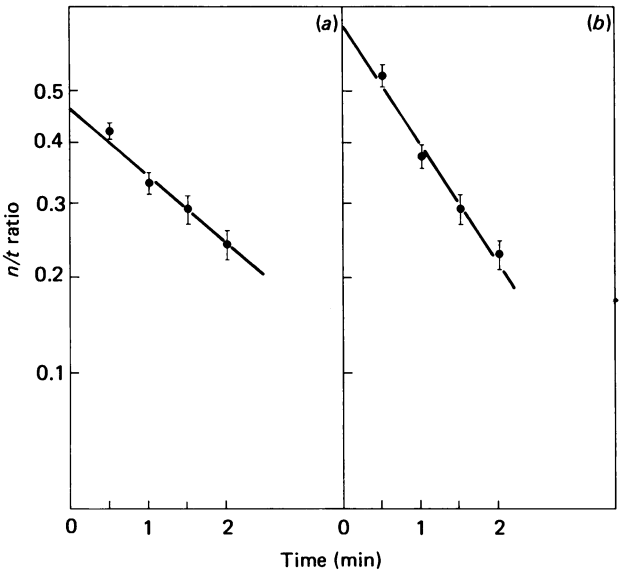

Fig. 3. Effect of the administration of alanine to starved rats on the rate of decay of the hepatic *n*/*t* ratio

Rats starved for 48 h received an intraperitoneal injection of 0.5 ml of saline solution containing no addition (*a*) or 1 mmol of alanine (*b*); 10 min thereafter liver biopsies were taken and processed as described in the Experimental section for determination of the rate of peptide-chain elongation. The points are means ± S.E.M. for seven different experiments. The rate of decay was calculated by linear regression analysis performed by the least-squares method. The ribosomal transit time was calculated from this rate of decay as the time needed for the *n*/*t* ratio to be decreased by 50%, and was 1.85 min in control animals and 1.28 min in alanine-treated animals.

To clarify this point further, experiments were carried out under more controlled conditions, with liver cells as the experimental model. Concentrations of the inhibitor as low as 0.1 mM produced a 75% inhibition of alanine utilization (results not shown). In Fig. 4 the rates of protein labelling and glucose production in liver cells as a function of cycloserine concentration are represented. A near-saturating concentration of the radiolabelled precursor was used in order to prevent artifacts owing to changes in specific radioactivity of the precursor secondary to dilution of the radioisotope [20]. The data in Fig. 4 show that alanine stimulates protein labelling even at concentrations of the inhibitor which fully prevented its conversion into glucose. This clear-cut dissociation between the gluconeogenic capacity of alanine and its ability to stimulate protein labelling offers firm experimental support for the conclusion that this amino acid acts as a positive effector of protein synthesis, regardless of its metabolic fate.

Ornithine is able to stimulate protein labelling in isolated liver cells by 32%. This stimulatory effect is absent in the presence of L-cycloserine (results not shown). This result offers further support to the

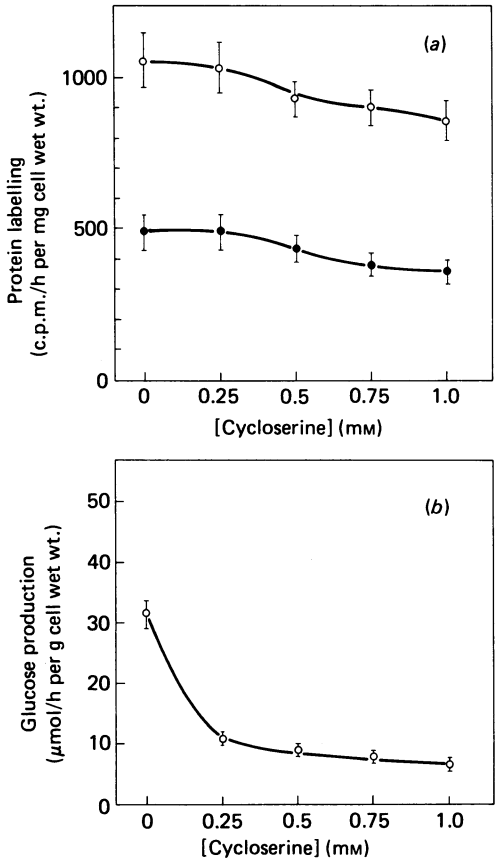

Fig. 4. Effect of L-cycloserine on the rates of protein labelling and gluconeogenesis by isolated liver cells

Liver cells were obtained from 48 h-starved rats and incubated for 1 h as described in the Experimental section. The rate of protein labelling was determined in the absence (●) or in the presence (○) of 10 mM-alanine and with the indicated concentrations of L-cycloserine. The rate of gluconeogenesis was determined with 10 mM-L-alanine as substrate and the indicated concentrations of L-cycloserine. The values are means ± S.E.M. for nine (*a*) and four (*b*) different experiments.

conclusion that the effect of ornithine is mediated by its metabolism, probably through an increase in alanine concentration.

