Comparative effects of tumour necrosis factor- α (cachectin), interleukin-1- β and tumour growth on amino acid metabolism in the rat *in vivo*

Absorption and tissue uptake of α -amino[1- 14 C]isobutyrate

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The effects of acute administration of either tumour necrosis factor- α (cachectin) (TNF) or interleukin-1- β (IL-1), or of tumour growth (Walker-256 carcinosarcoma), on blood amino acid concentrations and tissue α -amino[1- 14 C]isobutyrate (AIB) uptake in virgin and lactating rats were compared. Both monokines decreased the blood concentrations of those amino acids (serine, glycine, alanine and proline) transported via the A system. Tumour growth decreased the blood concentrations of serine, proline and histidine, whereas the concentrations of glutamine and leucine were increased. IL-1 decreased the intestinal absorption of AIB in all groups studied; TNF or tumour growth had no effect. Tissue AIB uptake was increased (1.5–2.5-fold) in liver, whereas it was decreased in heart and skeletal muscle of the three treatment groups (except skeletal muscle of the IL-1-treated rats). Lactating rats had lower hepatic uptake of AIB compared with livers of virgin rats. IL-1 increased the hepatic uptake of AIB in lactating rats, but not to the values seen in virgin rats treated with IL-1; there was no effect of the cytokine on muscle or mammary-gland uptake. In adrenalectomized rats, the stimulatory effect of IL-1 on hepatic AIB uptake was diminished, whereas that of TNF still persisted. IL-1 caused a marked decrease of AIB uptake in muscle and heart of adrenalectomized rats, which was accompanied by an increase in the blood concentrations of branched-chain amino acids. These effects did not occur with TNF. It is concluded that the effects of the cytokines on tissue amino acid metabolism may depend on a differential endocrine response involving glucagon and/or glucocorticoids.

INTRODUCTION

The acute response to tissue injury or sepsis is characterized by a shift in energy metabolism from storage to utilization and involving both fat and protein. In severe situations the overall result is a negative nitrogen balance. The metabolic changes are in part brought about by alterations in the relative concentrations or effectiveness of insulin versus the 'stress' hormones, glucagon, catecholamines and corticosteroids (see Wilmore, 1976; Frayn, 1986). Another class of inter-cellular mediators, the cytokines, polypeptides released by stimulated macrophages or lymphocytes, may also play an important role in the acute metabolic response to trauma, sepsis and tumour growth (see Dinarello & Mier, 1986; Beutler & Cerami, 1987, 1988). The metabolic effects of two of these cytokines, interleukin-1 (IL-1) and tumour necrosis factor- α (cachectin, TNF) are varied and include: (a) suppression of lipoprotein lipase active *in vitro* in 3T3-L1 adipocytes (Price *et al.*, 1986; Kawakami *et al.*, 1987) and *in vivo* in adipose tissue (Semb *et al.*, 1987; Evans & Williamson, 1988b), with resultant hypertriglyceridaemia (Evans & Williamson, 1988b); (b) stimulation of glycolysis in isolated cells (Taylor *et al.*, 1988); (c) stimulation of amino acid release

from muscle and increased uptake of amino acids by the liver (Roh *et al.*, 1986; Warren *et al.*, 1988); (d) increased hepatic synthesis of the acute-phase-reaction proteins (Sipe *et al.*, 1982). Many of these metabolic changes occur not only in trauma and sepsis but also in neoplastic diseases, and therefore it has been suggested that IL-1 and TNF play a role in cancer-induced cachexia (Beutler & Cerami, 1988), which is characterized by loss of muscle and adipose-tissue mass. Recent experiments from our laboratory have indicated that both cytokines have acute effects on exogenous lipid metabolism (Evans & Williamson, 1988b; Argilés *et al.*, 1989), which in part mimic those of rapidly growing tumour in the pre-anorexic phase (Evans & Williamson, 1988a). The aim of the present experiments was to compare the effects of recombinant IL-1, TNF (at an equimolar dose) and rapid tumour growth (Walker-256 carcinosarcoma) on blood amino acid concentrations and tissue uptake of amino acids as indicated by the accumulation of α -amino[1- 14 C]isobutyrate (AIB), a non-metabolizable analogue of alanine. The studies were mainly on virgin rats, but the effect of IL-1 was also studied in lactating rats to see whether the cytokines can affect mammary-gland amino acid metabolism.

