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Ornithine α-ketoglutarate metabolism in the healthy rat in the postabsorptive state

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Abstract

To gain further insight into the ability of ornithine α-ketoglutarate (OKG) to generate key metabolites, the aim of this work was to study the short-term metabolism, that is, 1 hour after administration, of OKG in plasma and tissues. Particular attention was paid to keto acids (α-ketoglutarate and branched-chain keto acids). Young (3 weeks old) male Wistar rats in the postabsorptive state received either 1.5 g/kg of monohydrated OKG (OKG group, n = 8) diluted in distilled water or an equivalent volume of saline solution at 0.9% (control group, n = 8) by gavage and were killed 1 hour later. Plasma, liver, jejunal and ileal mucosa, and the extensor digitorum longus muscle were removed to analyze amino and keto acid contents. Major metabolites detected after OKG ingestion (ornithine [ORN], α-ketoglutarate, proline and glutamate; OKG vs control, $P < .05$) and the absence of increased arginine (and even a decrease in jejunum and muscle) and citrulline levels suggested that ORN was mainly metabolized by the ORN aminotransferase pathway. In addition, significantly decreased plasma branched-chain keto acids and increased hepatic branched-chain amino acids (OKG vs control, $P < .05$) were observed upon OKG ingestion. Finally, glutamine accumulation restricted to the intestine, as evidenced in this short-term study, suggests that the effects of OKG on glutamine pools in other tissues in various pathological states after several days of treatment, as observed in previous studies, may be related to a long-term induction of glutamine synthetase.

1. Introduction

Ornithine α-ketoglutarate (OKG) is a part of the immunopharmaconutrient family. This salt, formed of 2 molecules of ornithine (ORN) and 1 molecule of α-ketoglutarate (αKG), has been used successfully via oral, enteral, and parenteral routes to improve protein status in chronically and acutely protein-depleted patients (see Refs [1,2] for recent reviews on the topic). At doses of 10 to 25 g/d, OKG administration improves nitrogen balance and attenuates protein catabolism by limiting muscular proteolysis in hypercatabolic states such as burn patients [3-5], surgical patients [6], and traumatized and septic patients [7,8]. In addition, it has been demonstrated that OKG administration improves wound healing in burn patients [3-5]. In elderly patients, OKG treatment improves nutritional status and quality of life and reduces recovery time from infection or surgery [2,9].

The effects of OKG on protein metabolism were also studied in animal models: 1.5 to 5 g/kg per day of OKG modulates protein metabolism by decreasing urinary nitrogen excretion in traumatized rats [10], by increasing hepatic and jejunal protein synthesis, and by inhibiting myofibrillar degradation, total proteolysis [11], and muscular glutamine depletion induced by burn injury [12] or trauma [10]. OKG has also been shown to have immunomodulatory properties in several stress situations [13-15].

OKG administration results in increased catabolism of branched-chain amino acids (BCAAs) to their corresponding α-keto acids (branched-chain keto acids [BCKAs]) and leads to the production of key metabolites such as glutamine, arginine, proline, and polyamines that may be involved in its mechanism of action (see Ref [1] for a recent review). Recent studies [14,16] using metabolic inhibitors strongly support the idea of glutamine and arginine involvement in the mechanism of action of OKG; however, further progress requires a better understanding of OKG metabolism in vivo. Thus, the effects of OKG

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administration on the plasma amino acid profile of healthy volunteers, compared with the administration of αKG or ORN alone [17], led us to conclude that the common metabolic pathway for αKG and ORN, with a specific interaction between αKG and ORN, is a key factor in explaining the effects of OKG [18]. Through this common metabolic pathway, the urea cycle (via the ORN moiety of OKG) and the Krebs cycle (via the αKG moiety of OKG) are linked, with ORN leading to αKG generation and αKG leading to ORN synthesis. Indeed, ORN aminotransferase can metabolize ORN into glutamate semialdehyde (GSA) and vice versa according to the organ. GSA can be converted into glutamyl-phosphate with co-formation of ATP from ADP, and then into glutamate with cooxidation of $NADP^+$ into NADPH. In turn, glutamate can be transaminated into αKG which, at the end, is oxidized in the Krebs cycle (see Ref [1] for a figure describing these pathways). Because the in vivo activities of some transaminases are so rapid [19], high amounts of αKG may alter amino acid metabolism. In addition, all the reactions cited above are reversible, either enzymatically driven or chemically instable, and the transamination of ORN into glutamate almost reaches equilibrium in vivo [18,20,21]. Thus, the administration of both αKG and ORN as OKG salt diverts these molecules and their direct metabolites toward other pathways generating glutamine (from glutamate, by the action of glutamine synthetase), arginine (from ORN via citrulline and argininosuccinate), proline (from GSA), and polyamines (as a result of decarboxylation of ORN), and all of these substrates play an important role in maintaining protein homeostasis [18].

