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# Effect of recombinant human interleukin 1β (rhIL-1β) on amino acid flux in the isolated perfused rat liver

**S. K. Lim[1], J. P. De Bandt[1], F. Ballet[2], C. Rey[2], C. Coudray-Lucas[1], F. Blonde-Cynober[1], J. Giboudeau[1], and L. Cynober[1]**

[1] Laboratoire de Biochimie A and [2] INSERM U 181 Hôpital Saint Antoine, Paris, France

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**Summary.** We studied the effect of recombinant human IL-1$\beta$ (rhIL-1$\beta$) on hepatic amino acid (AA) flux in the isolated perfused rat liver model. Two experimental groups were used – a control group ($n = 5$) and a rhIL-1$\beta$-treated group ($n = 5$). IL-1 was added to the perfusate in two successive boluses of 0.1 $\mu$g and 0.9 $\mu$g, respectively 35 min (final concentration 0.67 ng/ml) and 60 min (6 ng/ml) after beginning the perfusion. In the IL-1 treated group, a reduction in flux was observed for only three AA, alanine, phenylalanine and serine. Glucose and urea production in the IL-1-treated group was slightly but not-significantly lower than in the controls.

rhIL-1$\beta$ thus has only minor direct effects on AA flux and gluconeogenesis in the liver and cannot therefore be held responsible for the increase in hepatic amino acid uptake during stress.

**Keywords:** Amino acids – Interleukin-1 – Liver

## Introduction

The metabolic response to stress induces enhanced hepatic uptake of amino acids (AA), mainly gluconeogenic AA [1–3] and accelerated hepatic protein synthesis and gluconeogenesis [4] . This rapid adaptation of AA uptake by the liver results, in part, from hormonal changes, with increased secretion of glucagon, catecholamines and glucocorticoids [5–7]. Recently, attention has been focused on the possible role of cytokines, produced by cells of the reticuloendothelial system. Initial studies have shown that monocyte-conditioned medium is able to induce *in vivo* the entire spectrum of the host response to trauma [8]. Moreover, the same type of crude monocyte medium has been shown to increase AA uptake by the isolated perfused rat liver [9]. Among the large variety of cytokines contained in this medium, particular attention has been paid to

interleukin-1 (IL-1); indeed, the *in vivo* administration of semi-purified IL-1 is followed by enhanced AA uptake by the liver [10]. However, neither semi-purified IL-1 [10] nor recombinant IL-1 [11] has been shown to modify AA transport in isolated rat hepatocytes. Evidence for the direct involvement of IL-1 in injury-mediated changes in hepatic AA transport is thus lacking. The above apparent discrepancies could be due to an absence of the cooperation between periportal and perivenous hepatocytes which is required for the metabolism of some amino acids [12] and/or to a lack of cell cooperation in isolated hepatocytes. For example, it is well known [13] that IL-1 and TNF activate Kupffer cells that synthesize IL-6 which, in turn, induces acute-phase protein synthesis in hepatocytes.

We therefore evaluated the short-term effects of recombinant human IL-1$\beta$ (rhIL-1$\beta$) on AA flux in the isolated perfused rat liver, an experimental model in which interactions between different cell types and the functional heterogeneity of the liver lobule are maintained.

## Materials and methods

### *1. Reagents*

Recombinant human IL-1$\beta$ (rhIL-1$\beta$) was a generous gift from Eli Lilly laboratories (Indianopolis, USA). Crystallized AA and AA solutions (BME ref. B6766 and MEM ref. M7145) were purchased from Sigma (La Verpillière, France). Albumin fraction V was obtained from Calbiochem (Paris-France).

