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Does the ornithine–α-ketoglutarate ratio influence ornithine α-ketoglutarate metabolism in healthy rats?

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Abstract

Ornithine α-ketoglutarate (OKG) is a salt composed of 2 molecules of ornithine (ORN) and one molecule of α-ketoglutarate (αKG). OKG has been used successfully via oral, enteral, and parenteral routes to improve protein status in patients with chronic and acute protein depletion, but its mechanism of action, which is probably multifactorial, is still unclear. A specific metabolic interaction between αKG and ORN has been shown to be a key factor in the effects of OKG, but the impact of the ORN/αKG ratio (2 molecules of ORN for 1 molecule of αKG) has never been discussed. To clarify this point, young (3 weeks old) male Wistar rats in the postabsorptive state received 5 g/kg of either OKG or a mono-ornithine αKG (MOKG) salt (ORN/αKG ratio = 1:1) in amounts that were either isonitrogenous or isomolar to OKG, or a saline solution (controls) and were killed 1 hour later. In a second experiment, a kinetic study was performed in which rats were killed 1, 2, 3, or 6 hours after OKG, MOKG, or saline administration. Amino acid contents were analyzed in the plasma, liver, jejunal and ileal mucosae, and the extensor digitorum longus (EDL) muscle. The major metabolites detected after intake of OKG or MOKG (ie, ORN, proline [PRO], and glutamate; OKG and MOKG vs control, $P < .05$) together with the absence of increased arginine and citrulline levels suggested that ORN was mainly metabolized by the ORN aminotransferase pathway, leading to glutamate and PRO production with accumulation persisting at 6 hours postadministration. This study provides new and important data on the influence of the ORN/αKG ratio on OKG metabolism: MOKG-treated rats presented less intestinal ORN than OKG-treated rats (MOKG vs OKG, $P < .05$), suggesting that ORN/αKG ratio influences the rate of ORN availability and metabolism. In addition, the metabolic interaction between ORN and αKG (ie, in the presence of αKG, ORN metabolism is partially diverted toward PRO production), which is characteristic of OKG metabolism, still takes place even if the salt contains only 1 molecule of ORN instead of two.
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1. Introduction

Ornithine α-ketoglutarate (OKG) belongs to a family called immunopharmaconutrients and has been used successfully via oral, enteral, and parenteral routes to improve protein status in patients with chronic and acute protein depletion (see references [1,2] for recent reviews on the topic). The mechanism of action of OKG is probably linked to a stimulated secretion of anabolic hormones (insulin, insulin-like growth factor, growth hormone) and to the production of OKG metabolites (glutamine [GLN], arginine [ARG], proline [PRO], and polyamines) [1]. In addition, it is well established that the presence of ornithine (ORN) and α-ketoglutarate (αKG) is required for OKG to be effective. Molimard et al [3] have treated cirrhotic patients with OKG, ORN chloride, or sodium αKG. They noted a reduction in ammonemia and normalization of the aminoacidogram after administration of OKG only, suggesting that both ORN and αKG must be simultaneously present to be effective. Similarly, Cynober et al [4] showed different metabolic responses after ORN chloride, calcium αKG, or OKG administration in healthy subjects. When ORN was administered as a chloride salt, it led to a more rapid increase followed by a more rapid decrease of ornithinemia than when administered as OKG. Plasma αKG increased only

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slightly after the OKG and αKG loads and did not vary after the ORN load. The most important differences are that (1) when healthy subjects received OKG, the increase in plasma glutamate remained limited compared to when they received ORN or αKG salts separately, and (2) ARG, citrulline [CIT], and PRO concentrations only increased in plasma and urine after OKG administration, suggesting that ORN metabolism was diverted in the presence of αKG.

The effects of OKG on amino acid and protein metabolism have also been studied in animal models of stress. In traumatized rats (bilateral femur fracture), supplementation with OKG was more effective in improving nitrogen retention, stemming the decrease in muscle GLN and promoting a preferential use of branched-chain amino acids for protein synthesis than ORN or αKG alone [5]. Le Boucher et al [6] compared the effects of OKG and ARG αKG in burn-injured rats. They observed that OKG treatment alone led to a decrease in plasma phenylalanine (suggesting a decrease in protein catabolism) and an increase in plasma and muscle GLN, with a correlation between these 2 parameters. The authors concluded that (1) the action of OKG as a GLN precursor cannot be ascribed solely to αKG because ARG αKG did not exhibit this effect to the same extent and (2) the action of OKG is not due to its nitrogen content because isonitrogenous ARG αKG did not reproduce the effects of OKG.

Hence, a specific metabolic interaction between αKG and ORN exists. Indeed, αKG is involved in the Krebs cycle, whereas ORN belongs to the urea cycle. When both αKG and ORN are present, their metabolism is diverted toward production of GLN, PRO, ARG, and polyamine. This interaction between αKG and ORN is thus a key factor in explaining the effects of OKG [7]. Commercially available OKG takes the form of a salt containing 1 molecule of αKG and 2 molecules of ORN. This molecular form was chosen for chemical reasons (ie, dictated by chemical constraints to ensure stability of the salt and a pH close to physiologic ranges), but there are no available data on whether the molecular ratio of ORN to αKG influences the properties of OKG. This may be important because OKG metabolism depends on the fact that in the presence of αKG, ORN metabolism is partially diverted toward PRO production, and the ORN/αKG ratio could be a determining factor. Therefore, we addressed the issue of whether a salt formed with 1 molecule each of ORN and αKG is metabolized in a similar fashion as OKG. We thus synthesized the mono-ornithine αKG (MOKG) salt (ORN/αKG = 1:1) and compared its metabolism to that of OKG (ORN/αKG = 2/1) in healthy rats. In the first experiment, the quantity of synthesized salt supplied was either isomolar or isonitrogenous to OKG, and tissue and plasma samples were collected 1 hour after administration. When the quantity of synthesized salt administered was isomolar to OKG, it supplied an identical amount of αKG but 2-fold less ORN than OKG. Conversely, when the quantity of synthesized salt administered was isonitrogenous to OKG, it supplied an identical amount of ORN but 2-fold more αKG than OKG. A kinetic study was also performed to assess the effects of MOKG (isonitrogenous to OKG) on tissue and plasma amino acid patterns at 1 to 6 hours after MOKG administration. This study is thus the first to discuss the importance of the ORN/αKG ratio in the OKG salt.

