Leucine Metabolism in Rat Liver After a Bolus Injection of Endotoxin

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To evaluate the contribution of hepatic tissue to alterations in the metabolism of proteins and the branched-chain amino acids (BCAA) leucine, isoleucine, and valine in systemic inflammatory response syndrome, we studied the changes of leucine metabolism in isolated perfused liver (IPL) of endotoxin-treated rats. Male albino rats were injected with the endotoxin of *Salmonella enteritidis* (5 mg · kg^{-1}) or saline (control). Four hours later, leucine and ketoisocaproate (KIC) oxidation and incorporation into liver proteins were determined in IPL using the single-pass liver perfusion technique. L-[1-^{14}C]leucine and α-keto[1-^{14}C]isocaproic acid were used as a tracer in two separate experiments. Endotoxin treatment resulted in a decrease of plasma BCAA levels, an increase of leucine oxidation, and a decrease of KIC oxidation by IPL. Leucine incorporation into liver proteins was lower in endotoxin-treated rats, and we did not find measurable incorporation of the labeled carbon of KIC in liver proteins in either group of animals. The sum of individual amino acid concentrations in the effluent perfusate was higher in endotoxin-treated animals, although only leucine and phenylalanine increased significantly. The decrease in KIC oxidation indicates a decreased capacity of hepatic tissue to oxidize branched-chain ketoacids (BCKA). The increase in leucine oxidation by IPL of endotoxin-treated rats indicates an increase in BCAA aminotransferase activity. These changes demonstrate an important response of the body that enables the resynthesis of essential BCAA from their ketoanalogs delivered to the liver from peripheral tissues, particularly muscle.

ENDOTOXIN TREATMENT induces a marked production of cytokines, which are the principal mediators of the changes known as the systemic inflammatory response syndrome often observed in sepsis, trauma, burn injury, and cancer. Cytokines affect the metabolism of almost all tissues, and their effect is generally favorable for the body. For instance, skeletal muscle proteolysis and the consequent enhanced release of amino acids into the blood stream induced by tumor necrosis factor alpha and by some other cytokines enable the enhanced synthesis of immunoglobulins and acute-phase proteins in visceral tissues. However, the effect of cytokines on lean muscle tissue may be disastrous if it is excessive or lasts for a long time. Pronounced proteolysis of lean muscle tissue induced by cytokines and their anorectic action are the principle mechanisms for development of life-threatening muscle wasting and cachexia.

The branched-chain amino acids (BCAA) leucine, isoleucine, and valine are indispensable amino acids of special interest. In sepsis and trauma, insulin resistance develops in skeletal muscle to spare glucose for use by other tissues.[1] BCAA are used as an alternate energy source and for glutamine and alanine synthesis. Enhanced rates of BCAA oxidation and glutamine utilization have been observed in the whole body and in skeletal muscle in sepsis or trauma and after endotoxin or tumor necrosis factor treatment.[2-8] However, the contribution of visceral tissues to the alterations of BCAA metabolism in the systemic inflammatory response is unknown.

In our recent study using labeled ketoisocaproic acid (KIC), we observed a decrease of branched-chain ketoacid (BCKA) dehydrogenase (the key mitochondrial enzyme regulating BCAA oxidation) activity in isolated perfused liver (IPL) after administration of the endotoxin of *Salmonella enteritidis* in perfusion solution.[9] We hypothesized that the observed decrease of hepatic KIC oxidation demonstrates an important metabolic response that can resupply essential BCAA to the body. In this study, we investigated whether similar changes in hepatic KIC oxidation may also be observed in IPL of endotoxemic rats. In addition, we evaluated changes in the activity of BCAA aminotransferase, the catalyst for reversible transamination of BCAA.

MATERIALS AND METHODS

Animals

Male Wistar rats were obtained from VELAZ (Prague, Czech Republic). Rats were housed in standardized cages in quarters with a controlled temperature and 12-hour light-dark cycle and received Velaz-Altromin 1320 (VELAZ) laboratory chow and drinking water ad libitium. All procedures involving the animals were performed according to guidelines set by the Institutional Animal Use and Care Committee of Charles University.