Abbreviations used: TNF, tumour necrosis factor- α (cachectin); IL-1, interleukin-1- β ; AIB, α -amino[1- 14 C]isobutyrate.

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MATERIALS AND METHODS

Rats

Rats were fed *ad libitum* on a chow diet consisting (by wt.) of 52 % carbohydrate, 21 % protein and 4 % fat (the residue was non-digestible material; Special Diet Services, Witham, Essex, U.K.), with free access to drinking water, and were maintained at an ambient temperature at $22 \pm 2^\circ\text{C}$ with a 12 h-light/12 h-dark cycle (lights on from 07:00 h). Four groups of Wistar rats were studied: virgin rats (body wt. 207 ± 4 g, food intake 17 ± 1 g/day), virgin tumour-bearing rats (body wt. 206 ± 7 g, food intake 14 ± 1 g/day), lactating rats 10–13 days *post partum* with 9–10 pups (270 ± 20 g, food intake 48 ± 4 g/day) and virgin adrenalectomized rats (202 ± 6 g, food intake 14 ± 2 g/day).

Adrenalectomy was carried out under anaesthetic by standard procedures (Zarrow *et al.*, 1964). The animals were allowed to recover from the surgical trauma for 1 week before the experiments were carried out. They were also provided with 1 % NaCl solution as a drinking fluid to compensate for the loss of Na^+ owing to the lack of kidney reabsorption of this ion after adrenalectomy.

Biochemicals

All enzymes and coenzymes were obtained from Boehringer Corp. (London), Lewes, Sussex, U.K. Recombinant-derived human TNF- α was generously given by BASF/Knoll A.G., Ludwigshafen, Germany, and recombinant-derived IL-1 by Eli Lilly Co., Indianapolis, IN, U.S.A. AIB (sp. radioactivity 59 mCi/mmol) was obtained from Amersham International, Amersham, Bucks., U.K.

Cytokine administration

The animals were injected with 6 nmol of either human recombinant-derived TNF or IL-1/kg body wt. 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 administered between 08:00 and 09:00 h on the day of the experiment, with the animals being killed 2.5 h later; food was available during this 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 days (Herzfeld & Greengard, 1972); the amount implanted ensured that the tumour mass was 2–5 % of carcass weight at the time of the experiments (5–7 days after implantation).

Measurement of AIB absorption and tissue uptake

At 1 h after cytokine administration, both the control and the tumour-bearing group were sham-injected with physiological saline. The animals received 2 μCi (about 0.04 μmol) of AIB dissolved in 0.5 ml of distilled water enterally by gastric intubation, without anaesthetic but with minimal stress to the animal. The animals were killed 90 min after the AIB administration under pentobarbital anaesthesia, and samples of arterial blood were taken by means of a heparinized syringe, and samples of liver, white and brown adipose tissue, hind-leg muscle,

heart and, where appropriate, mammary gland or tumour were rapidly excised and freeze-clamped (Wollenberger *et al.*, 1960). The tissues were powdered in liquid N_2 and deproteinized in 4 vol. of cold 6 % (w/v) HClO_4 . After homogenization and centrifugation at 17000 rev./min for 15 min, the acid supernatants were neutralized with 30 % (w/v) KOH. After centrifugation at 3000 rev./min for 10 min, the clear neutral supernatants were used for measurement of ^{14}C radioactivity with a liquid-scintillation spectrometer.

Amino acid analysis

This was carried out with a LDC/Milton Roy high-pressure liquid chromatograph, with dimethylaminoazobenzenesulphonyl chloride to form derivatives (Chang *et al.*, 1982).

Plasma corticosterone

This was measured with an immunoassay kit (Immunodiagnostic Systems, Washington, Co. Durham, U.K.).