2. Materials and methods

2.1. Synthesis of a disodium salt of L-ornithine α-ketoglutarate chloride

L-Ornithine hydrochloride (3 g, 17.8 mmol) was dissolved in water (18 mL) at 0°C. α-Ketoglutaric acid (2.6 g, 17.8 mmol) was added, followed by sodium hydroxide (1.2 g, 30 mmol). After complete dissolution, the pH was adjusted to 7.2 by addition of sodium hydroxide (1 mol/L solution in water). The solution was freeze-dried to yield the salt (9.5 g, 99%) as a white powder.

2.2. Animals and experimental protocol

Three-week-old male Wistar rats (Charles River, L'Arbresle, France) were housed in a controlled environment (21°C ± 1°C) with a 12-hour light-dark cycle, and maintained on standard rat chow (A04, UAR, Epinay-sur-Orge, France) and water ad libitum until the day of the study.

This study complied with our institution's guidelines for animal care, and one member of the study team (LC) has French government authorization to use animal models of stress (No. 75.461).

2.3. Study design

In the first set of experiments, the rats were fasted overnight and then randomized to receive 5 g/kg of OKG (OKG group, n = 8), 7 g/kg of MOKG (MOKG-isonitrogenous [MOKG-isoN] group, n = 8), 3.5 g/kg of MOKG (MOKG-isomolar [MOKG-isoM] group, n = 8), or 1.1 g/kg of NaCl (NaCl group, n = 8). Hence, the rats in the MOKG-isoN group received identical amounts of nitrogen and ORN but 2-fold more αKG than rats in the OKG group (Table 1). The rats in the MOKG-isoM group received the MOKG in isomolar amounts of OKG (ie, identical amount of αKG, but 2-fold less ORN) than OKG-treated rats. The rats in the NaCl group received the same amount of sodium as the MOKG-isoN group. Solution characteristics are given in Table 1. The salts were diluted in distilled water before

Table 1
Solution characteristics

	NaCl	OKG	MOKG-isoN	MOKG-isoM
ORN/α-KG ratio	–	2:1	1:1	1:1
Grams per kilogram administered	1.1	5	7	3.5
Nitrogen (g)	–	0.65	0.65	0.33
ORN (mmol)	–	23	23	12
α-KG (mmol)	–	12	23	12

Table 2
Tissue OKG metabolite concentrations in the short-term experiment.

	NaCl	OKG	MOKG-isoN	MOKG-isoM
Ornithine				
Jejunal mucosa	0.78 ± 0.24[a]	22.27 ± 2.36[b]	8.08 ± 0.67[c]	5.72 ± 0.83[c]
Ileal mucosa	0.20 ± 0.02[a]	8.71 ± 0.90[b]	4.82 ± 0.90[c]	4.37 ± 0.45[c]
Liver	0.26 ± 0.01[a]	3.27 ± 0.29[b]	2.01 ± 0.15[c]	1.42 ± 0.12[c]
Plasma	355 ± 298[a]	2604 ± 164[b]	1832 ± 272[b]	1745 ± 83[b]
EDL	0.11 ± 0.01[a]	1.31 ± 0.07[b]	1.15 ± 0.12[b,c]	0.88 ± 0.09[c]
Glutamate				
Jejunal mucosa	2.76 ± 0.17[a]	7.09 ± 0.45[b]	6.08 ± 0.55[b]	5.80 ± 0.55[b]
Ileal mucosa	2.42 ± 0.11[a]	5.43 ± 0.58[b]	5.39 ± 0.49[b]	5.27 ± 0.37[b]
Liver	2.27 ± 0.10[a]	4.19 ± 0.24[b]	4.20 ± 0.08[b]	3.87 ± 0.15[b]
Plasma	76 ± 7[a]	210 ± 24[b]	158 ± 16[b,c]	120 ± 7[c]
EDL	1.96 ± 0.08	2.22 ± 0.19	2.21 ± 0.22	2.01 ± 0.16
Glutamine				
Jejunal mucosa	0.70 ± 0.05[a]	1.10 ± 0.07[b]	1.10 ± 0.05[b]	1.07 ± 0.09[b]
Ileal mucosa	0.48 ± 0.03[a]	1.25 ± 0.17[b]	1.00 ± 0.12[b]	1.07 ± 0.10[b]
Liver	6.01 ± 0.45	7.28 ± 0.45	7.95 ± 0.58	7.09 ± 0.53
Plasma	691 ± 40	829 ± 24	785 ± 30	740 ± 44
EDL	7.73 ± 0.22	7.25 ± 0.22	6.94 ± 0.53	6.55 ± 0.51
Proline				
Jejunal mucosa	0.43 ± 0.02[a]	0.97 ± 0.06[b]	0.81 ± 0.04[b]	0.79 ± 0.06[b]
Ileal mucosa	0.41 ± 0.03[a]	1.41 ± 0.23[b]	0.92 ± 0.18[ab]	1.08 ± 0.09[b]
Plasma	179 ± 24[a]	456 ± 20[b]	354 ± 26[c]	324 ± 22[c]
EDL	0.28 ± 0.02[a]	0.54 ± 0.04[b]	0.46 ± 0.05[b]	0.45 ± 0.03[b]
Arginine				
Jejunal mucosa	0.38 ± 0.07	0.40 ± 0.05	0.29 ± 0.03	0.25 ± 0.03
Ileal mucosa	0.21 ± 0.07	0.29 ± 0.12	0.15 ± 0.01	0.14 ± 0.01
Plasma	129 ± 8	117 ± 5	126 ± 8	125 ± 10
EDL	0.20 ± 0.02	0.12 ± 0.01	0.19 ± 0.02	0.17 ± 0.03
Citrulline				
Jejunal mucosa	0.26 ± 0.02	0.30 ± 0.03	0.27 ± 0.02	0.32 ± 0.03
Ileal mucosa	0.28 ± 0.01	0.29 ± 0.02	0.23 ± 0.02	0.28 ± 0.03
Liver	0.08 ± 0.01[a]	0.12 ± 0.01[b]	0.10 ± 0.01[a,b]	0.09 ± 0.01[a,b]
Plasma	194 ± 6	218 ± 8	210 ± 8	194 ± 11
EDL	0.74 ± 0.02	0.64 ± 0.05	0.69 ± 0.06	0.66 ± 0.04