In addition, the duration of OKG treatment may influence the action of OKG. For example, the increase in glutamine pools has only been demonstrated when patients or animals were treated for several days [6,11,12]. Hence, we failed to observe any increase in plasma glutamine content when OKG was administered as a single bolus to healthy subjects [17], whereas the same type of pharmacokinetic study indicated a clear increase in plasma glutamine in burn patients after 7 days of treatment [22]. Reasons for this discrepancy may be related to (i) the induction of enzymes with time of exposure to OKG: glutaminase, ORN aminotransferase, and enzymes of the urea cycle are known to be inducible by nutritional manipulations [23]; and (ii) the fact that the size of the glutamine pool may simply be related to the anabolic state of the tissue, as glutamine deficiency correlates with tissue atrophy.

In fact, few studies have investigated OKG metabolism in the short term [17]. In addition, the studies by Cynober et al [17] and Le Bricon et al [22] were restricted to the measurement of plasma amino acid concentrations. The study by Winkler et al [24], only published in abstract form, was limited to the splanchnic area. Finally, Vaubourdolle et al evaluated ORN and αKG metabolites 30 minutes after parenterally administered labeled ORN or αKG [25] and 60 minutes after enterally administered labeled ORN in the presence or absence of unlabeled αKG [26] in healthy rats. However, the method used provided qualitative data only. There are almost no data on αKG tissue pools after OKG administration, and data concerning BCKA remain anecdotal [27], despite the fact that BCKA production has been suggested to be involved in the mechanism of action of OKG [1]. This lack of data may be explained by the fact that it is difficult to perform accurate keto acid measurement at the tissue level.

Therefore, to gain further insight into the ability of OKG to generate key metabolites, the aim of this work was to study the short-term metabolism of OKG in plasma and tissue. For reasons explained above, particular attention was paid to keto acids (ie, αKG and BCKA). This study was performed on rats in the postabsorptive state to limit interferences related to nutrient intake. The time course of 1 hour was chosen because it corresponds to the plateau of appearance of ORN after enteral bolus administration in the presence of αKG in healthy rats [26].

2. Materials and methods

2.1. Animals and experimental protocol

Sixteen young (3 weeks old) male Wistar rats (Charles River, L'Arbresle, France) were housed individually in a controlled temperature environment (21°C ± 1°C), with a 12-hour light/dark cycle, and maintained on rat chow (A04, UAR, Epinay sur Orge, France) and water ad libitum for a 5-day acclimatization period. Young rats were used in this study because their protein turnover is faster and more sensitive to diet variations than that of older rats.

This study complied with the guidelines of our institution for animal care, and 1 member of the study team has French government authorization to use this animal model (LC, no. 75.461).

After a 5-hour fast, rats were randomized to receive either 1.5 g/kg of monohydrated OKG (OKG group, n = 8) diluted in distilled water or an equivalent volume of 0.9% saline solution (control group, n = 8) by gavage. Doses of 1.5 to 5 g of OKG per kilogram have been used in experiments on rats [10-15]. In the present study, as rat metabolism is roughly 10 times faster than that of humans, the dose of 1.5 g of OKG/kg was chosen because it represents, when extrapolated, the dose (10 g) usually administered in patients as a single bolus without side effects such as diarrhea [1,2].

One hour after gavage, the rats were anesthetized with isoflurane (3% in oxygen, using a Minerve apparatus, Esternay, France) before sacrifice by decapitation. Blood was collected into heparin-containing tubes which were immediately centrifuged (10 minutes, 2500 g, +4°C). The plasma was divided into 2 samples: one for keto acid quantification was stored at −80°C until analysis, and the other for the amino acid analysis was deproteinized (with

sulfosalicylic acid, 30 mg/mL) before being stored at −80°C until analysis. Twenty centimeters (2 × 10 cm) of proximal jejunum and proximal ileum was promptly removed. They were washed with ice-cold 0.9% NaCl (wt/wt) through the lumen and turned over to collect the mucosa by scraping with blades of glass. The intestine mucosa, 2 pieces of liver, and the extensor digitorum longus muscles (right and left) were promptly removed, weighed, frozen, and stored at −80°C until analysis. One half of the samples was intended for amino acid analysis, and the other was used to quantify the keto acids.

2.2. Measurements

2.2.1. Amino acid analysis

The frozen tissues were homogenized in ice-cold 10% trichloroacetic acid containing 0.5 mmol/L EDTA, and norvaline was used as a sample preparation internal control. The acid-soluble fraction containing free amino acids was separated by centrifugation from precipitated proteins (10 minutes, 2500 g, +4°C). Amino acid concentrations in tissues and deproteinized plasma were determined by ion-exchange chromatography with ninhydrin detection (AminoTac JLC-500V, Jeol, Tokyo, Japan) [28].

Our laboratory is registered to the European Quality Control Program. Our results ensure the reliability of measurements for all the amino acids studied. Note that citrulline concentration is shown only for the plasma because of an unsatisfactory quantification in some tissue samples. Arginine is not detectable in the liver. Results are expressed in micromolar per liter of plasma or micromolar per gram of tissue.