RESULTS AND DISCUSSION

Effects of IL-1, TNF and tumour growth on blood amino acid concentrations in virgin rats

Acute treatment with both IL-1 and TNF decreased the blood concentration of most gluconeogenic amino acids (Table 1), i.e. serine (36 % and 29 % decrease respectively), glycine (27 % and 24 %), alanine (38 % and 22 %) and proline (56 % and 40 %). These results are in agreement with the observation of enhanced hepatic gluconeogenesis from L-[U- ^{14}C]alanine after IL-1 treatment of rats (Roh *et al.*, 1986). In contrast, tumour growth resulted in smaller decreases in the concentrations of serine (13 %) and proline (24 %), and a larger decrease in histidine (60 %), whereas those of glutamine (50 %) and leucine (37 %) were significantly increased (Table 1). 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).

Effects of IL-1, TNF and tumour growth on tissue AIB uptake in virgin rats

As most of the amino acids affected by either TNF or IL-1 treatment can be transported across the cell membrane by the A system (glycine, serine, alanine, proline and other neutral amino acids), we decided to determine the tissue uptake of a trace amount of AIB as a measure of the net influx into tissues (Walker & Whitfield, 1978). This compound is a non-metabolizable analogue of alanine, the principal amino acid released by muscle in catabolic states and the major gluconeogenic amino acid in the liver (see Beisel, 1970). The net influx of AIB is the result of a carrier-mediated uphill influx and a passive AIB efflux out of the tissue (Cooper & Kohn, 1980).

We have previously reported that IL-1 administration decreases intestinal absorption of triacylglycerols and glucose (Argilés *et al.*, 1989) and that TNF (at a higher dose) has a similar effect on triacylglycerol absorption

Table 1. Effects of IL-1, TNF and tumour growth on the arterial blood concentrations of selected amino acids in virgin rats

For full details see the Materials and methods section. Concentrations are in nmol/ml and are mean values $\pm$ S.E.M. The numbers of animals are indicated in parentheses. Total amino acid concentration was calculated by summation of the individual amino acid concentration values, including those not listed here, i.e. citrulline, arginine, taurine, cysteine, ornithine and tyrosine. 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	Experimental group			
	Virgin, control (5)	Virgin, IL-1 (5)	Virgin, TNF (4)	Virgin, tumour (5)
Glutamate	89 $\pm$ 1.4	70 $\pm$ 17	71 $\pm$ 8.7	65 $\pm$ 6.2**
Glutamine	420 $\pm$ 35	370 $\pm$ 62	475 $\pm$ 47	630 $\pm$ 67*
Serine	158 $\pm$ 4.2	102 $\pm$ 11***	144 $\pm$ 12**	138 $\pm$ 7.8*
Threonine	145 $\pm$ 11	111 $\pm$ 12	112 $\pm$ 19	139 $\pm$ 6.8
Glycine	235 $\pm$ 13	172 $\pm$ 15**	179 $\pm$ 15*	287 $\pm$ 18
Alanine	362 $\pm$ 21	224 $\pm$ 24**	284 $\pm$ 24*	340 $\pm$ 33
Proline	141 $\pm$ 8.7	62 $\pm$ 6.6***	85 $\pm$ 13**	108 $\pm$ 5.7**
Valine	130 $\pm$ 7.1	140 $\pm$ 16	151 $\pm$ 13	145 $\pm$ 11
Isoleucine	67 $\pm$ 4.7	60 $\pm$ 8.2	65 $\pm$ 2.3	85 $\pm$ 12
Leucine	104 $\pm$ 8.7	104 $\pm$ 12	104 $\pm$ 7.9	143 $\pm$ 10*
Tryptophan	47 $\pm$ 4.7	32 $\pm$ 4.7*	33 $\pm$ 2.1*	41 $\pm$ 4.1
Phenylalanine	44 $\pm$ 2.8	42 $\pm$ 4.8	44 $\pm$ 2.2	64 $\pm$ 3.8**
Lysine	409 $\pm$ 29	276 $\pm$ 52	435 $\pm$ 24	431 $\pm$ 26
Histidine	69 $\pm$ 7.7	52 $\pm$ 7.4	41 $\pm$ 8.7*	28 $\pm$ 2.7***
Total amino acids	3070 $\pm$ 210	2453 $\pm$ 325	2871 $\pm$ 261	3322 $\pm$ 255

(Evans & Williamson, 1988b). For this reason we used the enteral route of administration of AIB in the present experiments to examine whether the cytokines also had an effect on amino acid absorption.