DISCUSSION

Correlation between the ability of individual amino acids to restore protein synthesis and their hepatic content

The ability of alanine aminotransferase to maintain its reactants at near-equilibrium [12,43] explains the increase in the hepatic steady-state concentrations of alanine after administration of glucose, pyruvate or ornithine. The last would increase alanine through an increased glutamate formation by ornithine transaminase [44]. Glucose can increase alanine formation in at least two different ways: by decreasing the flux of alanine to glucose and/or by increasing pyruvate formation. A positive correlation between pyruvate availability and alanine formation in liver has been previously reported [45]. According to these considerations, it seems plausible to conclude that, under physiological conditions, in which neither total amino acid supply nor energy availability is limiting, the rate of hepatic protein synthesis can be finely controlled by the availability of some key amino acids, particularly alanine. This conclusion seems to be supported by the observations that in hypophysectomized animals [46], as well as in those maintained on a protein-free diet [21], situations characterized by a drastic decrease in protein synthesis, alanine was the amino acid which showed the most profound decrease in hepatic content.

Mechanism of alanine action on hepatic protein synthesis

The observation that the administration of a single amino acid, alanine, increases protein synthesis does not support the idea that amino acids in general can enhance this process when their supply is limiting. It could be argued that the amounts of alanine administered were high enough to raise the steady-state concentrations of other amino acids; nevertheless, similar effects on protein synthesis were observed after administration of pyruvate or glucose, which increased the hepatic alanine content without affecting the total amino acid content. Furthermore, when alanine transamination was prevented with cycloserine, this amino acid was similarly effective in increasing protein synthesis either *in vivo* or in isolated liver cells. The latter observation also rules out the possibility that amino acids could perturb protein synthesis by increasing energy supply. If alanine does not need to be metabolized in order to exert its effect, this means that it is probably acting as an allosteric effector on some step(s) involved in protein synthesis. The observations that alanine acts as an allosteric effector of pyruvate kinase [47] and that it also stimulates glycogen synthesis by a mechanism independent of the incorporation of its carbon moieties [48] seem to support the latter possibility. The potential role of specific amino acids as metabolic effectors is also illustrated by a report that leucine stimulates skeletal-muscle protein synthesis by a mechanism which is not related to its metabolic fate [49].

The evidence in the present paper (Table 1), in conjunction with the observation that in perfused isolated liver deprived of amino acids a decreased rate of formation of 40S initiation complexes is detected [50], strongly suggests that alanine exerts its action at the initiation step. A similar conclusion has been reached by using tumour cells deprived of amino acids [51,52]. The observation that the alanine effect *in vivo* on the initiation step is accompanied by a stimulation of peptide-chain elongation (Fig. 3) raises the question of whether a coupling exists between these two steps. However, the possibility that both steps could be simultaneously controlled by alanine, or by a mediator sensitive to alanine, cannot be excluded.

Regarding the effect of alanine on the elongation step, work in liver [49,53] and in other systems [51,54] supports the conclusion that no correlation seems to exist between protein-synthesis activity and amount of aminoacylated tRNA. In any case it is likely that tRNA is fully charged under any physiological condition, with the result that uncharged tRNA amounts never become high enough to perturb protein synthesis when amino acid availability is decreased. However, the finding of an increased phosphorylation of the tRNA synthetase complex in starved animals [55] raises the question of whether the extent of charging of tRNA might be involved in the control of protein synthesis during

starvation. If this is the case, alanine could perhaps interact at this level.

