The effects of tumour necrosis factor-α (cachectin) and tumour growth on hepatic amino acid utilization in the rat

Josep M. ARGILÉS* and Francisco J. LÓPEZ-SORIANO
Departament de Bioquímica i Fisiologia, Unitat B, Facultat de Biologia, Universitat de Barcelona,
Diagonal 645, 08071-Barcelona, Spain

The effects of acute administration of tumour necrosis factor-α (cachectin) (TNF-α) or of malignant tumour growth (Walker-256 carcinosarcoma) on hepatic availability and uptake of individual amino acids were compared. The results show that, in spite of lowering the hepatic availability of alanine, aspartate, serine, glycine and proline, the cytokine increased both the total amino acid hepatic uptake and the individual uptakes of alanine, glutamate, serine, threonine, proline, lysine and arginine, while decreasing those of leucine, isoleucine and phenylalanine. Tumour burden resulted in an increase in the hepatic availability of glutamine, threonine, glycine, lysine, leucine, isoleucine, valine and phenylalanine. Total liver amino acid uptake was unaffected, whereas the individual uptakes of alanine, threonine and proline were increased and those of glutamate, glutamine, serine and leucine were decreased. When effects of the cytokine are compared with those induced by tumour growth, there are similar increases in net utilization for alanine, proline and leucine, and a 3-fold difference in the increase observed for threonine. Unmatched effects are seen for glutamate, glutamine, aspartate, glycine, lysine, arginine, valine, phenylalanine and serine.

INTRODUCTION

The metabolic response to a number of invasive stimuli involves the secretion of cytokines by a variety of cells such as macrophages and lymphocytes (Beutler & Cerami, 1988; Dinarello, 1988). Among these compounds, tumour necrosis factor-α (TNF-α; cachectin) has been shown to inhibit the synthesis of lipoprotein lipase in transformed mouse fibroblast and adipocyte (3T3-L1) cells (Price et al., 1986; Kawakami et al., 1987) and also to stimulate lipolysis in these cells. It has also been shown that TNF-α is able to decrease lipid absorption and tissue uptake and oxidation in vivo, with resultant hyper-triglyceridaemia (Evans & Williamson, 1988b). This cytokine also stimulates amino acid release (Mahony et al., 1988) and uptake from muscle (Argilés et al., 1989), increased uptake of amino acids by the liver (Roh et al., 1986; Warren et al., 1987, 1988; Argilés et al., 1989), and increased hepatic synthesis of acute-phase-reaction proteins (Darlington et al., 1986; Perlmutter et al., 1986). These metabolic alterations are associated not only with infectious diseases but also with malignant tumour growth, which is characterized by muscle and adipose tissue wasting, which leads to weight loss and anorexia through accelerated muscle proteolysis and adipose-tissue lipolysis, enhanced rates of hepatic fatty acid oxidation and gluconeogenesis from lactate, amino acids and glycerol (Shapot, 1979; Lawson et al., 1982; Argilés & Azcón-Bieto, 1988). Several studies have attributed TNF-α a central role in cancer-induced cachexia (Oliff et al., 1987; Stovroff et al., 1988; Tracey et al., 1988). Indeed, recent work has shown that TNF-α has acute effects on exogenous lipid metabolism (Evans & Williamson, 1988b; Argilés et al., 1989) which in part mimic those of rapidly growing tumours in the pre-anorexic phase (Evans & Williamson, 1988a).

The aim of the present study was to compare the effects of recombinant TNF-α and malignant tumour growth (Walker-256 carcinosarcoma) on the hepatic availability and utilization of individual amino acids in the rat.

EXPERIMENTAL

Animals

In all experiments, female Wistar rats weighing 180–200 g were used. The animals were bred in the animal house of the Faculty of Biology of the University of Barcelona, where they were kept individually in polypropylene cages and kept under conditions of controlled temperature (20–22 °C) and light cycle (light on from 08:00 to 20:00 h). They were fed ad libitum on standard laboratory chow (Panlab, Barcelona, Spain) containing 54 % carbohydrate, 17 % protein and 5 % fat (the residue was non-digestible material), with free access to drinking water.