Intestinal AIB absorption was significantly decreased (60%) by IL-1, whereas TNF treatment or tumour growth had no significant effect, although there was a tendency for decreased absorption with TNF (Table 2). It is clear that the effects of cytokines on intestinal absorption, which may be due to a delay in gastric emptying (Patton *et al.*, 1987), are important in relation to delivery of dietary nutrients. Because of these differences in absorption of AIB between the experimental groups, we have expressed AIB uptake in two ways: (a) as a percentage of the absorbed dose per g of tissue or per ml of plasma and (b) as a tissue/plasma ratio.

IL-1 and TNF increased AIB uptake by the liver, expressed in either of these ways (Table 2). The tissue/plasma ratio was increased 2.5- and 1.5-fold for IL-1 and TNF respectively. The effect of tumour burden on the host was also to increase the liver/plasma ratio for AIB (1.5-fold) and the percentage of the accumulated absorbed dose (Table 2). These results for the cytokines agree with previous observations where both IL-1 and TNF have been shown to stimulate hepatic AIB transport *in vivo* (Warren *et al.*, 1988) or *in vitro* after administration *in vivo* (Roh *et al.*, 1986). Although there was a tendency for decreased uptake of AIB in both white and brown adipose tissues of the TNF-treated and tumour-growth groups, no significant changes were observed, except for a decreased tissue/plasma ratio of AIB in brown adipose tissue of the TNF-treated group (Table 2). The situation was different in heart and skeletal muscle, where significant decreases in the tissue/plasma ratios of AIB occurred as a result of either cytokine treatment or tumour growth, the only exception being in the AIB ratio

of skeletal muscle after IL-1 treatment. It is of interest that, on a per-gram basis, the tumour accumulated more AIB, and had a higher tissue/plasma ratio of AIB (Table 2) than did the peripheral tissues studied. This would confirm the established view that amino acid uptake is an important aspect of tumour metabolism (see Argilés & Azcon-Bieto, 1988).

Effects of IL-1 on blood amino acid concentrations and AIB uptake in lactating rats

Lactation is associated with hyperphagia and increased uptake of amino acids into mammary gland, which is the main site of amino acid metabolism at peak lactation (see Williamson, 1986). Therefore we have examined the effects of IL-1 in the lactating rat. In the control lactating rats, the previously observed increase in arterial alanine and the decrease in glutamine (Viña & Williamson, 1981) compared with virgin rats were confirmed. The only effect of IL-1 on the blood concentrations of amino acids was to increase significantly the branched-chain amino acids, isoleucine (169%) and leucine (155%); no change was observed in the concentration of gluconeogenic or total blood amino acids (results not shown).

As in virgin rats, IL-1 significantly decreased intestinal AIB absorption in the lactating rats. In the control lactating rat the liver AIB uptake was low (50%) compared with that of the virgin animal (tissue/plasma ratios of 2.42 ± 0.24 and 4.94 ± 0.27 respectively; *P* < 0.001), which suggests that hepatic uptake of amino acids via the A transport system is decreased during lactation in the rat. This result is in contrast with the findings by Casado *et al.* (1987) of increased net hepatic extraction *in vivo* of amino acids (including alanine and serine) in lactation.