Physiological significance of the alanine effect on hepatic protein synthesis

In the starved state, survival relies mainly on the ability of the liver to increase its glucose production in order to meet the requirements of the central nervous system. Since glucose can only be formed *de novo* from amino acids, it is obvious that a co-ordination between the metabolic pathways leading to their production and utilization is required. The finding that exogenously added alanine or the rise in its concentration by other means leads to a restoration of hepatic protein synthesis in starved rats indicates that a decrease in its concentration during starvation plays the important role of channelling the carbon skeletons of amino acids towards the vital function of glucose production. Since alanine also inhibits hepatic proteolysis [12], its decrease in the starved state creates optimal conditions for gluconeogenesis by increasing the amino acid supply from protein breakdown and preventing their use for protein synthesis. From the above considerations it seems logical to conclude that those mechanisms controlling the alanine production and/or utilization might indirectly control hepatic protein synthesis. In the starved animal about 50% of the hepatic glucose output comes from alanine [41,56], and this amino acid is also quantitatively the most important released by skeletal muscle [57,58]. Since alanine is formed from glucose moieties in skeletal muscle [59], a glucose–alanine cycle occurs between liver and skeletal muscle [41,60]. This cycle implies no net transport of carbon; apparently it serves the function of transporting ammonia from muscle to liver for its further conversion into urea. From the results presented herein, it is obvious that this cycle, by setting the steady-state concentrations of alanine, is actually controlling the rate of hepatic protein synthesis.

We are grateful to Dr. V. Pain for helpful discussions and criticism, and to Mrs Ljubica Franic for her help in the preparation of the manuscript. The skilful technical assistance of Ms. Ana Rodriguez and Ms. Maria José Arias Salgado is greatly appreciated. This work has been supported in part by grants from the Spanish Comisión Asesora de Investigación Científicia y Técnica (174 and 1674) and Fondo de Investigaciones Sanitarias (85/839 and 85/1321). D.P.-S. is the recipient of a fellowship from the Ministry of Education and Science.

REFERENCES

1. Cotman, C. W., Foster, A. & Lantham, T. (1981) Adv. Biochem. Psychopharmacol. **27**, 1–41
2. Fajans, S. S., Floyd, J. C., Jr., Knopt, R. F. & Conn, J. W. (1967) Recent Prog. Horm. Res. **23**, 617–662
3. Ohneda, A., Parada, E., Eisentraut, A. M. & Unger, R. H. (1968) J. Clin. Invest. **47**, 2305–2322
4. Okajima, F. & Katz, J. (1979) Biochem. Biophys. Res. Commun. **87**, 155–162
5. Friedrich, D. (1975) Biochem. Biophys. Acta **392**, 255–270
6. Stubbs, M. & Krebs, H. (1975) Biochem. J. **150**, 41–45
7. Cornell, N. W., Lund, P. & Krebs, H. A. (1974) Biochem. J. **142**, 327–337
8. Krebs, H. A., Hems, R. & Lund, P. (1973) Adv. Enzyme Regul. **11**, 361–377
9. Grendell, J. H., Tseng, H. C. & Rothman, S. S. (1984) Am. J. Physiol. **246**, 445–450
10. Austin, S. A. & Clemens, M. J. (1981) Biosci. Rep. **1**, 35–44
11. Munro, H. H., Hubert, C. & Baliga, B. S. (1975) in Alcohol and Abnormal Protein Biosynthesis: Biochemical and Clinical (Rothschild, M. A., Oratz, M. & Schreiber, S. S., eds.), pp. 33–66, Pergamon Press, New York
12. Parrilla, R. & Goodman, M. N. (1974) Biochem. J. **138**, 341–348
13. Woodside, K. H. & Mortimore, G. E. (1972) J. Biol. Chem. **247**, 6474–6481
14. Parrilla, R. (1978) Pflugers Arch. **374**, 3–7
15. Kirsten, E., Kirsten, R., Hohorst, H. J. & Bucher, Th. (1961) Biochem. Biophys. Res. Commun. **4**, 169–174
16. Bromer, W. W. & Chance, R. E. (1969) Diabetes **18**, 748–754
17. Manchester, K. L. (1970) Biochem. Actions Horm. **1**, 267–320
18. Jefferson, L. S. & Korner, A. (1969) Biochem. J. **111**, 703–712
19. Seglen, P. O. & Solheim, A. E. (1978) Biochim. Biophys. Acta **520**, 630–641
20. Girbes, T., Susin, A., Ayuso, M. S. & Parrilla, R. (1983) Arch. Biochem. Biophys. **226**, 37–49
21. Wannemacher, R. W. & Allison, J. B. (1968) in Protein Nutrition and Free Amino Acid Patterns (Leathem, J. H., ed.), pp. 206–227, Rutgers University Press, New Brunswick, NJ
22. Girbes, T., Susin, A., Ayuso-Parrilla, M. S. & Parrilla, R. (1984) in Recent Advances in Obesity and Diabetes Research (Melchionda, N., ed.), pp. 261–267, Raven Press, New York
23. Scornik, O. A. (1974) J. Biol. Chem. **249**, 3876–3883
24. Ayuso-Parrilla, M. S., Martin-Requero, A., Perez-Diaz, J. & Parrilla, R. (1976) J. Biol. Chem. **251**, 7785–7790
25. Wollenberger, A., Ristau, O. & Schoffa, G. (1960) Pflugers Arch. Gesamte Physiol. Menschen Tiere **270**, 399–412
26. Roth, J. S. (1958) J. Biol. Chem. **231**, 1085–1095
27. Shortman, K. (1962) Biochem. Biophys. Acta **55**, 88–96
28. Berry, M. N. & Friend, D. S. (1969) J. Cell Biol. **43**, 506–520
29. Mortimore, G. E. & Mondon, C. E. (1970) J. Biol. Chem. **245**, 2375–2383
30. Bergmeyer, H. U. (ed.) (1965) Methods of Enzymatic Analysis, Academic Press, New York
31. Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971) J. Biol. Chem. **246**, 436–446
32. Perez-Sala, M. D. & Parrilla, R. (1985) Congr. Spanish Biochem. Soc. 12th, abstr. no. 167
33. Li, J. B., Higgins, J. E. & Jefferson, L. S. (1979) Am. J. Physiol. **236**, E222–E228
34. Harmon, C. S., Proud, C. G. & Pain, V. M. (1984) Biochem. J. **223**, 687–690
35. Cahill, G. F., Jr. (1970) N. Engl. J. Med. **282**, 668–675
36. Felig, P. (1975) Annu. Rev. Biochem. **44**, 933–955
37. Wunner, W. H., Bell, J. & Munro, H. N. (1966) Biochem. J. **101**, 417–428
38. Sidransky, H., Sarma, D. S. R., Bongiorno, M. & Verney, E. (1968) J. Biol. Chem. **243**, 1123–1132
39. Ip, C. C. Y. & Harper, A. E. (1973) Biochim. Biophys. Acta **331**, 251–263
40. Henderson, L. M., Schurr, P. E. & Elvehjem, C. A. (1949) J. Biol. Chem. **177**, 815–823
41. Felig, P., Marliss, E. & Cahill, G. F., Jr. (1970) Science **167**, 1003–1004
42. Braunstein, A. E. (1961) Proc. Int. Congr. Biochem. 5th, **4**, 280–294
43. Brosnan, J. T., Krebs, H. A. & Williamson, D. H. (1967) Biochem. J. **104**, 43P–44P
44. McGivan, J. D., Bradford, N. M. & Beavis, A. D. (1977) Biochem. J. **162**, 147–156
45. Parrilla, R., Goodman, M. N. & Toews, C. J. (1977) Pflugers Arch. **369**, 167–175
46. Tolman, E. L., Schworer, C. M. & Jefferson, L. S. (1973) J. Biol. Chem. **248**, 4552–4559