2.2.2. Keto acid analysis

Tissue αKG and plasma keto acid concentrations were determined by reversed-phase high-performance liquid chromatography with fluorimetric detection after a derivatization to the corresponding quinoxalinol. The technique used is an adaptation of that developed by Pailla et al [29]. Briefly, the frozen tissues were first homogenized in saline solution (0.9%, 1 mL for 100 mg of tissue). Internal standard (ketovalerate [Sigma, Saint Quentin-Fallavier, France], 180 μmol/L) and 1 mL of the fluorogenic agent (*o*-phenylenediamine [Sigma], prepared by dissolving 133 mg of *o*-phenylenediamine in 100 mL of 3 mol/L hydrochloridric acid) were added to 100 μL of plasma and 100 μL of saline solution (0.9%), or to 200 μL of homogenized tissue. The derivatization was performed by heating the mixtures at 80°C for 20 minutes. The samples were then cooled in an ice bath. Sodium sulfate (0.5 g) was added to each tube, and the quinoxalinol derivatives were twice extracted with 1 mL of ethyl acetate. The organic phases were mixed and evaporated to dryness under a nitrogen stream at 37°C. The residue was then dissolved in 500 μL of a buffer solution containing 75% Na_2HPO_4 (50 mmol/L, pH 6.7) and EDTA-Na_2 (0.27 mmol/L) and 25% of methanol. The samples were then filtered on a 0.45-μm filter.

Samples were chromatographed at room temperature at a flow rate of 1.2 mL/min (pump: Spectra System P1000 XR), using a Nucleosil CN column (150 × 4.6 mm; 5 μm particles) followed by a Kromasil C18 column (150 × 4.6 mm; 5-μm particles, Touzard et Matignon, Paris,

Table 1
Tissue OKG metabolites, alanine, and BCAA concentrations

	Control group	OKG group
Jejunal mucosa (μmol/g)		
Ornithine	0.11 ± 0.04[a]	4.24 ± 0.64[b]
Proline	0.41 ± 0.07[a]	0.83 ± 0.03[b]
Glutamate	2.05 ± 0.09[a]	3.21 ± 0.20[b]
Glutamine	0.39 ± 0.05[a]	1.32 ± 0.13[b]
Arginine	0.20 ± 0.04[a]	0.08 ± 0.01[b]
Alanine	2.23 ± 0.13[a]	3.08 ± 0.22[b]
Valine	0.29 ± 0.05	0.25 ± 0.02
Isoleucine	0.18 ± 0.04	0.12 ± 0.02
Leucine	0.31 ± 0.07	0.22 ± 0.02
Ileal mucosa (μmol/g)		
Ornithine	0.14 ± 0.04[a]	8.03 ± 0.90[b]
Proline	0.37 ± 0.02[a]	0.76 ± 0.05[b]
Glutamate	2.29 ± 0.26[a]	4.21 ± 0.22[b]
Glutamine	0.55 ± 0.06[a]	1.56 ± 0.11[b]
Arginine	0.19 ± 0.02[a]	0.18 ± 0.03[b]
Alanine	1.76 ± 0.03[a]	2.50 ± 0.23[b]
Valine	0.26 ± 0.01	0.22 ± 0.02
Isoleucine	0.16 ± 0.01[a]	0.09 ± 0.01[b]
Leucine	0.26 ± 0.01[a]	0.18 ± 0.03[b]
Liver (μmol/g)		
Ornithine	0.38 ± 0.02[a]	3.12 ± 0.41[b]
Proline	0.21 ± 0.02[a]	0.67 ± 0.10[b]
Glutamate	2.30 ± 0.16[a]	5.02 ± 0.39[b]
Glutamine	6.83 ± 0.22	5.97 ± 0.36
Alanine	2.44 ± 0.19[a]	4.42 ± 0.22[b]
Valine	0.28 ± 0.01[a]	0.73 ± 0.10[b]
Isoleucine	0.15 ± 0.01[a]	0.31 ± 0.05[b]
Leucine	0.28 ± 0.01[a]	0.84 ± 0.12[b]
Plasma (μmol/L)		
Ornithine	73 ± 7[a]	2251 ± 216[b]
Proline	208 ± 19[a]	421 ± 37[b]
Glutamate	91 ± 5[a]	182 ± 16[b]
Glutamine	746 ± 27	723 ± 33
Arginine	190 ± 28	118 ± 12
Citrulline	262 ± 15[a]	193 ± 22[b]
Alanine	536 ± 40[a]	902 ± 98[b]
Valine	187 ± 12	176 ± 11
Isoleucine	88 ± 8	84 ± 7
Leucine	99 ± 7	98 ± 7
EDL (μmol/g)		
Ornithine	0.13 ± 0.02[a]	1.01 ± 0.15[b]
Proline	0.28 ± 0.05	0.42 ± 0.05
Glutamate	1.84 ± 0.26	1.75 ± 0.21
Glutamine	5.23 ± 0.67	5.12 ± 0.33
Arginine	0.19 ± 0.04[a]	0.06 ± 0.01[b]
Alanine	2.21 ± 0.32[a]	3.62 ± 0.39[b]
Valine	0.15 ± 0.02	0.14 ± 0.02
Isoleucine	0.07 ± 0.01	0.04 ± 0.01
Leucine	0.10 ± 0.01	0.24 ± 0.13

Results are expressed as means ± SEM. Line values with different superscripts are significantly different ($P < .05$). EDL indicates extensor digitorum longus muscle.