After IL-1 treatment, the liver AIB uptake increased significantly, but only to reach a similar tissue/plasma ratio to that found in control virgin rats. No significant

Table 2. Effects of IL-1, TNF and tumour growth on the intestinal absorption and tissue uptake of AIB in virgin rats

For full details see the Materials and methods section. Tissue AIB uptake is expressed both as a percentage of the absorbed dose and also as the tissue/plasma ratio. The results are mean values $\pm$ S.E.M., for the numbers of animals indicated in parentheses. Values that are significantly different by Student's *t* test from the control values are indicated by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Tissue AIB uptake	Experimental group					
	Virgin, control (5)		Virgin, IL-1 (5)		Virgin, TNF (4)	
	(% of absorbed dose/g)	(tissue/plasma)	(% of absorbed dose/g)	(tissue/plasma)	(% of absorbed dose/g)	(tissue/plasma)
Liver	1.30 $\pm$ 0.20	4.94 $\pm$ 0.27	4.36 $\pm$ 1.33*	12.3 $\pm$ 1.91**	3.05 $\pm$ 0.50**	7.47 $\pm$ 0.51**
White adipose tissue	0.18 $\pm$ 0.08	0.64 $\pm$ 0.23	0.19 $\pm$ 0.05	0.74 $\pm$ 0.34	0.13 $\pm$ 0.02	0.29 $\pm$ 0.03
Brown adipose tissue	0.52 $\pm$ 0.11	1.94 $\pm$ 0.31	0.77 $\pm$ 0.24	2.36 $\pm$ 0.52	0.33 $\pm$ 0.06	0.83 $\pm$ 0.09*
Heart	0.82 $\pm$ 0.13	3.04 $\pm$ 0.12	0.79 $\pm$ 0.33	2.02 $\pm$ 0.32*	0.70 $\pm$ 0.13	1.72 $\pm$ 0.10*
Skeletal muscle	0.23 $\pm$ 0.05	0.88 $\pm$ 0.09	0.22 $\pm$ 0.08	0.60 $\pm$ 0.10	0.21 $\pm$ 0.03	0.53 $\pm$ 0.04*
Tumour	—	—	—	—	—	—
Plasma	0.28 $\pm$ 0.03	1.00	0.35 $\pm$ 0.02	1.00	0.40 $\pm$ 0.05	1.00
Intestinal absorption of AIB (%)	70.9 $\pm$ 3.67	—	28.3 $\pm$ 9.62**	—	44.6 $\pm$ 15.7	75.1 $\pm$ 2.41

Table 3. Effects of IL-1 and TNF on the arterial blood concentration of selected amino acids in virgin adrenalectomized rats

For full details, see the Materials and methods section. Concentrations are in nmol/ml and represent mean values $\pm$ S.E.M. for four animals per group. Total amino acid concentration was calculated by adding all the individual amino acid concentration values, including those not listed here, i.e. citrulline, arginine, taurine, cysteine, ornithine and tyrosine. Values that are significantly different by Student's *t* test from the control group are indicated by **P* < 0.05, ***P* < 0.01.

Amino acid	Experimental group		
	Adrenal-ectomized, control	Adrenal-ectomized, IL-1	Adrenal-ectomized, TNF
Glutamate	84 $\pm$ 11	94 $\pm$ 6	73 $\pm$ 14
Glutamine	512 $\pm$ 47	344 $\pm$ 40*	405 $\pm$ 33
Serine	143 $\pm$ 11	99 $\pm$ 9.3*	132 $\pm$ 15
Threonine	94 $\pm$ 11	78 $\pm$ 5.8	109 $\pm$ 13
Glycine	255 $\pm$ 30	193 $\pm$ 16	259 $\pm$ 9.5
Alanine	252 $\pm$ 35	177 $\pm$ 12	213 $\pm$ 18
Proline	140 $\pm$ 20	72 $\pm$ 6.6*	99 $\pm$ 7.7
Valine	153 $\pm$ 12	279 $\pm$ 34*	150 $\pm$ 10
Isoleucine	73 $\pm$ 11	139 $\pm$ 20**	77 $\pm$ 7.5
Leucine	107 $\pm$ 11	228 $\pm$ 33*	112 $\pm$ 8.4
Tryptophan	31 $\pm$ 4.8	26 $\pm$ 2.4	30 $\pm$ 3.1
Phenyl-alanine	44 $\pm$ 4.1	48 $\pm$ 3.8	49 $\pm$ 2.9
Lysine	572 $\pm$ 85	437 $\pm$ 79	458 $\pm$ 46
Histidine	48 $\pm$ 6.4	49 $\pm$ 10	48 $\pm$ 5.9
Total amino acids	3322 $\pm$ 422	3208 $\pm$ 429	2961 $\pm$ 254