Materials

L-[1-^{14}C]leucine and α-keto[1-^{14}C]isocaproate were obtained from Amersham International (Buckinghamshire, UK). Leucine, sodium salt of α-ketoisocaproic acid (KIC), lipopolysaccharide from *S enteritidis* (endotoxin), methylbenzethonium hydroxide, Folin-Ciocalteu phenol reagent, and bovine serum albumin were purchased from Sigma Chemical (St Louis, MO). The amino acid solution AMINO-MEL 10% pure was obtained from Leopold Pharma (Graz, Austria). The remaining chemicals were obtained from Lachema (Brno, Czech Republic).

Preliminary Study

In a preliminary study, the effect of endotoxin treatment on the change in some metabolic parameters in blood was estimated. The overnight-fasted rats were injected intraperitoneally with saline (control group) or lipopolysaccharide of *S enteritidis* at a dose of 5 mg · kg^{-1} (endotoxin group). Rats were killed by exsanguination via the abdominal aorta, and blood was collected for biochemical analyses 4 hours later. The dose of endotoxin and the time of death were based on data from Nawabi et al,[3] who observed the activation of muscle BCKA

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dehydrogenase in rats treated with this dose of endotoxin of *S enteritidis,* and our previous study.[10] For hematological examination, blood was mixed in a tube containing K_3EDTA (1.5 mg · mL^{-1} blood). The blood cell count was evaluated using a Coulter Counter JT3 blood particle analyzer (Coulter Electronics, Luton, UK). Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gammaglutamyltransferase (GMT), triglycerides, cholesterol, creatinine, and urea were measured using commercial tests (UV-KIN tests; Elitech, Sées, France; and Boehringer, Mannheim, Germany) on a Hitachi (Tokyo, Japan) 704 analyzer. Amino acid concentrations were determined in deproteinized blood plasma on a high-performance liquid chromatograph (Waters, Milford, MA) after precolumn derivatization with orthophthaldialdehyde.

Study Protocol

In the two separate experiments, we evaluated the effect of endotoxemia on leucine and KIC oxidation and incorporation into liver protein in IPL using the single-pass perfusion technique.[11] Four hours after endotoxin or saline treatment, the rats were anesthetized with sodium pentobarbital (35 mg · kg body weight $[BW]^{-1}$ intraperitoneally) and the livers prepared for perfusion. Briefly, after laparotomy, the bile duct was cannulated and heparin 1,000 IU · kg^{-1} was injected into the saphenous vein. Then, the portal vein was cannulated with a polyethylene catheter (ID 1.5 mm), and the hepatic artery was ligated. During portal perfusion with Krebs-Henseleit solution (20°C), the liver was quickly removed. Perfusion was performed at 37°C in a thermostatically controlled cabinet. A peristaltic pump moved the perfusate from the reservoir through an oxygenator and a bubble trap to the liver. Flow rates were maintained at 3.5 mL · g $liver^{-1}$ · min^{-1}. The membrane oxygenator was made from thin-walled silicone tubing (Silastic, ID 0.058 in, OD 0.077 in) 25 ft in length enclosed in a glass cylinder continually gassed with a mixture of O_2 and CO_2 (95%:5%) at a flow rate of 500 mL · min^{-1}. The glass bubble trap also served as a peristaltic wave compensator. Viability of the perfused livers was monitored by their appearance and the stability of the bile flow.