### *2. Liver perfusion*

Male Sprague Dawley rats (323 $\pm$ 65 g) fed "ad libitum" on laboratory chow (UAR $AO_3$) were fasted for 16 hours prior to experimentation, with free access to a glucose solution (50 g/l). Livers were prepared for the perfusion according to the method described by Miller [14]. Briefly, the rats were first anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally). After catheterization of the bile duct and portal vein, the livers were immediately perfused via the portal vein and removed. They were then perfused in a recirculating system at constant pressure (9.5 cm $H_2O$) at 37°C in a thermostatically controlled cabinet. The perfusate was oxygenated with a 95% $O_2$/5% $CO_2$ mixture. The perfusion medium (150 ml) was composed of Krebs Ringer buffer (pH 7.40) enriched with bovine serum albumin (30 g/l), glucose (8.5 mmol/l), calcium (1.5 mmol/l) and eight AA known for their antiproteolytic activities (alanine, proline, glutamine, methionine, leucine, phenylalanine, tryptophan and histidine) at twice the normal rat plasma concentration [15–17]. After a 30-minute equilibration period during which hepatic flow stabilized, a complete AA solution (aspartate, serine, asparagine, glutamate, proline, glycine and alanine at 333 $\mu$mol/l, lysine at 208 $\mu$mol/l, threonine, valine, isoleucine and leucine at 166 $\mu$mol/l, arginine, phenylalanine, tyrosine at 83 $\mu$mol/l, cysteine, methionine and histidine at 41 $\mu$mol/l, and tryptophan at 16 $\mu$mol/l) and 2 ml of sodium bicarbonate solution (50 g/l) were added, the latter in order to maintain the pH within the physiological range. This perfusion design ("two-step AA loading") has previously been shown to be suitable for the measurement of AA exchanges in the isolated perfused rat liver [15]. The following modifications of the original technique were made: i) use of a blood-free buffer to avoid interference by mediators contained in the blood; ii) enrichment of the complete AA solution with gluconeogenic AA to enhance gluconeogenic flux.

### 3. *Experimental design*

Two sets of perfusions were performed – a control group ($n = 5$) and an IL-1-treated group ($n = 5$). The study period was 60 minutes (total perfusion time 90 minutes). When used, IL-1 was added to the perfusate in two boluses of 0.1 $\mu$g and 0.9 $\mu$g at the 35th* and 60th minute of perfusion (final concentrations 0.67 ng/ml and 6 ng/ml, respectively). Samples were taken for the measurement of AA, glucose, urea and enzyme activities at 35 and 60 minutes (just before the administration of IL-1), and at 90 minutes; portal flow was measured after each sampling.

Bile production was measured over three periods of 30 minutes, i.e. during equilibration (0 to 30 min) and then during the experimental period (30 to 60 and 60 to 90 min). At the end of the perfusion, a specimen of each liver was immediately frozen in liquid nitrogen using the freeze clamp technique and stored at $-80°C$ for measurement of intrahepatic AA.

### 4. *Analytical methods*

The activities of ASAT, ALAT and LDH were measured using standard methods according to the French Society of Biological Chemistry (SFBC) recommendations, using a Kone autoanalyser [18]. Glucose and urea were measured using the glucose oxidase and urease methods, routinely performed with an Astra 8 analyser (Beckman, USA).

AA concentrations in the perfusate were determined by means of ion-exchange chromatography (Chromakon 500 Kontron, Switzerland) after deproteinization of samples with sulfosalicylic acid (50 mg/ml) [19].

The frozen liver specimens (0.5 g) were homogenized in 5 ml of cold 4% perchloric acid/0.5 mM EDTA and centrifuged. The supernatant was used for the determination of AA using the method described above.

### 5. *Calculation*

All results are expressed per gram of liver (wet weight). The amounts of AA, urea and glucose released (+) or taken up (−) by the liver were calculated according to the following formula:

$$F = \frac{C2 - C1}{t2 - t1} \times \frac{V}{W}$$

where C1 and C2 are the concentrations at times t1 and t2, respectively, V the volume of the perfusate and W the liver weight. The results are expressed as $nmol.min^{-1}.g^{-1}$ and presented as mean ± SEM. The two groups were compared using the non-parametric Mann and Whitney U-test.

## Results

Liver viability [assessed in terms of enzyme activities, hepatic perfusate flow and bile production (Table 1)] was similar in the two groups and values were close to those previously reported [20].

Glucose and urea production, although slightly reduced in the IL-1 treated group, were not statistically different between the two groups (Table 2).

AA fluxes were not affected by IL-1 except for decreases in the uptake of alanine and phenylalanine after the first bolus of IL-1 and serine after the 2nd bolus (Table 3). Intrahepatic AA content was not different between the two groups, except for leucine which was decreased after IL-1 treatment (Table 4).

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* All the times quoted are relative to the beginning of the perfusion.