France). The composition of the mobile phase was as follows: the starting mobile phase consisted of 85% of solution A (Na_2HPO_4 [50 mmol/L, pH 6.7] and EDTA-Na_2 [0.27 mmol/L]) and 15% of solution B (acetonitrile). This was changed by a linear gradient to 79% of A and 21% of B for 5.5 minutes, then to 65% of A and 35% of B for 2.7 minutes, to 60% of A and 40% of B for 9.8 minutes. This composition was maintained for 2 minutes. The initial conditions were then applied from the 20th to the 21st minute.

The quinoxalinol derivatives were detected by monitoring the fluorescence emission at 410 nm (with excitation at 350 nm) using a fluorescence detector (Shimadzu RF-535, distributed by Touzard et Matignon).

2.3. Statistical analysis

Data are presented as means ± SEM. Data were analyzed using the Student *t* test (PCSM software, Deltasoft, Meylan, France).

3. Results

3.1. Amino acids related to OKG metabolism

One hour after OKG ingestion, OKG-treated rats exhibited higher ORN concentrations in all the tissues studied in comparison to controls (OKG vs control, $P < .05$) (Table 1). Proline and glutamate concentrations (Fig. 1) were higher in intestinal mucosa, liver, and plasma (OKG vs control, $P < .05$). Glutamine concentrations were higher in intestinal mucosa (OKG vs control, $P < .05$), whereas arginine concentrations were lower in jejunal mucosa and muscle (OKG vs control, $P < .05$). In addition, interestingly, OKG-treated rats exhibited remarkably higher alanine concentration levels (OKG vs control, $P < .05$) in all the tissues studied (Fig. 1).

3.2. Plasma and tissue α-keto acid content

In comparison with the control group, αKG concentrations were higher in the plasma and intestinal mucosa of