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer, 10 mmol/L glucose, and amino acids at approximately the normal plasma concentration in rats (996 mL Krebs-Henseleit solution mixed with 4 mL AMINO-MEL 10% pure), pH 7.4. In the first experiment, the perfusion solution contained 1.26 mmol/L leucine, 0 mmol/L KIC, and [1-^{14}C]leucine (10 μgCi · L^{-1}) as a tracer. In the second study, the perfusion solution contained 0.26 mmol/L leucine, 1 mmol/L KIC, and α-keto[1-^{14}C]isocaproate (2 μCi · L^{-1}). Before starting the experimental protocol, the livers were perfused with tracer-free perfusion medium for 15 minutes to ensure stabilization of the liver and washout of endogenous hormones. At minute 16, the perfusion medium containing a tracer was infused for 10 minutes, and samples of the effluent perfusate were collected in 20-mL flasks equipped with stoppers and center wells containing 0.4 mL methylbenzethonium hydroxide at 1-minute intervals to monitor $^{14}CO_2$ production. At the end of the second study (experiment with perfusion solution containing 1 mmol/L KIC), samples of the effluent perfusate were collected for analysis of amino acid concentrations. Labeled carbon dioxide in the perfusate that was produced from the infused 1-^{14}C-labeled substrates was released by injecting 0.5 mL 5N sulfuric acid through the stopper into the flasks. At the end of perfusion, the liver was quickly frozen in liquid nitrogen and saved for the measurement of leucine incorporation into protein. Oxidation rates of leucine and KIC were calculated as $O = (R \cdot F)/(SA \cdot W)$, where O is the substrate oxidation (micromoles of substrate oxidized per gram of dry liver per hour), R is the radioactivity of $^{14}CO_2$ in the effluent perfusate (dpm per milliliter), F is the flow rate of perfusion medium through the liver (milliliters per hour), SA is the specific activity of Leu or KIC in the perfusion medium (dpm per micromole), and W is the liver dry weight (grams).

For assessment of leucine incorporation into protein, small pieces of liver tissue (~0.5 g) were homogenized in perchloric acid (2%). The precipitated proteins were collected by centrifugation and washed three times. The pellet was hydrolyzed in 2N NaOH. Aliquots were taken for protein analysis[12] and radioactivity measurement. Radioactivity of the samples was measured with the LS 6000 liquid scintillation radioactivity counter (Beckman Instruments, Fullerton, CA). Incorporation of leucine and/or KIC into the protein of IPL was calculated by dividing the [^{14}C]leucine radioactivity in liver protein (dpm per milligram of protein) by the specific activity of substrate in the perfusion medium (dpm per micromole) and by the time of liver perfusion with the label (hours).

Statistical Analysis

Results are presented as the mean ± SE. The significance of differences in mean values was checked using the F test and Student's *t* test for unpaired data, with *P* less than .05 regarded as significant.

RESULTS

Within 1 hour after injection of endotoxin, the animals began to show signs of acute illness such as lethargy, piloerection, and diarrhea. In our recent study, we observed that these signs are not present at 16 hours and no deaths occurred.[10] Tables 1 to 3 present the evaluated parameters for control and experimental animals. The red blood cell count, hemoglobin concentration, hematocrit, and leukocyte count were not significantly different. However, endotoxin treatment resulted in a significant decrease in platelet count in the blood, probably caused by disseminated intravascular coagulation.[13] The insignificant differences in ALT, AST, GMT, urea, and creatinine levels demonstrate an intact function of the liver and kidney, respectively. However, the decreased plasma glucose and increased triglyceride concentrations demonstrate marked metabolic perturbations (Table 1). Plasma levels of BCAA and most of the other amino acids decreased or tended to decrease in endotoxin-treated rats (Table 2). The insignificant differences in the wet and dry weight of the liver, concentration of liver protein, and bile flow demonstrate identical experimental conditions during the liver perfusions (Table 3).

In endotoxin-treated rats, an increase in leucine oxidation and a decrease in KIC oxidation were observed (Figs 1 and 2). The

Table 1. Characteristics of the Experimental Animals (preliminary study)

Characteristic	Control (n = 8)	Endotoxin (n = 8)
Blood		
Red blood cell count (×10^{12} · L^{-1})	7.62 ± 0.19	7.84 ± 0.14
Hemoglobin (g · L^{-1})	151.8 ± 4.7	157.8 ± 1.6
Hematocrit (%)	43.7 ± 1.1	44.3 ± 0.55
White blood cell count (×10^9 · L^{-1})	4.99 ± 0.37	4.61 ± 0.30
Platelets (×10^9 · L^{-1})	885 ± 68	625 ± 76*
Plasma		
AST (μkat · L^{-1})	1.51 ± 0.06	1.57 ± 0.08
ALT (μkat · L^{-1})	0.59 ± 0.04	0.56 ± 0.04
GMT (μkat · L^{-1})	0.21 ± 0.01	0.23 ± 0.01
Urea (mmol · L^{-1})	6.84 ± 0.29	6.96 ± 0.42
Creatinine (μmol · L^{-1})	43.25 ± 1.15	41.75 ± 1.39
Glucose (mmol · L^{-1})	6.4 ± 0.54	4.76 ± 0.27*
Triglycerides (mmol · L^{-1})	0.41 ± 0.07	0.77 ± 0.08*
Cholesterol (mmol · L^{-1})	1.40 ± 0.07	1.25 ± 0.06