differences exist between the two values (5.04 $\pm$ 0.79 and 4.94 $\pm$ 0.27 for lactating IL-1-treated and virgin control respectively). IL-1 treatment did not alter AIB uptake into any other tissue studied, including the mammary gland (results not shown).

Effects of cytokines in adrenalectomized rats

IL-1 and TNF cause acute increases in plasma corticosterone (del Rey & Besedovsky, 1987; Warren *et al.*, 1988). In the present experiments there were significant increases in plasma corticosterone in IL-1- and TNF-treated rats [controls 450 $\pm$ 29 (5) μ g/l; IL-1-treated 603 $\pm$ 56 (5) μ g/l, *P* < 0.05; TNF-treated 683 $\pm$ 49 (4) μ g/l, *P* < 0.01]. We have therefore measured blood amino acid concentrations and the tissue AIB uptake in adrenalectomized rats treated with the cytokines. All the adrenalectomized rats had plasma corticosterone values below 20 μ g/l. Adrenalectomy resulted in a few significant changes (*P* < 0.05) in whole-blood amino acids in the control rats (Tables 1 and 3); glutamine (22 %) was increased, whereas threonine (35 %), alanine (31 %) and tryptophan (35 %) were decreased. Treatment with IL-1 significantly decreased the arterial concentrations of glutamine (33 %), serine (31 %) and proline (49 %), whereas it had no effect on the concentration of either alanine or glycine (Table 3). More striking were the significant increases in the branched-chain amino acids (valine 82 %, isoleucine 90 % and leucine 113 %

Table 4. Effects of IL-1 and TNF on the tissue uptake and intestinal absorption of AIB in virgin adrenalectomized rats

For full details, see the Materials and methods section. Tissue AIB uptake is expressed both as a percentage of the absorbed dose and as the tissue/plasma ratio. The results are mean values $\pm$ S.E.M. for four animals per group. Values that are significantly different by Student's *t* test from the control values are indicated by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Tissue AIB uptake	Experimental group					
	Adrenalectomized, control		Adrenalectomized, IL-1		Adrenalectomized, TNF	
	(% of absorbed dose/g)	(tissue/plasma)	(% of absorbed dose/g)	(tissue/plasma)	(% of absorbed dose/g)	(tissue/plasma)
Liver	1.30 $\pm$ 4.46	4.46 $\pm$ 0.62	1.98 $\pm$ 0.70	6.48 $\pm$ 0.61	2.02 $\pm$ 0.16	7.60 $\pm$ 0.77*
White adipose tissue	0.17 $\pm$ 0.01	0.51 $\pm$ 0.12	0.10 $\pm$ 0.01*	0.41 $\pm$ 0.09	0.24 $\pm$ 0.03	0.88 $\pm$ 0.12
Brown adipose tissue	0.75 $\pm$ 0.16	2.16 $\pm$ 0.53	0.14 $\pm$ 0.04*	0.64 $\pm$ 0.25*	0.53 $\pm$ 0.09	1.97 $\pm$ 0.33
Heart	0.40 $\pm$ 0.04	1.11 $\pm$ 0.10	0.22 $\pm$ 0.04*	0.80 $\pm$ 0.10	0.36 $\pm$ 0.11	1.32 $\pm$ 0.34
Skeletal muscle	0.22 $\pm$ 0.02	0.56 $\pm$ 0.02	0.06 $\pm$ 0.01***	0.19 $\pm$ 0.06***	0.86 $\pm$ 0.20*	0.86 $\pm$ 0.20
Plasma	0.35 $\pm$ 0.02	1.00	0.31 $\pm$ 0.10	1.00	0.27 $\pm$ 0.01	1.00
Intestinal absorption of AIB (%)	64.7 $\pm$ 7.31		23.9 $\pm$ 7.98**		51.1 $\pm$ 5.76	