**Table 1.** Action of rhIL-1$\beta$ on liver viability

| | Times (min) | Group C (control) $n = 5$ | Group I (IL-1) $n = 5$ |
|---|---|---|---|
| ASAT | 35 | 7 ± 4* | 5 ± 1 |
| UI/I | 60 | 15 ± 2 | 9 ± 3 |
| | 90 | 36 ± 8 | 27 ± 3 |
| ALAT | 35 | 5 ± 1 | 5 ± 2 |
| UI/I | 60 | 5 ± 1 | 5 ± 2 |
| | 90 | 18 ± 8 | 14 ± 9 |
| LDH | 35 | 29 ± 8 | 25 ± 6 |
| UI/I | 60 | 94 ± 26 | 88 ± 7 |
| | 90 | 241 ± 64 | 296 ± 43 |
| Hepatic flow rate $ml \cdot min^{-1} \cdot g^{-1}$ | | 3.67 ± 0.12 | 3.62 ± 0.14 |
| Bile production $\mu g \cdot min^{-1} \cdot g^{-1}$ | 0–35 | 533 ± 50 | 442 ± 42 |
| | 35–60 | 504 ± 62 | 466 ± 51 |
| | 60–90 | 488 ± 75 | 557 ± 45 |

* Mean ± SEM

**Table 2.** Effect of rhIL-1$\beta$ on hepatic production of glucose and urea ($nmol \cdot min^{-1} \cdot g^{-1}$; mean ± SEM)

| | IL-1 Concentration (ng/ml) | Group C (control) $n = 5$ | Group I (IL-1) $n = 5$ |
|---|---|---|---|
| Glucose | 0.67 | 155 ± 119 | 112 ± 67 |
| | 6 | 193 ± 66 | 68 ± 34 |
| Urea | 0.67 | 294 ± 67 | 260 ± 59 |
| | 6 | 309 ± 12 | 217 ± 48 |

## Discussion

The results of this study investigating the short-term effects of IL-1 on liver AA metabolism show that rhIL-1$\beta$ only induces minor changes in AA flux in the isolated perfused rat liver.

The lack of any major effect does not seem to be due to inadequate concentrations of IL-1, for the following reasons:

- The two concentrations used can be considered largely supraphysiologic, with final concentrations respectively 10- and 100-fold higher than circulating levels in healthy subjects [21].

**Table 3.** Effect of rhIL-1$\beta$ on hepatic amino acid fluxes ($nmol \cdot min^{-1} \cdot g^{-1}$; mean ± SEM)

| | IL-1 Concentration (ng/ml) | Group C (control) $n = 5$ | Group I (IL-1) $n = 5$ |
|---|---|---|---|
| Threonine | 0.67 | −12.0 ± 5.9 | −4.0 ± 2.1 |
| | 6 | −13.4 ± 5.4 | −7.0 ± 0.3 |
| Serine | 0.67 | −56.4 ± 12.7 | −35.0 ± 3.8 |
| | 6 | −52.8 ± 6.9 | −33.4 ± 2.3* |
| Glutamate | 0.67 | 39.6 ± 3.8 | 37.7 ± 10.7 |
| | 6 | 56.6 ± 23.2 | 32.8 ± 6.6 |
| Glutamine | 0.67 | −24.8 ± 15.8 | −17.0 ± 11.0 |
| | 6 | −36.0 ± 7.1 | −49.8 ± 20.8 |
| Proline | 0.67 | −57.8 ± 14.3 | −44.0 ± 9.7 |
| | 6 | −59.4 ± 8.4 | −40.4 ± 1.5 |
| Glycine | 0.67 | −19.8 ± 7.1 | −22.7 ± 10.2 |
| | 6 | −5.2 ± 6.1 | −18.2 ± 3.7 |
| Alanine | 0.67 | −144.2 ± 15.1 | −97.2 ± 6.1* |
| | 6 | −71.6 ± 13.6 | −81.0 ± 10.7 |
| Valine | 0.67 | 5.2 ± 4.8 | 7.5 ± 6.8 |
| | 6 | −1.8 ± 5.3 | −0.4 ± 4.4 |
| Isoleucine | 0.67 | 1.2 ± 1.6 | 2.2 ± 3.1 |
| | 6 | −2.6 ± 3.8 | −1.6 ± 2.6 |
| Leucine | 0.67 | 1.8 ± 8.2 | 2.5 ± 10.7 |
| | 6 | −9.0 ± 8.3 | −5.8 ± 7.3 |
| Tyrosine | 0.67 | −1.0 ± 3.3 | 1.7 ± 2.3 |
| | 6 | −10.0 ± 2.5 | −12.7 ± 4.8 |
| Phenylalanine | 0.67 | −24.8 ± 4.3 | −13.7 ± 1.9* |
| | 6 | −16.8 ± 3.5 | −16.8 ± 1.5 |
| Lysine | 0.67 | −4.0 ± 4.0 | −1.7 ± 3.3 |
| | 6 | −8.0 ± 3.6 | −11.6 ± 3.5 |

Negative values = uptake; positive values = release
* $p < 0.05$ versus control group

- The biological activity of the batch of IL-1 used has been assessed on target cells (synovial cells) and is highly effective on 2-deoxyglucose transport, the effect being apparent at a concentration as low as 0.1 pg/ml [22].
- A number of studies have shown that rIL-1 has an endocrine and/or metabolic effect after administration to mice at doses as low as 5 pg [23], 5 ng [24] and 10 ng [25].