Biochemicals

Recombinant-derived human TNF-α (specific activity 8.1×10^6 units/mg of protein) was generously given by BASF/Knoll A.G., Ludwigshafen, Germany. p-Amino-[^{3}H]hippuric acid (sp. radioactivity 447 mCi/mmol) was obtained from Amersham International, Amersham, Bucks., U.K.

Cytokine administration

The animals were injected with 20 μg of human recombinant-derived TNF-α in 0.5 ml of Krebs–Henseleit (1932) saline intravenously through the dorsal tail vein under light diethyl ether anaesthesia; control animals received 0.5 ml of vehicle. All injections were adminis-

Abbreviation used: TNF-α, tumour necrosis factor-α (cachectin).
* To whom correspondence should be addressed.

tered between 09:00 and 10:00 h on the day of the experiment. They were killed 2 h later, and food was available during the intervening period.

Tumour inoculation

A Walker-256 carcinosarcoma cell suspension [approx. 2×10^7 cells in 0.5 ml of Krebs–Henseleit (1932) saline] was injected subcutaneously on the left flank of the rats under light diethyl ether anaesthesia. The Walker-256 carcinosarcoma is a rapidly growing tumour with a doubling time of 0.86 day (Herzfeld & Greengard, 1972). The amount implanted ensured that the tumour mass was 2–5 % of carcass weight at the time of the experiments (7–10 days after implantation).

Experimental design

The different experimental groups, control (saline-injected), TNF-injected and tumour-bearing, were each randomly divided into two different subgroups for two different sets of experiments. In the first, the portal and hepatic blood flows were measured. In the second, the arteriovenous amino acid concentration differences across the liver were estimated.

Blood-flow determinations

The portal and hepatic blood flows were estimated by a modification of the indicator-dilution method (Katz & Bergman, 1969; Casado et al., 1987) involving p-amino-[^{3}H]hippuric acid. Steady-state arterial concentrations of the indicator were obtained after 5 min of infusion of the tracer in a mesenteric vein (results not shown). After the infusion period, blood was sampled from the hepatic and portal veins and from the aorta as rapidly as possible, haemorrhage and hypovolaemic shock being avoided and haematocrit values remaining unchanged (Pastor-Anglada et al., 1987). Blood samples were deproteinized by adding 6% (w/v) $HClO_4$. After centrifugation (2000 g, 5 min), supernatants were used for the measurement of ^{3}H in a liquid-scintillation spectrometer.

Amino acid analysis and calculations

This was carried out in deproteinized blood samples from the portal and hepatic veins and aorta with an automatic amino acid analyser (Lee & Drescher, 1978). Amino acid availability was calculated as follows:

$$A = (F_p \times C_p) + (F_a \times C_a)$$

where F_p and F_a represent the portal and hepatic artery blood flows, and C_p and C_a the substrate concentrations in the portal vein and aorta respectively. Liver net amino acid utilization (U) was calculated as:

$$U = A - (F_h \times C_h)$$

where $F_h = F_p + F_a$, and C_h is the amino acid concentration in the hepatic vein.

All results are expressed as means ± S.E.M., and statistical comparisons were carried out by Student's t test.

RESULTS AND DISCUSSION

Body and liver weights

The body weights of the different experimental groups were: control 201±4 g, TNF-treated 206 ±8 g and tumour-bearing 212±10 g. Liver weights were 8.22 ±0.35 g, 8.36±0.15 g and 9.92±0.62 g respectively.

Table 1. Effects of TNF-α and tumour growth on the arterial, portal and venous blood concentrations of selected amino acids in the rat

For full details see the Experimental section. Concentrations are in nmol/ml and are mean values ±S.E.M. The number of animals per group was five. Total amino acid concentration was calculated by summation of the individual amino acid concentration values, including those not listed here because of the lack of statistical differences between groups. The control values for these amino acids were, for arterial, portal and venous blood respectively; tryptophan 32±2, 38±3, 31±4; citrulline 40±10, 52±8, 48±6; histidine 66±6, 63±7, 54±8; ornithine 47±5, 49±4, 41±10; arginine 185±19, 184±13, 180±18; and tyrosine 110±24, 106±20, 82±8. Values that are significantly different by Student's t test from the control group are indicated by *P < 0.05, **P < 0.01, ***P < 0.001.