Animals were killed 1 hour after gavage with 1.1 g/kg of NaCl, 5 g/kg of OKG, 7 g/kg of MOKG (isonitrogenous to OKG; MOKG-isoN group), or 3.5 g/kg of MOKG (isomolar to OKG; MOKG-isoM group). Results are expressed in micromoles per gram of tissue or micromoles per liter of plasma as means ± SEM. Values with different superscript letters in the same row are significantly different (a ≠ b ≠ c at $P < .05$).

administration by gavage, and the rats were then killed 1 hour after administration.

Because no difference was observed between the MOKG-isoN and the MOKG-isoM groups in the first experiment, only MOKG-isoN was compared to OKG in the second (kinetic study) experiment. Thus, MOKG-treated rats received an identical amount of ORN but 2-fold more αKG than the OKG-treated rats. The rats were fasted overnight then randomized to receive 5 g/kg of OKG (OKG group, n = 32), 7 g/kg of MOKG (MOKG-isoN group, n = 32), or 1.1 g/kg of NaCl (NaCl group, n = 32). Each group was then divided into 4 subgroups killed at 1 to 6 hours after treatment (n = 8 in each subgroup).

2.4. Tissue collection

One hour after the gavage for the first set of experiments, or at different times for the kinetic study, the rats were anesthetized with isoflurane (3% in oxygen; Minerve, Esternay, France) before killing (by decapitation). Blood was collected into heparin-containing tubes and immediately centrifuged (10 minutes, 2500*g*, 4°C). The plasma was deproteinized with sulfosalicylic acid (30 mg/mL) before storing at −80°C for amino acid analysis. As mentioned by Neveux et al [8], the deproteinized samples can be stored at −70°C until analysis without any loss of amino acids, including GLN, for at least several months to 1 year. In the present study, samples were analyzed within 1 month of collection. In addition, 10 cm of proximal jejunum and proximal ileum were promptly removed, washed with ice-cold 0.9% NaCl (wt/wt) through the lumen, and then inverted to collect the mucosa by using glass scrapers. The intestinal mucosae, a piece of liver, and the left extensor digitorum longus (EDL) were promptly removed, weighed, frozen, and stored at −80°C until amino acid analysis.

2.5. Measurements

2.5.1. Amino acid analysis

The frozen tissues were homogenized in ice-cold 10% trichloroacetic acid containing 0.5 mmol/L EDTA and norvaline (internal standard, 200 μM). Free amino acids

were separated from precipitated proteins by centrifugation (10 minutes, 2500*g*, 4°C). Free amino acid concentrations in tissues and plasma were determined by ion exchange chromatography with ninhydrin detection (AminoTac JLC-500 V, Jeol, Tokyo, Japan) [8].

Our laboratory is registered with the European quality control program ERNDIM, ensuring the reliability of measurements for all the amino acids studied. Results are expressed in micromoles per liter of plasma or micromoles per gram of tissue.

2.6. Statistical analysis

Data are presented as means ± SEM. Results of the first experiment were analyzed by 1-way analysis of variance with an a posteriori Bonferroni-Dunn test. Results of the second experiment were analyzed by 2-way analysis of variance to test diet-time interaction followed by an a posteriori Bonferroni-Dunn test (Statview software, Abacus Concepts, Berkeley, CA).

3. Results

3.1. First experiment

Ornithine concentrations were significantly higher in all tissues studied after OKG and MOKG administration compared with the control (NaCl) group (OKG, MOKG-isoN, and MOKG-isoM vs NaCl, $P < .05$) (Table 2). Compared with the OKG group, this increase in ORN concentration was less pronounced in the intestinal mucosae and the liver when ORN was given in the form of the MOKG salt (MOKG-isoN and MOKG-isoM vs OKG, $P < .05$), independently of the quantity of MOKG administered (ie, isomolar or isonitrogenous to OKG).