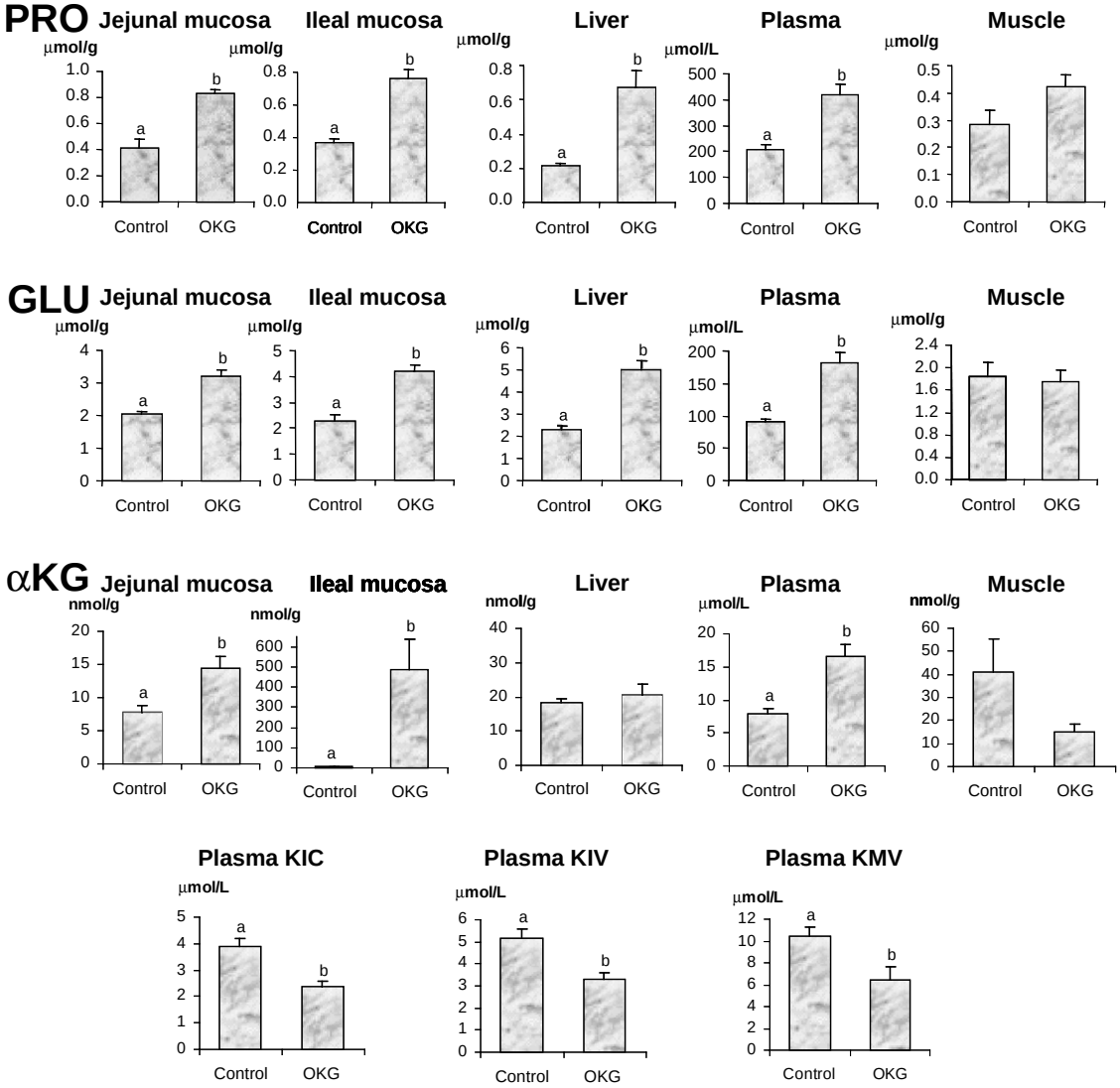

Fig. 1. Tissue proline (PRO), glutamate (GLU), and αKG concentrations and plasma keto acid concentrations (ketoisocaproate [KIC], ketoisovalerate [KIV], ketomethylvalerate [KMV]). Bars with different superscripts are statistically different ($P < .05$).

OKG-treated rats (OKG vs control, $P < .05$) (Fig. 1). αKG concentrations were not significantly different from control in the liver and in the muscle. The other keto acid plasma concentrations were decreased (OKG vs control, $P < .05$).

4. Discussion

In the intestinal mucosa, 1 hour after the ingestion of OKG, the concentrations of αKG, ORN, glutamate, glutamine, and proline were higher in the OKG-treated rats than in the control group. These compounds are produced by the metabolism of αKG and ORN. Indeed, it has been shown that, 60 minutes after an enteral administration of ^{14}C-ORN (without αKG) in healthy rats, radiolabeled ORN, proline, and glutamate can be found in the intestine [26]. Similarly, 30 minutes after a parenteral administration of ^{14}C-αKG (without ORN) in healthy rats, radiolabeled αKG, ORN, glutamate, glutamine, and proline were found in the intestinal mucosa [25]. The higher glutamine levels may have 2 origins: first, glutamine could be the result of glutamate amidation in the intestine. It is to be noted that young rats have a more active intestinal glutamine synthetase than adults [30]. Second, the accumulation of ORN may block the reaction mediated by ORN aminotransferase (a fully reversible enzyme), leading to glutamate accumulation and to an increase in glutamine content [1]. Indeed, ORN aminotransferase activity is very active in the intestine [23] and specifically works from glutamate to ORN [21] to ensure the flux from glutamine to citrulline [23], and also probably because of a rapid use of ORN for polyamine synthesis.

The higher levels of alanine together with lower levels of leucine and isoleucine than in controls in ileal mucosa are consistent with the cycling of αKG/glutamate in a number of transamination reactions (ie, alanine and BCAA transaminases) [31]. This is supported by the fact that, in this tissue, combined pyruvate plus oxaloacetate concentration (our technique did not allow us to separate these 2 compounds, probably due to decarboxylation of oxaloacetate) was higher in the OKG-treated rats than in the controls (data not shown) because increased pyruvate might further favor the increase in the alanine pool. It is likely that pyruvate represents the bulk because oxaloacetate tissue levels are very low [32]. The determination of the source of pyruvate is beyond the scope of this paper but again underlines how OKG can modify the intermediary metabolism.

On the other hand, the low arginine content in the jejunal mucosa is an intriguing finding, in contradiction with the results of other experimental studies performed in injured rats treated with OKG for several days, in which arginine was either unchanged compared with control [15,33-35] or increased [36]. At this time, we can offer no clear explanation of this phenomenon. Clearly, it is not a question of dose since Lasnier et al [36] observed an arginine increase with OKG treatment providing only 0.5 g/kg per day. However, in this latter study, OKG was administered over 2 days.

In the liver, the observed pattern (higher ORN, proline, and glutamate in the OKG-treated rats than in controls) reflects the flux of molecules that presumably come from the intestine via the portal vein. In addition, proline and glutamate could be synthesized from αKG and ORN because the liver content of these amino acids increases when isolated rat liver is perfused with OKG [37]. The fact that hepatic αKG concentrations did not differ from controls may be due to a limited uptake by the liver, an idea supported by the fact that plasma concentrations of αKG increase after OKG administration. Indeed, little is known about this process which occurs in perivenous hepatocytes [38].

It is also likely that a part of the glutamine produced by the intestine may be released into the portal vein and taken up by the liver after OKG treatment. Knowing that periportal glutaminase and perivenous glutamine synthetase are simultaneously active, resulting in an intercellular glutamine cycle [39], the fact that no changes in glutamine concentrations were observed in the liver supports the argument for a very intensive and rapid metabolism of this amino acid by periportal hepatocytes, once taken up by these cells [40].