after IL-1 treatment. In contrast, none of these effects were observed after TNF administration (Table 3). Although both IL-1 and TNF treatments resulted in higher tissue/plasma AIB ratios for liver, this was only significantly increased in the TNF-treated group (Table 4). In fact, the IL-1 response was decreased by 50% (*P* < 0.05) compared with its effect in non-adrenalectomized rats. In addition, IL-1 caused a marked decrease in AIB ratios in all the other tissues (except white adipose tissue) studied, particularly in skeletal muscle (Table 4). In contrast, TNF administration significantly increased AIB accumulation in skeletal muscle, and had no effect on uptake by the other tissues (Table 4).

The intestinal absorption of AIB was significantly decreased by IL-1 and unchanged by the corresponding equimolar dose of TNF (Table 4), thus again demonstrating the greater effect of IL-1 on this process (see Tables 2 and 4).

The above results suggest that the glucocorticoid response may be involved in the mechanism whereby IL-1 elicits its effects in liver, whereas the effects of TNF appear to be independent of increased corticosterone, as indicated by the ability of TNF still to increase the tissue/plasma ratio of AIB in livers of adrenalectomized rats. Studies *in vitro* with cultured hepatocytes have ruled out a direct effect of TNF on amino acid uptake (Warren *et al.*, 1987a). TNF administration increases plasma glucagon (Warren *et al.*, 1987a, 1988), and this is a well-established inducer of the A transport system in liver (see Kilberg *et al.*, 1985).

Furthermore, Warren *et al.* (1987a) have demonstrated that TNF can have a permissive effect in enhancing the stimulation of amino acid uptake by glucagon. Apparently IL-1 does not exert a permissive or a direct effect on hepatic AIB transport (Warren *et al.*, 1987a), and therefore the hepatic effects observed here in rats with intact adrenals may be mediated via the transitory increase in plasma corticosterone associated with IL-1

administration (Warren *et al.*, 1988), or by a combination of increased glucagon and corticosterone.

The decreased uptake of AIB into heart and skeletal muscle in normal rats treated with IL-1 or TNF may also be related to the increase in plasma corticosterone. Corticosterone administration to humans increases net efflux of alanine, glutamine and total amino acids from muscle (Legaspi *et al.*, 1985), as does TNF administration (Warren *et al.*, 1987b). This postulate is supported by the finding that in adrenalectomized rats TNF does not decrease muscle AIB uptake. A novel and surprising finding in the present study is that IL-1 is more effective in decreasing AIB uptake in skeletal muscle of adrenalectomized rats, suggesting either that IL-1 is able to act directly or that it can act via a mediator other than plasma corticosterone. It is of interest in this respect that impure preparations of IL-1 (compared with the recombinant material used in the present experiments) stimulate muscle protein breakdown and prostaglandin E₂ release *in vitro* (Baracos *et al.*, 1983). However, recent experiments with recombinant peptides appear to rule out a direct effect *in vitro* of IL-1 or several other cytokines (Moldawer *et al.*, 1987a; Goldberg *et al.*, 1988) on muscle protein metabolism. Whatever the mechanism whereby IL-1 exerts its action, it would seem that this may be modified by the corticosteroid status of the animal. It is also noteworthy that the effects of IL-1 to decrease AIB accumulation in muscle of adrenalectomized rats are accompanied by increases in the branched-chain amino acids (Table 3), which are normally utilized by skeletal muscle.

Experiments on tumour-bearing mice (methylcholanthrene-induced fibrosarcoma) have not detected the appearance of IL-1 or TNF in the circulation (Moldawer *et al.*, 1987b). If the same holds for the rats bearing the Walker 256 carcinosarcoma, this suggests that the effects of the tumour on amino acid metabolism are brought about by other mediators, as appears to be the case with regard to lipid metabolism (Evans & Williamson,

1988a,b), or that effects are the result of the action of cytokines produced locally.

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