NOTE. Data are the mean ± SE. The F test and Student's *t* test were used for unpaired data.

*$P < .05$ *v* control.

Table 2. Blood Plasma Amino Acid Levels 4 Hours After Endotoxin Treatment (preliminary study)

Amino Acid	Control (n = 8)	Endotoxin (n = 8)
Taurine	62 ± 4	76 ± 4*
Aspartate	26 ± 2	26 ± 3
Threonine	129 ± 9	100 ± 9*
Serine	99 ± 5	71 ± 5*
Asparagine	54 ± 4	44 ± 4
Glutamate	86 ± 10	54 ± 4*
Glutamine	238 ± 13	167 ± 16*
Glycine	72 ± 6	69 ± 2
Alanine	136 ± 12	90 ± 5*
Valine	91 ± 6	78 ± 4
Methionine	33 ± 2	44 ± 5
Isoleucine	81 ± 5	51 ± 3*
Leucine	94 ± 9	60 ± 3*
Tyrosine	53 ± 5	51 ± 2
Phenylalanine	54 ± 4	52 ± 2
Tryptophane	52 ± 4	61 ± 5
Ornithine	16 ± 2	15 ± 2
Lysine	121 ± 11	138 ± 23
Histidine	23 ± 2	25 ± 1
Arginine	31 ± 2	28 ± 2
Derived values		
BCAA	266 ± 15	190 ± 7*
Total amino acids	1,550 ± 60	1,299 ± 64*

NOTE. Data are the mean ± SE. The F test and Student's *t* test were used for unpaired data.

*$P < .05$ *v* control.

values for leucine oxidation were approximately 25-fold (controls) and 16-fold (endotoxin-treated rats) less than for KIC oxidation. Table 4 shows the significant decrease in leucine incorporation into the proteins of IPL of endotoxin-treated rats. We did not find measurable incorporation of the labeled carbon of KIC into liver proteins in either group of rats. The sum of perfusate amino acid concentrations after liver perfusion was higher in endotoxin-treated animals, although only leucine and phenylalanine increased significantly (Table 5).

DISCUSSION

Changes in Plasma Amino Acid Levels

The decrease in most of the amino acid levels in endotoxin-treated rats, particularly BCAA, is consistent with a decrease of plasma amino acid levels in sepsis.[14] Considering that our results were obtained in fasted rats, the basic mechanism for the decrease of most of the plasma amino acids after endotoxin

Table 3. Characteristics of the Experimental Animals

Characteristic	Control (n = 18)	Endotoxin (n = 16)
Body weight (g)	238 ± 9	231 ± 8
Liver weight		
Wet (g · kg BW^{-1})	31.5 ± 1.2	33.6 ± 1.7
Dry (% of wet weight^{-1})	28.2 ± 0.5	27.7 ± 0.4
Liver proteins (mg · g dry liver^{-1})	503 ± 23	472 ± 14
Bile production (mg · g dry liver^{-1} · min^{-1})	294 ± 15	286 ± 20

NOTE. Data are the mean ± SE. The F test and Student's *t* test were used for unpaired data.

*$P < .05$ *v* control.