On the other hand, hepatic BCAAs increased by more than 200%. Because BCAA levels in the ileal mucosa of OKG-treated rats were low compared with controls, it is unlikely that BCAA accumulation in the liver came from the portal vein. BCAA may have 2 other sources: (i) proteolysis; an increase in proteolysis in the liver of OKG-treated rats seems very unlikely because it has been shown that OKG treatment increases net liver protein synthesis [11], or (ii) retransamination of BCKAs into BCAAs. This is a possible option, because this reaction is favored by high levels of glutamate. However, it has been suggested that BCAA transaminase activity is low in hepatocytes [41] with species differences [42]. Nevertheless, we cannot exclude that this reaction occurs in other cells such as Kupffer or endothelial cells [43].

In the plasma (obtained from mixed blood), the 2 components of the OKG salt (ORN and αKG) and their metabolites formed in the intestine and the liver (proline and glutamate) were recovered in higher concentrations in OKG-treated rats than in controls. These results are consistent with those obtained measuring amino acid flux in a model of isolated perfused rat liver [37]. In contrast, an unexpected decrease in BCKAs was observed, which is not in accordance with the suggested formation of these compounds in healthy volunteers after OKG administration (increase in the plasma concentration of glutamate and a decrease in that of leucine [17]), nor with the observed increase in plasma concentrations of ketoisocaproate in burn patients treated with OKG [27]. However, in the latter study, patients were in fed state. The low plasma BCKAs observed in OKG-treated rats compared with controls could be the result of a decreased muscular BCKA release or an increased uptake by the liver. This second hypothesis could explain why BCAA levels were so high in the livers of OKG-treated rats.

In muscle, only ORN was higher than in controls, coming probably from the accumulation of this amino acid in the plasma. Contrary to what was observed in all the other tissues studied, proline and glutamate were not altered in muscle after the OKG treatment. This is not surprising, because in the postabsorptive state, proline is released by muscle as other major gluconeogenic amino acids. Glutamate is taken up but is rapidly metabolized to sustain the transamination of pyruvate into alanine. This is illustrated by the dramatic increase in muscular alanine content observed here. Furthermore, arginine levels decreased, and this is in contradiction with the literature in which OKG treatment either induced an increase in muscle arginine concentrations [34,36,44-46] or did not alter this parameter [15,33,47]. There is a strong rationale and background (see Ref [48] for a review) to support the idea that OKG administration increases muscle arginine levels. Dosage, duration of administration, and physiopathologic state are factors that may explain the discrepancy. Clearly, this point urgently deserves further investigation.

In conclusion, this study provides new and important data concerning the metabolism of OKG in the short term (especially for αKG and BCKA). In particular, it appears that, in these conditions, the major pathway of OKG metabolism is that involving ORN aminotransferase leading to glutamate and proline production. In previous experimental studies [11,12], it was hypothesized that the source of glutamine in the muscle after OKG treatment could result from either an increase in its synthesis via glutamine synthetase (from αKG or glutamate taken up by muscle) or a decrease in muscle glutamine release due to reduced intestinal requirements. If the glutamine accumulation in plasma and muscle observed in these studies [11,12] after several days of treatment is due to an increase in its synthesis via the glutamine synthetase, it probably results from long-term induction of this enzyme. Indeed, in the present short-term study, glutamine levels in most tissues were not altered by the single-bolus administration of OKG. Alternatively, the long-term action of OKG on glutamine pools could be due to ATP replenishment [41] or to a modification in ammonia disposal [49].

References

[1] Cynober L. Ornithine α-ketoglutarate. In: Cynober L, editor. Metabolic and therapeutic aspects of amino acids in clinical nutrition. Boca Raton: CRC Press; 2004. p. 633-46.

[2] Blonde-Cynober F, Aussel C, Cynober L. Use of ornithine alpha-ketoglutarate in clinical nutrition of elderly patients. Nutrition 2003;19: 73-5.

[3] Donati L, Ziegler F, Pongelli G, et al. Nutritional and clinical efficacy of ornithine alpha-ketoglutarate in severe burn patients. Clin Nutr 1999;18:307-11.

[4] Coudray-Lucas C, Le Bever H, Cynober L, et al. Ornithine α-ketoglutarate improves wound healing in severe burn patients. A prospective randomized double-trial versus isonitrogenous controls. Crit Care Med 2000;28:1772-6.

[5] De Bandt JP, Coudray-Lucas C, Lioret N, et al. A randomized controlled trial of the influence of the mode of enteral ornithine–α-ketoglutarate administration in burned patients. J Nutr 1998;128: 563-9.

[6] Hammarqvist F, Wernerman J, Ali R, et al. Effects of an amino acid solution enriched with either branched chain amino acids or ornithine–α-ketoglutarate on the postoperative intracellular amino acid concentration of skeletal muscle. Br J Surg 1990;77:214-8.