Amino acid	Control			TNF-α			Tumour		
	Arterial	Portal	Venous	Arterial	Portal	Venous	Arterial	Portal	Venous
Alanine	360±17	520±43	342±27	270±19**	512±62	140±21***	331±15	438±34	261±23*
Glutamate	100±12	126±13	117±12	81±9	124±14	56±2**	62±5*	80±7*	96±7
Glutamine	540±22	388±32	480±47	609±31	584±44**	668±72	801±47***	513±52	744±82*
Aspartate	30±3	41±2	28±4	26±1	38±4	24±2	24±3	20±1***	22±3
Serine	160±9	139±11	152±7	109±4***	160±21	92±11**	132±10	130±18	144±16
Threonine	160±18	141±18	135±13	121±12	157±9	94±7*	154±18	204±17*	90±6*
Glycine	216±13	238±15	151±17	165±8*	230±27	114±12	284±13**	345±44	296±24***
Proline	152±9	182±21	169±12	90±10**	143±16	76±8***	101±11**	115±17*	91±11***
Lysine	420±24	301±22	375±12	440±32	421±40*	370±16	432±21	402±15**	451±42
Leucine	102±10	98±19	87±12	100±21	125±22	119±22	162±14**	168±18*	184±17**
Isoleucine	60±17	66±6	54±6	66±4	72±8	74±4*	85±10	92±8*	74±2
Valine	130±15	117±20	110±11	149±18	164±18	162±3**	142±16	152±18	94±8*
Phenylalanine	46±5	48±5	30±2	44±3	42±2	44±6	65±3*	64±1*	39±7
Total amino acids	2956±262	3489±353	2896±265	2762±238	3607±394	2725±247	3203±236	3455±328	3371±321

Table 2. Effects of TNF-α and tumour growth on the hepatic availabilities of selected amino acids in the rat

For full details see the Experimental section. Only the availabilities of the amino acids showing high hepatic extraction are listed. They are expressed as μmol/min per 100 g body wt. and represent mean values $\pm$ s.e.m. Total amino acid availability was calculated by summation of the individual amino acid availability values, including those not listed here, i.e. glutamate, glutamine, aspartate, leucine, lysine, arginine, citrulline, ornithine, histidine, tryptophan and tyrosine. The number of animals per group was five. Values that are significantly different by Student's t test from control values are indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Amino acid	Control	TNF-α	Tumour
Alanine	6.39±0.45	4.57±0.46*	7.00±0.43
Serine	2.13±0.14	1.60±0.14*	2.21±0.24
Threonine	2.14±0.26	1.66±0.13	3.06±0.29*
Glycine	3.26±0.20	2.35±0.20*	5.36±0.51**
Proline	2.41±0.22	1.38±0.15**	1.83±0.24
Isoleucine	0.91±0.09	0.84±0.07	1.50±0.15**
Valine	1.76±0.25	1.89±0.22	2.49±0.29**
Phenylalanine	0.67±0.07	0.53±0.03	1.09±0.03***
Total amino acids	41.85±4.00	36.41±3.54	53.65±4.58

Table 3. Effects of TNF-α and tumour growth on the net hepatic utilization of selected amino acids in the rat

For full details see the Experimental section. Only amino acids showing statistically significant differences are listed. They are expressed as μmol/min per 100 g body wt. and represent mean values $\pm$ s.e.m. Total net amino acid utilization was calculated by summation of the individual amino acid values, including those not listed here, i.e. citrulline, ornithine, histidine and tryptophan. The number of animals per group was five. Values that are significantly different by Student's t test from control values are indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and from zero are indicated by $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$.