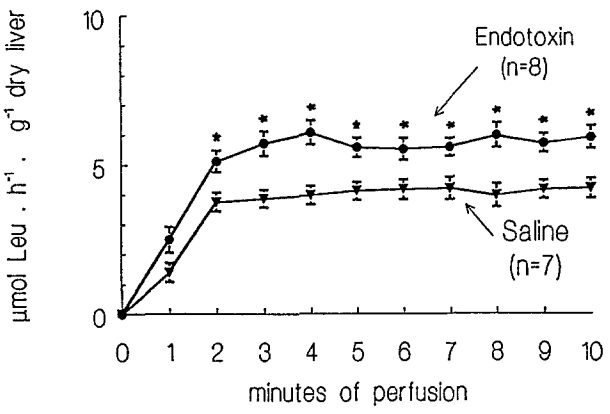

Fig 1. Oxidation of leucine in IPL of rats 4 hours after intraperitoneal injection of endotoxin. Data are the mean ± SE.*$P < .05$ *v* control rats.

treatment was a higher uptake versus release by body tissues. However, it should be noted that some studies have shown that BCAA levels are unchanged or even elevated in septic patients.[15] These findings demonstrating significant changes in the release and uptake of amino acids during disease development may have practical importance, as some studies reported a beneficial effect of BCAA administration in the treatment of sepsis or trauma.[16-19] In the case of a low plasma BCAA level, BCAA administration may have a therapeutic influence; however, if the plasma BCAA level is elevated, it should be assumed that the extra BCAA will not have a therapeutic effect. Unfortunately, there is little, if any, information regarding the therapeutic effect of BCAA administration in the systemic inflammatory response syndrome in relation to the actual levels of BCAA in plasma and/or tissues.

Changes in Hepatic Leucine Metabolism

Since BCAA aminotransferase activity in the liver is very low while BCKA dehydrogenase activity is high,[20,21] evaluation of CO_2 release using leucine as a substrate indicates changes in the flux of leucine through BCAA aminotransferase. Evaluation of CO_2 release using labeled KIC indicates changes in the flux through BCKA dehydrogenase.[11] In addition, the higher rate of

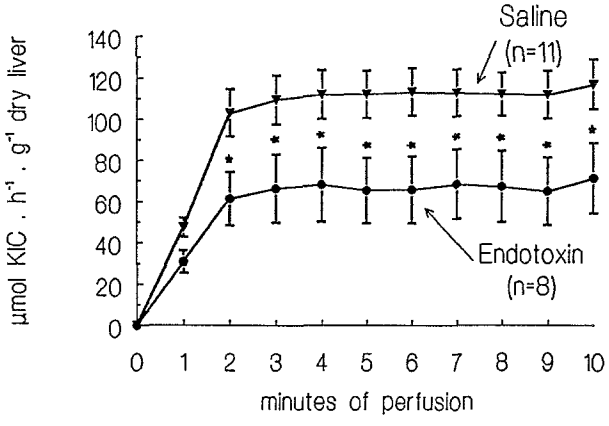

Fig 2. Oxidation of KIC in IPL of rats 4 hours after intraperitoneal injection of endotoxin. Data are the mean ± SE. *$P < .05$ *v* control rats.

Table 4. Effect of Bolus Injection of Endotoxin on Incorporation of Leucine and KIC Into Proteins of IPL

	Control	Endotoxin
Leucine	5.26 ± 0.42 (n = 7)	4.03 ± 0.28* (n = 8)
KIC	ND (n = 11)	ND (n = 8)

NOTE. Units are nmol · mg · protein^{-1} · h^{-1} and are the mean ± SE. The F test and Student's *t* test were used for unpaired data.

*$P < .05$ *v* saline control.

Table 5. Effect of Bolus Injection of Endotoxin on Perfusate Amino Acid Concentrations After the Liver

Amino Acid	Control (n = 11)	Endotoxin (n = 8)
Aspartate (230)	162 ± 13	171 ± 15
Threonine (134)	78 ± 12	87 ± 13
Serine (350)	176 ± 14	199 ± 14
Glycine (362)	228 ± 14	221 ± 15
Alanine (350)	254 ± 12	229 ± 16
Valine (191)	174 ± 12	185 ± 12
Methionine (107)	60 ± 7	81 ± 9
Isoleucine (134)	68 ± 7	80 ± 8
Leucine (262)	171 ± 11	245 ± 23*
Tyrosine (181)	110 ± 9	118 ± 9
Phenylalanine (111)	55 ± 5	81 ± 9*
Tryptophane (35)	28 ± 4	30 ± 3
Lysine (157)	95 ± 12	118 ± 10
Histidine (113)	96 ± 9	108 ± 5
Derived value		
BCAA	412 ± 15	590 ± 34*
Total amino acids	1,771 ± 52	1,950 ± 30*

NOTE. Units are μmol · L^{-1}. Values in parentheses indicate amino acid concentration in perfusion solution before the liver. Data are the mean ± SE. The F test and Student's *t* test were used for unpaired data.