[7] Demarcq JM, Delbar M, Trochu G, et al. Effets de l'alpha cétoglutarate d'ornithine sur l'état nutritionnel des malades de réanimation. Cah Anesthesiol 1984;32:3-6.

[8] Mertes N, Möllmann M, Pfisterer M, et al. Nitrogen sparing effect of Ornicetil-supplemented TPN in hypercatabolic septic or polytraumatized patients. In: Soeters PB, Wilson JHP, Meyer AJ, et al, editors. Advances in ammonia metabolism and hepatic encephalopathy. Amsterdam: Elsevier Science Publishers; 1988. p. 141-53.

[9] Brocker P, Vellas B, Albarede J-L, et al. A two-centre, randomized, double-blind trial of ornithine oxoglutarate in 194 elderly, ambulatory, convalescent subjects. Age Ageing 1994;23:303-6.

[10] Jeevanandam M, Holaday NJ, Petersen SR. Ornithine alpha-ketoglutarate (OKG) supplementation is more effective than its components in traumatized rats. J Nutr 1996;126:2141-50.

[11] Le Boucher J, Obled C, Farges MC, et al. Ornithine alpha-ketoglutarate modulates tissue protein metabolism in burn-injured rats. Am J Physiol 1997;273:E557-63.

[12] Vaubourdolle M, Coudray-Lucas C, Jardel A, et al. Action of enterally administered ornithine alpha-ketoglutarate on protein breakdown in skeletal muscle and liver of the burned rat. JPEN J Parenter Enteral Nutr 1991;15:517-20.

[13] Moinard C, Caldefie F, Walrand S, et al. Involvement of glutamine, arginine and polyamines in the action of ornithine α-ketoglutarate on macrophage functions in stressed rats. J Leucok Biol 2000;67: 834-40.

[14] Moinard C, Caldefie F, Walrand S, et al. Effects of ornithine 2-oxoglutarate on neutrophils in stressed rats: evidence for the involvement of nitric oxide and polyamines. Clin Sci 2002;102:287-95.

[15] Raul F, Gosse F, Galluser M, et al. Functional and metabolic changes in intestine mucosa of rats after enteral administration of ornithine alpha-ketoglutarate salt. JPEN J Parenter Enteral Nutr 1995;19:145-50.

[16] Schneid C, Darquy S, Cynober L, et al. Effects of ornithine alpha-ketoglutarate on insulin secretion in rat pancreatic islets: implication of nitric oxide synthase and glutamine synthetase pathways. Br J Nutr 2003;89:249-57.

[17] Cynober L, Coudray-Lucas C, De Bandt JP, et al. Action of ornithine α-ketoglutarate, ornithine hydrochloride, and calcium α-ketoglutarate on plasma amino acid and hormonal patterns in healthy subjects. J Am Coll Nutr 1990;9:2-12.

[18] Le Boucher J, Cynober L. Ornithine alpha-ketoglutarate: the puzzle. Nutrition 1998;14:870-3.

[19] Cooper AJ, Nieves E, Coleman AE, et al. Short-term metabolic fate of [^{13}N]ammonia in rat liver in vivo. J Biol Chem 1987;262:1073-80.

[20] O'sullivan D, Brosnan JT, Brosnan ME. Catabolism of arginine and ornithine in the perfused rat liver: effect of dietary protein and of glucagon. Am J Physiol Endocrinol Metab 2000;278:E516-21.

[21] Wu G, Haynes TE, Li H, et al. Glutamine metabolism in endothelial cells: ornithine synthesis from glutamine via pyrroline-5-carboxylate synthase. Comp Biochem Physiol A Mol Integr Physiol 2000;126: 115-23.

[22] Le Bricon T, Coudray-Lucas C, Lioret N, et al. Ornithine α-ketoglutarate metabolism after enteral administration in burn patients: bolus compared with continual infusion. Am J Clin Nutr 1997;65:512-8.

[23] Wakabayashi Y. The glutamate crossway. In: Cynober LA, editor. Amino acid metabolism and therapy in health and nutritional disease. Boca Raton: CRC Press; 1995. p. 89-98.

[24] Winkler S, Hotzenbein T, Karner J, et al. Kinetics of organ specific metabolism of a bolus injection into the jejunum of glutamine, alpha-