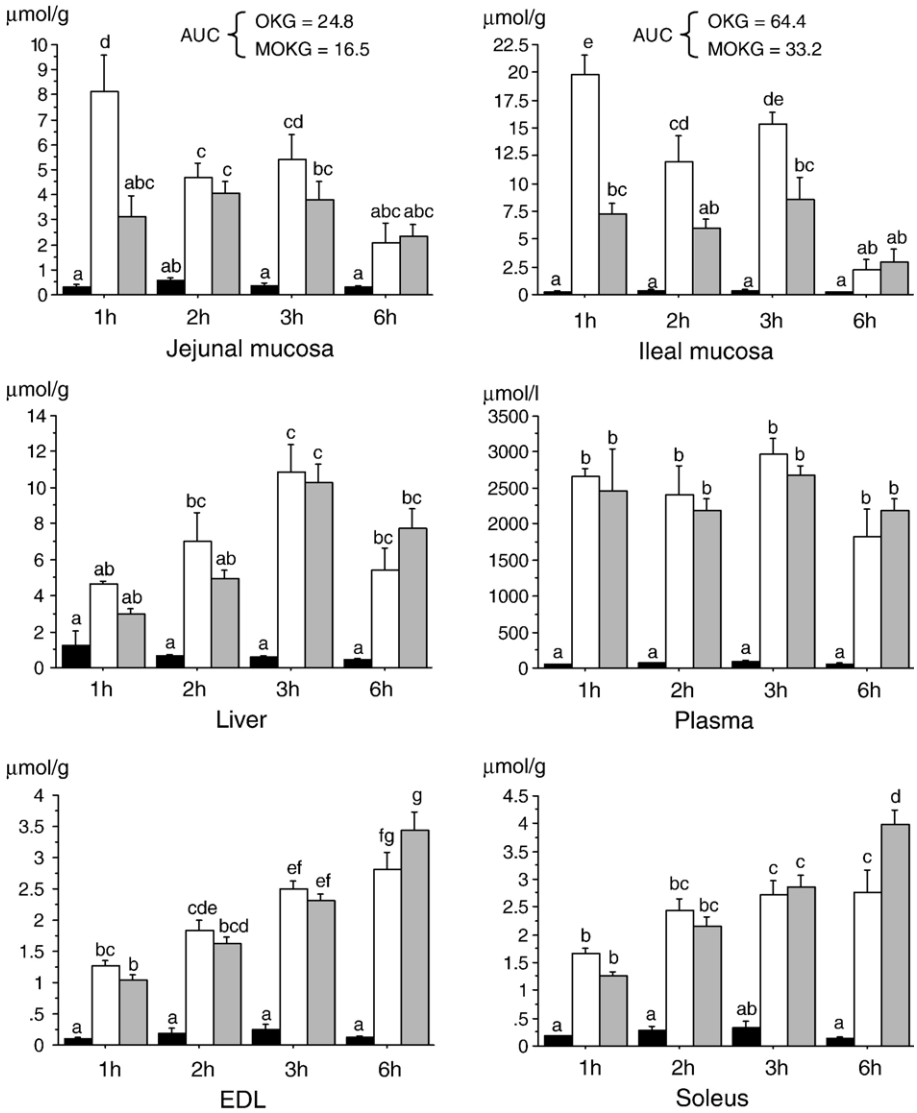

Fig. 1. Tissue ORN concentrations. Rats were killed 1, 2, 3, or 6 hours after administration of saline solution (dark bars), OKG (5 g/kg, white bars), or MOKG (7 g/kg, gray bars). Bars with different superscript letters are statistically different (a ≠ b ≠ c ≠ d ≠ e ≠ f ≠ g at $P < .05$). When 2 bars share the same letter, differences between the 2 groups concerned are not significant.

Glutamate (GLU) concentrations were higher in the intestinal mucosa, liver, and plasma after OKG or MOKG administration compared with the control (NaCl) group (OKG, MOKG-isoN, and MOKG-isoM vs NaCl, $P < .05$) (Table 2). Compared with the OKG group, this increase was less pronounced in plasma when ORN was given in the form of the MOKG salt, with a significant difference between the MOKG-isoM and OKG groups ($P < .05$). No differences were observed in the EDL between the 4 groups studied.

OKG and/or MOKG treatment induced an increase in GLN concentrations in the jejunal and ileal mucosae (OKG, MOKG-isoN, and MOKG-isoM vs NaCl, $P < .05$) (Table 2). The 3 treatments did not alter liver, plasma, or EDL GLN concentrations.

OKG and MOKG treatment induced an increase in PRO concentrations in the intestinal mucosae, EDL, and plasma (OKG, MOKG-isoN, and MOKG-isoM vs NaCl, $P < .05$), but to a lesser extent in plasma after MOKG administration (OKG vs MOKG-isoN and MOKG-isoM vs NaCl, $P < .05$) (Table 2).

No differences between groups were observed for ARG concentrations, and CIT concentrations only increased in the liver after OKG treatment (OKG vs NaCl, $P < .05$).

3.2. Second experiment

OKG and MOKG treatments resulted in an increase in ORN concentrations in all the tissues analyzed, but the 2 treatments led to different ORN profiles in the intestinal