Amino acid	Control	TNF-α	Tumour
Alanine	+1.50±0.06†††	+2.86±0.20***†††	+2.60±0.04***†††
Glutamate	−0.04±0.01††	+0.50±0.11**†	−0.41±0.02***†††
Glutamine	−0.32±0.28	−0.84±0.43	−1.74±0.54*†
Aspartate	+0.11±0.02††	+0.09±0.04†††	−0.00±0.02**
Serine	−0.05±0.04	+0.48±0.01***†††	−0.22±0.03*††
Threonine	+0.21±0.07†	+0.52±0.05**†††	+1.55±0.19***††
Glycine	+1.10±0.04†††	+0.97±0.05†††	+0.37±0.11***†
Proline	−0.10±0.05	+0.45±0.06***††	+0.30±0.06**††
Lysine	−0.28±0.16	+0.76±0.24**†	−0.60±0.41
Arginine	+0.06±0.03	+0.39±0.02***†††	−0.12±0.09
Leucine	+0.18±0.04†	−0.10±0.01***†††	−0.32±0.01***†††
Isoleucine	+0.13±0.01†††	−0.07±0.02***†	+0.25±0.12
Valine	+0.19±0.10	−0.08±0.18	+0.90±0.15**††
Phenylalanine	+0.25±0.04††	−0.10±0.04**	+0.43±0.09†††
Tyrosine	+0.37±0.20	+0.27±0.15	−0.08±0.08
Total amino acids	+3.69±0.45††	+6.22±0.80*††	+2.95±0.71†

Blood flows

The total hepatic blood flows were 3.5±0.3, 3.0±0.3 and 3.6±0.7 ml/min per g of liver for control, TNF-treated and tumour-bearing groups respectively. Although there were no statistical differences between these values, the portal blood flow was significantly lower ($P < 0.05$) in the TNF-treated group than that of the control group. The portal blood flows were 1.9±0.2, 1.3±0.1 and 2.0±0.4 ml/min per g of liver for control, TNF-treated and tumour-bearing groups respectively. TNF administration inhibits gastric emptying in the rat (Tracey et al., 1988), and this fact may possibly be related to a lower portal blood flow.

Amino acid concentrations

These are presented as nmol/ml in Table 1, which shows that administration of the cytokine decreased the arterial blood concentrations of alanine (25 %), serine (32 %), glycine (24 %) and proline (41 %). Tumour burden resulted in a lower arterial concentration of glutamate (36 %) and proline (34 %), whereas it increased those of glutamine (23 %), glycine (24 %), leucine (37 %) and phenylalanine (29 %). No significant changes in total amino acid concentration were found between the different groups studied. Others have reported that there were no significant changes in plasma amino acids in 24 h-starved tumour-bearing rats compared with non-

tumour-bearing controls when decreased food intake was not manifest (Arbeit *et al.*, 1985). These results fully agree with those reported with a similar experimental design (Argilés *et al.*, 1989).

TNF-α administration resulted in a significant increase in the portal concentrations of glutamine and lysine, but it decreased the venous concentrations of alanine, glutamate, serine, threonine, proline and isoleucine. On the other hand, tumour-bearing animals showed decreased portal concentrations of glutamate, aspartate and proline, but increased concentrations of threonine, lysine, leucine, isoleucine and phenylalanine. In the same experimental group there were significant decreases in the venous concentrations of alanine, threonine, proline and valine and increases in those of glutamine, glycine and leucine.

Amino acid availabilities

TNF treatment induced a decreased hepatic availability (μmol of amino acid reaching the liver/min per 100 g body wt.) for alanine (29 %), serine (25 %), glycine (28 %) and proline (48 %), but it did not affect the total amino acid availability (Table 2). In contrast, tumour growth increased the availabilities for threonine (43 %), glycine (65 %), isoleucine (65 %), valine (41 %) and phenylalanine (63 %). No significant changes were observed in total amino acid availability in the tumour-bearing animals.

Net hepatic utilization

TNF treatment resulted in an increased utilization of alanine, glutamate, serine, threonine, proline, lysine, and arginine, but decreased that of leucine, isoleucine and phenylalanine. Tumour-bearing animals showed an increased utilization of alanine, threonine and proline and a diminished utilization of glutamate, glutamine, serine and leucine.

Concluding remarks

The present results show that TNF administration to rats can mimic some of the effects of malignant tumour growth on hepatic amino acid utilization. This is especially the case for the amino acids that can be transported through the A system, some of them clearly gluconeogenic precursors: alanine, threonine, proline and other neutral ones. These results are in full agreement with the uptake results reported by us using α-amino-isobutyrate, a non-metabolizable analogue of alanine (Argilés *et al.*, 1989).