ketoglutarate ornithine and ornithine alpha-ketoglutarate. Clin Nutr 1993;12:56-7 [abstract].
[25] Vaubourdolle M, Jardel A, Coudray-Lucas C, et al. Metabolism and kinetics of parenterally administered ornithine and α-ketoglutarate in healthy and burned animals. Clin Nutr 1988;7:105-11.
[26] Vaubourdolle M, Jardel A, Coudray-Lucas C, et al. Fate of enterally administered ornithine in healthy animals: interactions with alpha-ketoglutarate. Nutrition 1989;5:183-7.
[27] Aussel C, Cynober L, Lioret N, et al. Branched-chain ketoacid plasma concentrations following burn injury: effects of enteral feeding supplemented with ornithine α-ketoglutarate. Proc 4th World congress on Intensive Critical Care Medecine Jerusalem; 1985.
[28] Neveux N, David P, Cynober L. Measurement of amino acid concentration in biological fluids and tissues using ion-exchange chromatography. In: Cynober L, editor. Metabolic and therapeutic aspects of amino acids in clinical nutrition. Boca Raton: CRC Press; 2004. p. 17-28.
[29] Pailla K, Blonde-Cynober F, Aussel C, et al. Branched-chain keto-acids and pyruvate in blood: measurement by HPLC with fluorimetric detection and changes in older subjects. Clin Chem 2000;46:848-53.
[30] Shenoy V, Roig JC, Chakrabarti R, et al. Ontogeny of glutamine synthetase in rat small intestine. Pediatr Res 1996;39:643-8.
[31] Abumrad NN, Williams P, Frexes-Steed M, et al. Inter-organ metabolism of amino acids in vivo. Diabetes Metab Rev 1989;5: 213-26.
[32] Howse DC, Duffy TE. Control of the redox state of the pyridine nucleotides in the rat cerebral cortex. Effect of electroshock-induced seizures. J Neurochem 1975;24:935-40.
[33] Dumas F, De Bandt JP, Colomb V, et al. Enteral ornithine alpha-ketoglutarate enhances intestinal adaptation to massive resection in rats. Metabolism 1998;47:1366-71.
[34] Le Boucher J, Farges MC, Minet R, et al. Modulation of immune response with ornithine alpha-ketoglutarate in burn injury: an arginine or glutamine dependency? Nutrition 1999;15:773-7.
[35] Schlegel L, Coudray-Lucas C, Barbut F, et al. Bacterial dissemination and metabolic changes in rats induced by endotoxemia following intestinal *E. coli* overgrowth are reduced by ornithine alpha-ketoglutarate administration. J Nutr 2000;130:2897-902.
[36] Lasnier E, Coudray-Lucas C, Le Boucher J, et al. Ornithine alpha-ketoglutarate counteracts thymus involution and glutamine depletion in endotoxemic rats. Clin Nutr 1996;77:197-200.
[37] De Bandt JP, Cynober L, Lim SK, et al. Metabolism of ornithine, alpha-ketoglutarate and arginine in isolated perfused rat liver. Br J Nutr 1995;73:227-39.
[38] Haüssinger D, Gerok W. Regulation of hepatic glutamate metabolism. Role of 2-oxoacids in glutamate release from isolated perfused rat liver. Eur J Biochem 1984;143:491-7.
[39] Haüssinger D. Glutamine and hepatic metabolism. In: Kinney JM, Tucker HN, editors. Organ metabolism and nutrition: ideas for future critical care. New York: Raven Press Ltd; 1994. p. 315-29.
[40] Souba WW. Glutamine: a key substrate for the splanchnic bed. Annu Rev Nutr 1991;11:285-308.
[41] Wagenmakers AJM, Soeters PB. Metabolism of branched-chain amino acids. In: Cynober LA, editor. Amino acid metabolism and therapy in health and nutritional disease. Boca Raton: CRC Press; 1995. p. 67-87.
[42] Suryawan A, Hawes JW, Harris RA, et al. A molecular model of human branched-chain amino acid metabolism. Am J Clin Nutr 1998;68:72-81.
[43] Blonde-Cynober F, Plassart F, De Bandt JP, et al. Metabolism of alpha-ketoisocaproic acid in isolated perfused liver of cirrhotic rats. Am J Physiol 1995;268:E298-E304.
[44] Le Bricon T, Cynober L, Field CJ, et al. Supplemental nutrition with ornithine α-ketoglutarate in rats with cancer-associated cachexia: surgical treatment of the tumor improves efficacy of nutritional support. J Nutr 1995;125:2999-3010.
[45] Roch-Arveiller M, Tissot M, Coudray-Lucas C, et al. Immunomodulatory effects of ornithine alpha-ketoglutarate in rats with burn injuries. Arch Surg 1996;131:718-23.
[46] Robinson LE, Bussière FI, Le Boucher J, et al. Amino acid nutrition and immune function in tumor bearing rats: a comparison of glutamine-, arginine- and ornithine α-ketoglutarate–supplemented diets. Clin Sci 1999;97:657-69.
[47] Le Boucher J, Coudray-Lucas C, Lasnier E, et al. Enteral administration of ornithine alpha-ketoglutarate or arginine alpha-ketoglutarate: a comparative study of their effects on glutamine pools in burn-injured rats. Crit Care Med 1997;25:293-8.
[48] Cynober L. Ornithine alpha-ketoglutarate as a potent precursor of arginine and nitric oxide: a new job for an old friend. J Nutr 2004;134: 2858S-62S.
[49] Leander U, Fürst P, Vesterberg K, et al. Nitrogen sparing effect of Ornicetil in the immediate postoperative state: clinical biochemistry and nitrogen balance. Clin Nutr 1985;4:43-51.