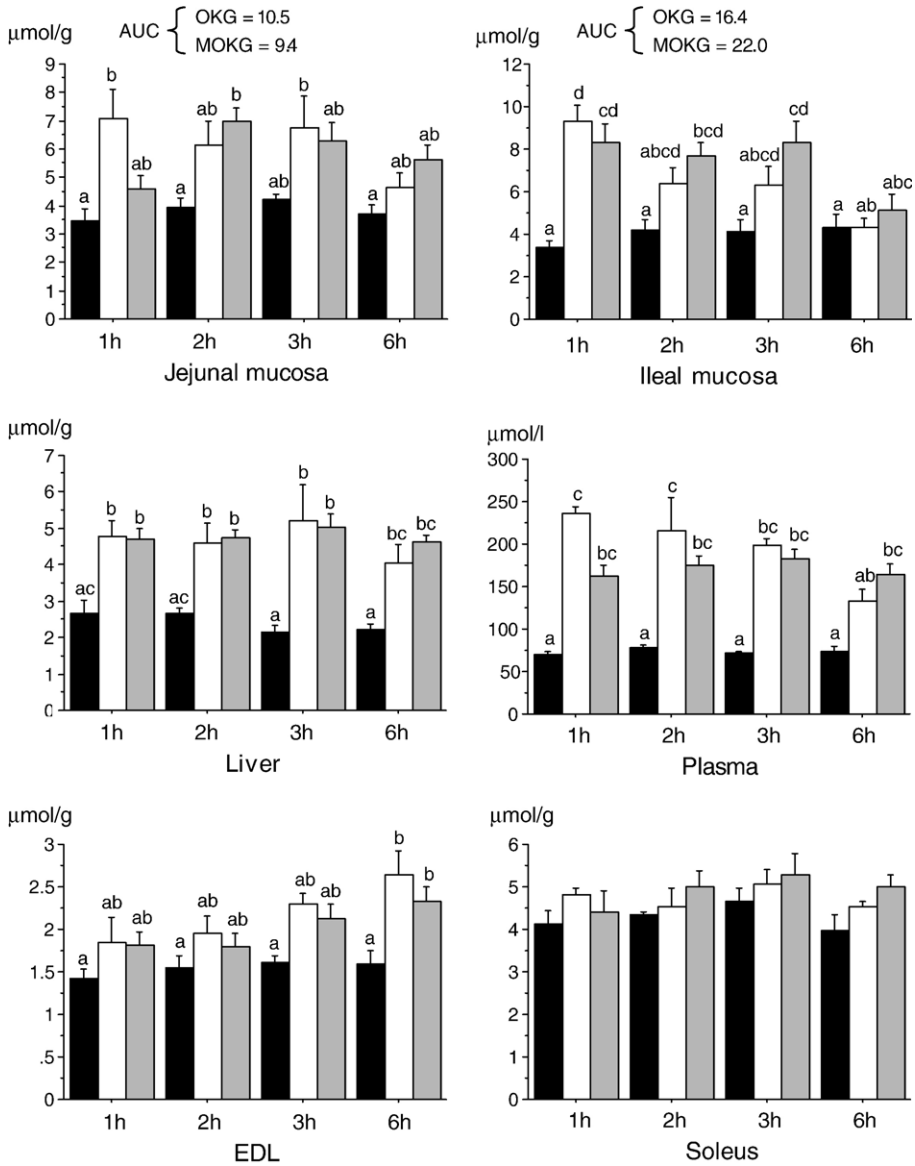

Fig. 2. Tissue GLU concentrations. Rats were killed 1, 2, 3, or 6 hours after administration of saline solution (dark bars), OKG (5 g/kg, white bars), or MOKG (7 g/kg, gray bars). Bars with different superscript letters are statistically different ($a \neq b \neq c \neq d$ at $P < .05$). When 2 bars share the same letter, differences between the 2 groups concerned are not significant.

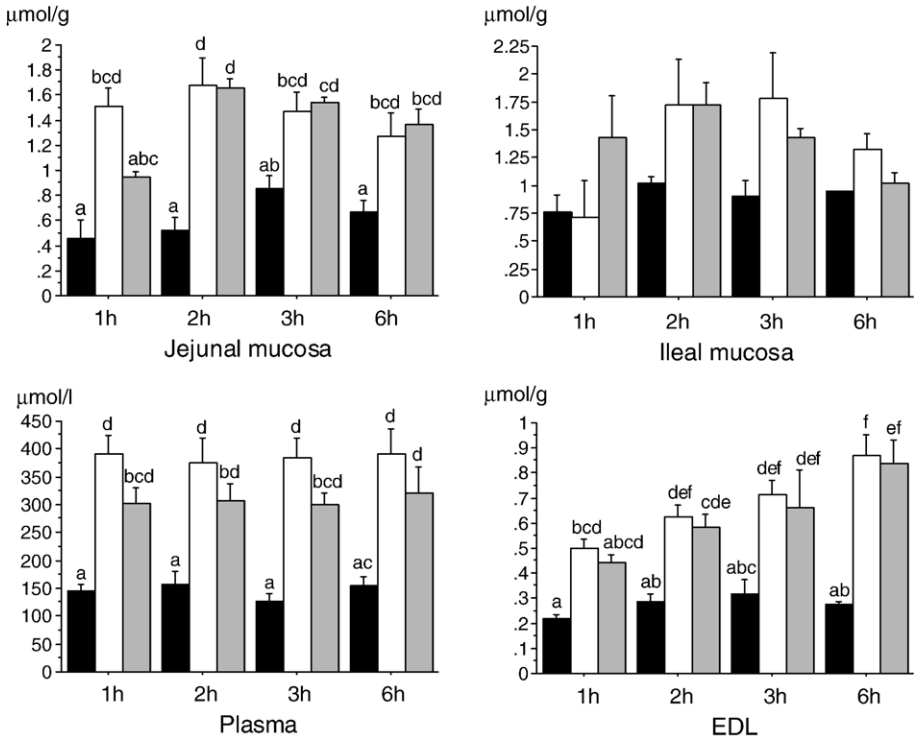

Fig. 3. Tissue PRO concentrations. Rats were killed 1, 2, 3, or 6 hours after administration of saline solution (dark bars), OKG (5 g/kg, white bars), or MOKG (7 g/kg, gray bars). Bars with different superscript letters are statistically different (a ≠ b ≠ c ≠ d≠ e ≠ f at $P < .05$). When 2 bars share the same letter, differences between the 2 groups concerned are not significant. Note that PRO concentrations are not shown for the liver due to unsatisfactory quantification.

mucosae (Fig. 1). Indeed, peak jejunal and ileal ORN concentrations were observed 1 hour after OKG treatment (OKG vs MOKG and NaCl, $P < .05$). In contrast, the MOKG treatment induced a less pronounced increase in ORN concentrations than the OKG treatment and remained stable throughout the study (MOKG vs NaCl, $P < .05$ at 2 and 3 hours in the jejunum and at 1 and 3 hours in the ileum). Six hours after the 2 treatments, ORN concentrations returned to