In the present study, not only did rhIL-1$\beta$ fail to enhance hepatic AA uptake but it also appeared to inhibit the uptake of certain gluconeogenic AA. It is noteworthy that the decrease in alanine and phenylalanine uptake occured after the first IL-1 bolus but not after the second which gave IL-1 concentrations nine-fold higher. A possible explanation is that the decrease in AA concentrations in the perfusate with time reduced the hepatic uptake of AA [15]. This could also account for the decreased uptake of the above two AA in the control group during the second half of the study period (Table 3).

**Table 4.** Effect of rhIL-1$\beta$ on intrahepatic amino acid content ($\mu$mol/g of liver; mean $\pm$ SEM)

| | Group C (control) $n = 5$ | Group I (IL-1) $n = 5$ |
|---|---|---|
| Taurine | 1.88 $\pm$ 0.34 | 1.66 $\pm$ 0.24 |
| Aspartate | 1.20 $\pm$ 0.17 | 0.95 $\pm$ 0.05 |
| Threonine | 0.20 $\pm$ 0.01 | 0.20 $\pm$ 0.01 |
| Serine | 0.30 $\pm$ 0.06 | 0.40 $\pm$ 0.05 |
| Glutamate | 3.25 $\pm$ 0.20 | 3.27 $\pm$ 0.32 |
| Glutamine | 1.93 $\pm$ 0.54 | 2.26 $\pm$ 0.49 |
| Proline | 0.30 $\pm$ 0.03 | 0.38 $\pm$ 0.02 |
| Glycine | 1.69 $\pm$ 0.17 | 1.46 $\pm$ 0.11 |
| Alanine | 0.99 $\pm$ 0.22 | 0.70 $\pm$ 0.11 |
| Valine | 0.17 $\pm$ 0.01 | 0.16 $\pm$ 0.01 |
| Isoleucine | 0.14 $\pm$ 0.01 | 0.13 $\pm$ 0.01 |
| Leucine | 0.38 $\pm$ 0.01 | 0.33 $\pm$ 0.02* |
| Ornithine | 0.12 $\pm$ 0.01 | 0.15 $\pm$ 0.02 |
| Lysine | 0.20 $\pm$ 0.02 | 0.21 $\pm$ 0.02 |
| Histidine | 0.27 $\pm$ 0.04 | 0.37 $\pm$ 0.04 |

* $p < 0.05$ versus group C

Glucose and urea production were slightly lower in the rhIL-1-treated group, especially with regard to glucose production after the second bolus (Table 2). Since in our experimental conditions the livers are glycogen-depleted (F. Blonde-Cynober, JP. De Bandt, F. Ballet et al., unpublished data), glucose release into the medium can only be attributed to gluconeogenesis. The apparently paradoxical notion that IL-1 may inhibit gluconeogenesis is supported by animal studies in which hypoglycemia (not always related to hormonal changes), is observed after IL-1 administration [24, 26]. One of these studies [26] showed that IL-1 purified from supernatants of cultured rabbit macrophages prevented the fasting-mediated induction of one of the key enzymes of gluconeogenesis, phosphoenolpyruvate carboxykinase. These data are compatible with a clinical situation, the terminal phase of septic shock, in which IL-1 production is strongly increased and gluconeogenesis is inhibited [27].

At all events, IL-1$\beta$ is not the key mediator in the increased hepatic AA uptake observed in sepsis and trauma, at least on a short-term basis.

However it cannot be ruled out that action of IL-1 on AA fluxes is only observed after a longer period. Indeed, this is the case for IL-6 which increases the uptake of $\alpha$-aminoisobutyrate (a non metabolizable analogue of alanine) by hepatocytes after a two-day incubation period [28].

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**Authors' address:** S. K. Lim, Laboratoire de Biochimie A, Hôpital Saint Antoine, 184, rue du Fbg Saint Antoine, F-75571 Paris Cedex 12, France.

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