47. Seubert, W. & Schoner, W. (1971) Curr. Top. Cell. Regul. **3**, 237–267
48. Katz, J., Golden, S. & Wals, P. A. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 3433–3437
49. Tischler, M. E., Desautels, M. & Goldberg, A. L. (1982) J. Biol. Chem. **257**, 1613–1621
50. Flaim, K. E., Peavy, D. E., Everson, W. V. & Jefferson, L. S. (1982) J. Biol. Chem. **257**, 2932–2938
51. Austin, S. A., Pain, V. M., Lewis, J. A. & Clemens, M. J. (1982) Eur. J. Biochem. **122**, 519–526
52. Pain, V. M., Lewis, J. A., Huvos, P., Henshaw, E. C. & Clemens, M. J. (1980) J. Biol. Chem. **255**, 1486–1491
53. Shenoy, S. T. & Rogers, Q. R. (1978) J. Nutr. **108**, 1412–1421
54. Morgan, H. E., Chua, B. H., Boyd, T. A. & Jefferson, L. S. (1981) Dev. Biochem. **18**, 217–226
55. Damuni, Z., Caudwell, F. B. & Cohen, P. (1982) Eur. J. Biochem. **129**, 57–65
56. Ishikawa, E., Aikawa, T. & Matsutaka, H. (1972) J. Biochem. (Tokyo) **71**, 1097–1099
57. London, D. R., Foley, T. H. & Webb, C. G. (1965) Nature (London) **208**, 588–589
58. Pozefsky, T., Felig, P., Tobin, J. D., Soeldner, J. S. & Cahill, G. F. (1969) J. Clin. Invest. **48**, 2273–2282
59. Chang, T. W. & Goldberg, A. L. (1978) J. Biol. Chem. **253** 3677–3684
60. Mallette, L. E., Exton, J. H. & Park, C. R. (1969) J. Biol. Chem. **244**, 5713–5723

Received 27 May 1986/29 August 1986; accepted 25 September 1986