Table 3
Tissue GLN concentrations in the kinetic study

Tissue	Time (h)	NaCl group	OKG group	MOKG group
Jejunal mucosa (µmol/g)	1	0.66 ± 0.10^{a}	1.27 ± 0.11^{ab}	0.98 ± 0.09^{a}
	2	0.70 ± 0.09^{a}	1.86 ± 0.34^{b}	1.59 ± 0.12^{b}
	3	0.88 ± 0.09^{a}	1.35 ± 0.11^{ab}	1.32 ± 0.12^{b}
	6	0.64 ± 0.06^{a}	1.09 ± 0.13^{a}	1.34 ± 0.11^{ab}
Ileal mucosa (µmol/g)	1	1.11 ± 0.21^{a}	1.31 ± 0.07^{a}	1.37 ± 0.14^{a}
	2	1.19 ± 0.14^{a}	1.58 ± 0.19^{ab}	1.77 ± 0.24^{ab}
	3	1.28 ± 0.18^{a}	2.47 ± 0.35^{b}	1.57 ± 0.15^{ab}
	6	1.42 ± 0.38^{a}	1.65 ± 0.18^{ab}	1.38 ± 0.15^{a}
Liver (µmol/g)	1	6.47 ± 0.39^{ab}	6.28 ± 0.29^{ab}	7.39 ± 0.66^{a}
	2	6.47 ± 0.32^{ab}	5.97 ± 0.42^{ab}	5.13 ± 0.73^{b}
	3	6.32 ± 0.29^{ab}	5.85 ± 0.30^{ab}	5.00 ± 0.25^{b}
	6	6.29 ± 0.29^{ab}	5.35 ± 0.62^{ab}	5.17 ± 0.41^{b}
Plasma (µmol/L)	1	731 ± 38	906 ± 37	844 ± 50
	2	765 ± 42	816 ± 74	793 ± 70
	3	718 ± 24	865 ± 20	773 ± 33
	6	736 ± 26	885 ± 50	746 ± 71
EDL (µmol/g)	1	5.77 ± 0.19	6.06 ± 0.19	6.04 ± 0.26
	2	5.76 ± 0.25	5.76 ± 0.26	5.96 ± 0.24
	3	5.42 ± 0.24	5.49 ± 0.30	5.53 ± 0.33
	6	5.39 ± 0.16	5.77 ± 0.31	5.40 ± 0.26

Results are expressed as means ± SEM. For each tissue, values with different superscript letters (both in rows and columns) are significantly different (a ≠ b ≠ c at $P < .05$).

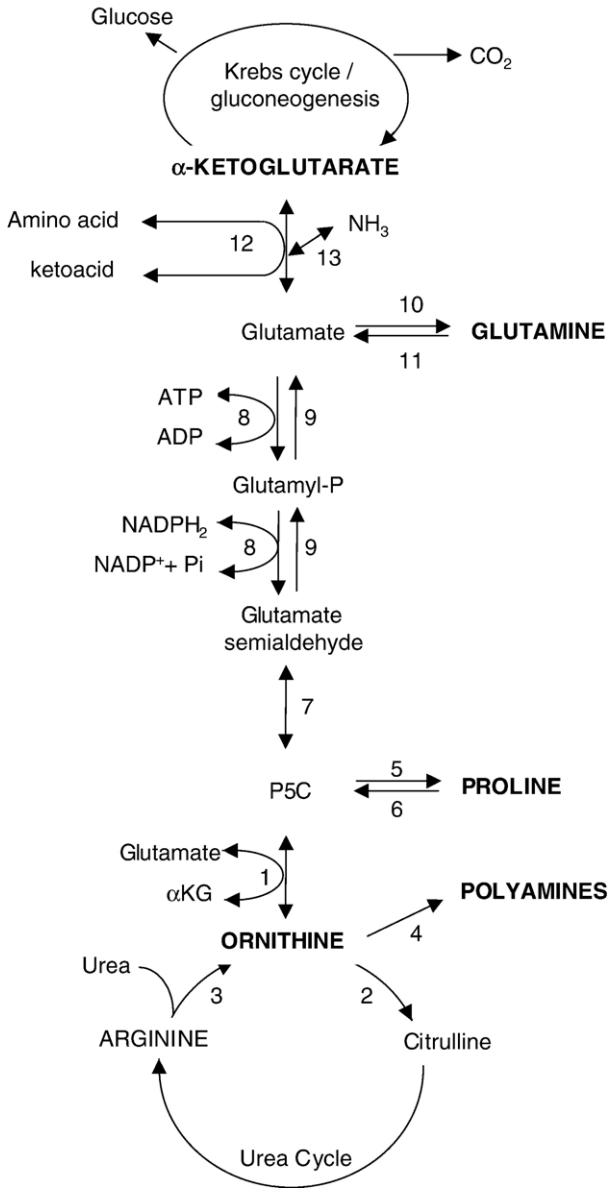

Fig. 4. ORN and αKG metabolic pathways. (1) ORN aminotransferase (EC 2.6.1.13), (2) ORN carbamoyltransferase (EC 2.1.3.3), (3) arginase (EC 3.5.3.1), (4) ORN decarboxylase (EC 4.1.1.17), (5) pyrroline-5-carboxylic acid reductase (EC 1.5.1.2), (6) PRO oxidase (EC 1.5.1.2), (7) spontaneous nonenzymatic reaction in chemical equilibrium, (8) ^{1}Δ-pyrroline-5-carboxylate synthase (EC not assigned), (9) ^{1}Δ-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12), (10) GLN synthetase (EC 6.3.1.2), (11) glutaminase (EC 3.5.1.2), (12) αKG-linked aminotransferases (EC 2.6.1 family), and (13) GLU dehydrogenase (EC 1.4.1.2).

values not significantly different from the baseline values in both groups of treated rats. We calculated areas under the curve to better evaluate the intestinal differences in ORN concentrations between these 2 groups. Interestingly, the area under the curve (AUC) was 2-fold higher after OKG treatment than after MOKG treatment (jejunum, 24.69 vs 16.47; ileum, 64.35 vs 33.2).