The enhanced hepatic utilization of gluconeogenic amino acids is indeed a well-known phenomenon in tumour-bearing animals (Shapot, 1979; Lawson *et al.*, 1982; Argilés & Azcón-Bieto, 1988) and has been related to the action of glucocorticoids on liver amino acid transport, the concentration of these hormones being specially high in the host (Saez, 1971). On the other hand, TNF administration also modifies the cortico-

steroid status of the animal (Argilés *et al.*, 1989) and this fact may explain the results obtained with the cytokine.

REFERENCES

Arbeit, J. M. Gorschboth, C. M. & Brennan, M. F. (1985) Cancer Res. **45**, 6296–6300

Argilés, J. M. & Azcón-Bieto, J. (1988) Mol. Cell. Biochem. **81**, 3–17

Argilés, J. M., López-Soriano, F. J., Wiggins, D. & Williamson, D. H. (1989) Biochem. J. **261**, 357–362

Beutler, B. & Cerami, A. (1988) Annu. Rev. Biochem. **57**, 505–518

Casado, J., Pastor-Anglada, M. & Remesar, X. (1987) Biochem. J. **245**, 297–300

Darlington, G. J., Wilson, D. R. & Lachman, L. B. (1986) J. Cell Biol. **103**, 787–793

Dinarello, C. A. (1988) FASEB J. **2**, 108–115

Evans, R. D. & Williamson, D. H. (1988a) Biochem. J. **252**, 65–72

Evans, R. D. & Williamson, D. H. (1988b) Biochem. J. **256**, 1055–1058

Herzfeld, A. & Greengard, O. (1972) Cancer Res. **32**, 1826–1832

Katz, M. L. & Bergman, E. N. (1969) Am. J. Physiol. **216**, 946–952

Kawakami, M., Murase, T., Ogawa, H., Ishibashi, S., Mori, N., Takaku, F. & Shibata, S. (1987) J. Biochem. (Tokyo) **101**, 331–338

Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66

Lawson, D. H., Richmond, A., Nixon, D. W. & Rudman, D. (1982) Annu. Rev. Nutr. **2**, 277–301

Lee, K. S. & Drescher, D. G. (1978) Int. J. Biochem. **9**, 457–467

Mahony, S. M., Beck, S. A. & Tisdale, M. J. (1988) Br. J. Cancer **57**, 385–389

Oliff, A., Defeo-Jones, D., Boyer, M., Martinez, D., Kiefer, D., Vuocolo, G., Wolfe, A. & Sochor, S. H. (1987) Cell **50**, 555–563

Pastor-Anglada, M., Remesar, X. & Bourdel, G. (1987) Am. J. Physiol. **252**, E408–E413

Perlmutter, D. H., Dinarello, C. A., Punsal, P. I. & Colten, H. R. (1986) J. Clin. Invest. **78**, 1349–1354

Price, S. R., Mizel, S. B. & Pekala, H. (1986) Biochim. Biophys. Acta **889**, 374–381

Roh, M. S., Moldawer, L. L., Ekman, L. G., Dinarello, C. A., Bistrian, B. R., Jeevanandam, M. & Brennan, M. F. (1986) Metab. Clin. Exp. **35**, 419–424

Saez, S. (1971) Eur. J. Cancer **7**, 381–387

Shapot, V. S. (1979) Adv. Cancer Res. **30**, 89–150

Stovroff, M. C., Fraker, D. L., Swedenborg, J. A. & Norton, J. A. (1988) Cancer Res. **48**, 4567–4572

Tracey, K. J., Wei, H., Manogue, K. R., Fong, Y., Hesse, D. G., Nguyen, H. T., Kuo, G. C., Beutler, B., Cotran, R. S., Cerami, A. & Lowry, S. F. (1988) J. Exp. Med. **167**, 1211–1227

Warren, R. S., Donner, D. B., Starnes, H. F., Jr. & Brennan, M. F. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 8619–8622

Warren, R. S., Starnes, F. H., Jr., Alcock, N., Calvano, S. & Brennan, M. F. (1988) Am. J. Physiol. **255**, E206–E212