*$P < .05$ *v* control.

hepatic KIC oxidation versus leucine oxidation supports the idea of interorgan cooperation in BCAA oxidation and demonstrates the crucial position of the liver in BCAA oxidation via decarboxylation of BCKA delivered to the liver by the bloodstream. Skeletal muscle, because of the high level of BCAA aminotransferase and low level of BCKA dehydrogenase, releases significant amounts of BCKA into the circulation.[20,22] These BCKA are oxidized in tissues with high BCKA dehydrogenase activity, primarily the liver and adipose tissue.[21]

The present study demonstrates the decreased rate of KIC oxidation in the IPL of endotoxin-treated rats, while leucine oxidation increased. The decrease of KIC oxidation in IPL of endotoxin-treated rats is in good agreement with the results of our previous study in which endotoxin was administered into the perfusion medium, and undoubtedly indicates the decreased capacity of hepatic tissue to oxidize BCKA.[9] Since BCAA transamination is reversible, the decreased oxidation of BCKA enables resynthesis of a larger amount of essential BCAA. The ability of the liver to reaminate BCKA was demonstrated by several investigators.[23,24] The direction of the flux depends on the supply of BCAA and BCKA, the rate of BCKA oxidation, and probably the rate of other processes as well.[25] We assume that the observed increase of leucine oxidation by IPL of endotoxin-treated animals indicates merely the increase of substrate flux through BCAA aminotransferase and not the increase of in vivo leucine oxidation. Thus, a decrease of BCAA concentration in the plasma together with a decrease of hepatic BCKA decarboxylation and an increase of hepatic BCAA aminotransferase activity may result in a marked increase of BCAA resynthesis from BCKA delivered into the liver by the bloodstream. These essential BCAA may be used in the liver and/or released into the bloodstream and then oxidized and/or used in protein synthesis by other tissues.

The significantly lower values for leucine incorporation into liver proteins of endotoxin-treated rats indicate decreased protein synthesis. A similar result was also obtained in our previous in vivo experiment.[10] This decrease in protein synthesis may be caused by a separate decrease in the synthesis of some tissue proteins (eg, membrane proteins) and/or a decrease in the synthesis of some secretory proteins (eg, albumin, fibrinogen, C-reactive protein, etc.). Unfortunately, we did not measure the incorporation of leucine into particular types of proteins in this study.

The decreased rate of protein synthesis in IPL of endotoxin-treated animals is in agreement with the observation of higher amino acid levels in the perfusate. Changes in the concentration of amino acids in the effluent perfusate are undoubtedly also affected by the endotoxin effect on protein breakdown. However, it should be noted that the increase in perfusate total amino acid concentrations in endotoxin-treated animals was caused mainly by leucine and that the measurements of perfusate amino acid levels were performed in the experiment using a high KIC concentration in the perfusion solution. This observation of a significant increase in leucine in the effluent perfusate of the IPL of endotoxin-treated rats supports our hypothesis of an increased reamination of BCKA in hepatic tissue in the systemic inflammatory response syndrome. We believe that the decreased rate of leucine incorporation and undetectable [^{14}C]KIC incorporation in liver proteins and the increased leucine concentration in the effluent perfusate after endotoxin treatment indicate that most of the BCAA reaminated from BCKA are released into the bloodstream.

Conclusion

On the basis of the results obtained, we conclude that the decrease in BCKA oxidation and the increase in BCKA flux through BCAA aminotransferase in the liver of endotoxin-treated rats enable resynthesis of a significant amount of BCAA from BCKA delivered to the liver by the bloodstream. This metabolic alteration enables the preservation of essential BCAA for the body's requirements.

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