In the liver, OKG and MOKG treatments resulted in an increase in ORN concentrations that peaked at 3 hours postadministration (OKG and MOKG vs NaCl, $P < .05$). ORN concentrations in the plasma increased dramatically and remained high throughout the study (OKG and MOKG vs NaCl, $P < .05$), whereas ORN concentrations in the muscles increased gradually to reach a peak at 6 hours postadministration (OKG and MOKG vs NaCl, $P < .05$). Interestingly, ORN levels at 6 hours in the soleus were significantly higher in the MOKG group than in the OKG group (MOKG vs OKG, $P < .05$).

GLU concentrations in the jejunum were higher in the first 3 hours postadministration, reaching a peak at 1 hour after OKG administration (OKG vs NaCl, $P < .05$) and between 2 and 3 hours after MOKG administration (MOKG vs NaCl, $P < .05$) (Fig. 2). In the ileum, the peak of GLU concentration was observed 1 hour after OKG treatment (OKG vs NaCl, $P < .05$) whereas it was higher during the first 3 hours after MOKG treatment (MOKG vs NaCl, $P < .05$). Six hours after administration, GLU concentrations returned to baseline values, giving a slightly higher AUC after MOKG treatment than after OKG treatment. In the liver and plasma, GLU concentrations increased and remained high throughout the study (OKG and MOKG vs NaCl, $P < .05$), whereas in the EDL, the increase in GLU concentrations only reached significance at 6 hours postadministration (OKG and MOKG vs NaCl, $P < .05$).

Fig. 3 shows an increase in jejunal PRO, peaking 2 hours after both treatments, as well as a stabilization of high PRO concentrations in plasma throughout the study and a gradual increase in EDL PRO, peaking 6 hours after both treatments (OKG and MOKG vs NaCl, $P < .05$).

Intestinal GLN concentrations increased after both treatments, peaking at 2 hours in the jejunum (OKG and MOKG vs NaCl, $P < .05$) and at 3 hours in the ileum (OKG vs NaCl, $P < .05$) and returning to baseline values at 6 hours postadministration (Table 3). In the liver of the rats administered MOKG, GLN concentrations were lower at 2, 3, and 6 hours postadministration than at 1 hour postadministration (MOKG, 2, 3, and 6 hours vs MOKG, 1 hour; $P < .05$), whereas no difference was observed in the other 2 groups.

Concerning tissue ARG content, the only effect observed was an increase in ARG in the ileum 3 hours after OKG administration (OKG, 3.21 ± 0.65 vs MOKG, 0.93 ± 0.21 and NaCl, 1.21 ± 0.26; $P < .05$). The other tissues showed no differences in ARG content among the 3 groups (data not shown). Similarly, an increase in CIT concentration was observed in the liver of rats treated with OKG and MOKG at the 3-hour time point (OKG, 0.31 ± 0.05 and MOKG, 0.36 ± 0.05 vs NaCl, 0.11 ± 0.00, $P < .05$), but no other differences in CIT were observed in the other tissues (data not shown).

4. Discussion

This study was performed to improve our understanding of the impact of the ORN/αKG ratio on OKG metabolism,

especially the metabolic interaction between ORN and αKG (discussed above).

The results of the first experiment showed that MOKG administration, like OKG administration, leads to an increase in tissue ORN, GLU, GLN, and PRO. These compounds are produced by the metabolism of αKG and ORN (Fig. 4), as described in the literature [9,10]: radiolabeled ORN, PRO, and GLU were found in healthy rat tissues 60 minutes after an enteral administration of [^{14}C]ORN, whereas radiolabeled αKG, ORN, GLU, GLN, and PRO were found 30 minutes after a parenteral administration of [^{14}C]αKG.

However, in this first experiment, less ORN was detected in the intestinal mucosae of rats treated with the MOKG salt than in those treated with OKG, regardless of whether the MOKG salt supplied was isonitrogenous (MOKG-isoN group) or isomolar (MOKG-isoM group) to OKG. This would appear logical for the MOKG-isoM group (3.5 g/kg of MOKG; Table 1) because these animals received the same amount of αKG but 2-fold less ORN than the OKG-treated rats (which were administered 5 g/kg of OKG; Table 1). However, the results are intriguing for the MOKG-isoN group (7.5 g/kg of MOKG), as these animals received the same amount of ORN and 2-fold more αKG than the rats treated with OKG (Table 1). We wondered whether these differences might be due to differences in solution osmolarities, as it is well known that hyperosmolar solutions can decrease absorption and induce osmotic diarrhea. However, intestinal ORN concentrations in the MOKG-isoM and MOKG-isoN groups were comparable, whereas the osmolarity of the MOKG-isoM solution (1888 mosm/L) was 2-fold less than that of the MOKG-isoN solution (>3000 mosm/L). Thus, the difference in intestinal ORN observed between MOKG-treated and OKG-treated rats was probably not the consequence of different osmolarities.

To explain the observed differences in intestinal ORN contents between the OKG and the MOKG-treated rats, a second experiment was performed to track amino acid (AA) metabolism until 6 hours postadministration.

The profiles of data taken at the same time points in experiments 1 and 2 are similar, but the absolute values are nevertheless different. The magnitude of these differences is characteristic of in vivo experiments. The most important feature is that these marginal differences between experiments did not change the overall interpretation.

In this second experiment, only MOKG supplied isonitrogenously to OKG was studied. Thus, MOKG-treated rats received an identical amount of ORN but 2-fold more αKG than OKG-treated rats. Intestinal ORN concentrations, particularly in the ileum, were lower in the MOKG-treated rats than in the OKG-treated rats until 3 hours after the treatment. The resulting areas under the curves showed almost 2-fold less ORN in the intestine of MOKG-treated rats compared with OKG-treated rats. This could be explained by the administration of a higher amount of αKG in the MOKG-treated rats (Table 1) that may have (1) competitively limited intestinal absorption of ORN or (2) stimulated ornithine aminotransferase (OAT) activity, promoting ORN metabolization (Fig. 4). The first hypothesis is supported by the study of Vaubourdolle et al [10] demonstrating that in healthy mice and rats, unlabeled αKG was able to strongly reduce the intestinal absorption rate of [^{14}C]ORN, whereas the inverse was not true (ie, no effect of ORN on intestinal absorption rate of αKG), thereby highlighting a direct interaction between αKG and ORN in the intestine. The second hypothesis is supported by the fact that lower ORN concentrations in MOKG-treated than in OKG-treated rats were only observed in the intestine, with no differences in liver, plasma, or muscle ORN concentrations. A further interesting point is that the AUC for GLU in the ileal mucosa was 34% higher after MOKG administration than after OKG administration. The OAT-catalyzed reaction generates GLU semialdehyde from ORN for eventual GLU synthesis in most tissues [11,12], as confirmed by the fact that OAT mutation (ie, gyral atrophy) leads to ORN accumulation. However, in the intestine, the OAT-catalyzed reaction is a net source of ORN [13] to ensure flux from GLN to CIT [14]. Furthermore, OAT activity is highly dependent on substrate availability. Hence, the large quantity of αKG introduced into the rats by the MOKG treatment may have influenced intestinal OAT activity, forcing it to generate GLU from ORN (Fig. 4), and thus leading to the observed lower ORN content. This is consistent with the results observed in mice overexpressing OAT in which higher OAT activity was associated with a decrease in intestinal and hepatic ORN content, whereas PRO, ARG, and GLU concentrations remained unchanged [15]. Further studies will be required to determine the relative contribution of the 2 above-mentioned hypotheses to ORN availability.

Excluding this difference in intestinal ORN metabolism, the MOKG and OKG treatments resulted in similar tissue AA profiles. Less hepatic ORN was detected 1 hour after MOKG administration than after OKG administration, but there was no difference thereafter. In addition, no difference in plasma and muscle ORN content was observed after MOKG or OKG treatment, suggesting that similar amounts of ORN originated from the intestine reached the liver via the portal vein and were then similarly metabolized in the liver and/or similarly recovered in the plasma and muscles. In the same way, compared to the administration of saline solution, MOKG and OKG treatments induced similar AA profiles in all the tissues studied.

First, in the intestinal mucosae, ORN was higher than in control rats until 3 hours after administration. This was associated with an increase in GLU and PRO (except in the ileum), further suggesting that ORN is used preferentially in the OAT-mediated pathway (Fig. 4). In addition, GLN

increased to a peak in the jejunum at 2 hours and in the ileum at 3 hours. These higher GLN levels may be the result of (1) GLU amidation in the intestine because young rats show more active intestinal GLN synthetase than adults [16], and (2) an accumulation of GLU derived from ORN and αKG, which may inhibit glutaminase [17], thus leading to GLN accumulation [1].

Second, in the liver, the fact that higher ORN and GLU concentrations were observed in the OKG- and MOKG-treated rats than in controls reflects a flux of molecules presumably derived from the intestine via the portal vein. GLU could also be synthesized directly in the liver because the hepatic content of GLU increases when isolated rat liver is perfused with OKG [18]. This hepatic synthesis of GLU could occur via 2 pathways: the first could be the transamination of ORN with αKG by the OAT enzyme (whose hepatic form is exclusively perivenous [12]), whereas the second pathway could involve $^{1}\Delta$-pyrroline-5-carboxylate dehydrogenase (Fig. 4). Indeed, the significant increase in PRO concentration suggests that the precursor $^{1}\Delta$-pyrroline-5-carboxylate is readily produced and is thus available to form GLU. In addition, no changes in GLN concentrations were observed in the liver, which strongly suggests a highly intensive and rapid metabolism of GLN by periportal hepatocytes once taken up by these cells [19].

Finally, in the muscle, ORN, PRO, and GLU increased gradually in OKG- and MOKG-treated rats compared with controls, with the only significant difference being for GLU in EDL at the 6-hour posttreatment time point. In the present study, muscle PRO and GLU probably came from their accumulation in the plasma, and their gradual increase until the end of the study was probably because they were present in sufficient amounts to cover muscle needs. Indeed, in the post-absorptive state, muscle usually releases gluconeogenic amino acids (such as PRO) and takes up GLU, which is then metabolized to sustain the transamination of pyruvate into alanine. On the other hand, muscle ARG was not affected by the treatments, as previously described [6,20,21], and GLN pools also remained unaffected, probably because there was not enough time to induce GLN synthetase. Indeed, this enzyme may need a long-term induction [22], as observed in previous experimental studies [23,24].

In conclusion, the major metabolites detected after OKG or MOKG intake together with the absence of increased ARG and CIT levels (except for CIT in the liver) suggest that ORN (from either OKG or MOKG) was mainly metabolized by the OAT pathway (Fig. 4), leading to GLU and PRO production, with accumulations persisting at 6 hours posttreatment.

This study provides new and important data concerning the influence of ORN/αKG ratio on OKG metabolism. It demonstrates that the molar ratios of ORN and αKG have a distinctive influence on ORN concentrations, especially in the jejunal and ileal mucosae, with a probable influence of αKG on ORN absorption and OAT activity in the intestine. In addition, the ORN-αKG metabolic interaction (ie, the fact that in the presence of αKG, ORN appears to be mainly metabolized by OAT, and the metabolism is thus partially diverted toward PRO production) still takes place even if the salt contains only one molecule of ORN instead of two. It would thus be of interest to test the effects of this MOKG salt in different stress situations such as burn injury, where the effect of OKG is well documented [1].

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