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Effect of α-ketoglutarate infusions on organ balances of glutamine and glutamate in anaesthetized dogs in the catabolic state

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SUMMARY

1. The salt complex of L-(+)-ornithine and α-ketoglutarate (2-oxoglutarate) has recently been proposed for the treatment of patients in the catabolic state. As yet, it is unclear which of the two substrates (ornithine or α-ketoglutarate) is responsible for the anticatabolic effect. We infused α-ketoglutarate into anaesthetized postoperative dogs in order to investigate whether infusion of α-ketoglutarate affects the flux of glutamine and glutamate between skeletal muscle and the splanchnic bed. We used three infusion rates: 3, 10 and 20 μmol min^{-1} kg^{-1}. A steady state of α-ketoglutarate concentration in arterial whole-blood was attained only when the infusion rate was 3 μmol min^{-1} kg^{-1}.

2. Arterial whole-blood concentrations of α-ketoglutarate were 8.8 ± 1.2 μmol/l in the basal period and rose to 208 ± 41, 344 ± 61 and 1418 ± 315 μmol/l after 60 min infusions of α-ketoglutarate at 3, 10 and 20 μmol min^{-1} kg^{-1}, respectively.

3. α-Ketoglutarate uptake was measured in skeletal muscle, liver, gut and kidneys in the basal period and during the infusion of α-ketoglutarate. The net uptake of infused α-ketoglutarate was highest in the skeletal muscle, followed by kidneys, liver and gut.

4. The α-ketoglutarate load increased the muscular tissue content of α-ketoglutarate from 49.5 ± 5 to 142 ± 15 nmol/g of dry substance ($P < 0.001$), but did not alter the muscular glutamate or glutamine contents.

5. Infusion of α-ketoglutarate had no effect on the plasma glutamine concentration, nor on the glutamine and glutamate balances across the skeletal muscle, liver and gut. However, α-ketoglutarate infusion significantly reduced the renal extraction of glutamine ($P < 0.05$) and enhanced the renal production of glutamate ($P < 0.05$).

6. We conclude that an intravenous α-ketoglutarate load affects the renal balances of glutamine and glutamate, but does not alter the nitrogen flux of glutamine and glutamate between skeletal muscle, liver and gut.

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Abbreviation: α-KG, α-ketoglutarate.

INTRODUCTION

Previous studies on the metabolic impact of exogenous α-ketoglutarate (α-KG; 2-oxoglutarate; Ornicetil) supply focused on its effect on renal ammonia metabolism [1–3]. Several authors have demonstrated that the infusion of α-KG depresses the rates of renal ammonia synthesis and glutamine extraction [1, 3]. Alternatively, infusion of α-KG may also influence the metabolism of glutamine and glutamate in various organs, such as skeletal muscle, liver or gut. This is especially important in view of the finding that both glutamine and α-KG play key roles in protein turnover during various catabolic conditions.

It is well established that the intracellular glutamine levels in skeletal muscle, but not in the liver, are decreased in catabolic states such as trauma, sepsis, acute pancreatitis or burn injury [4–7]. Glutamine is known as the predominant metabolite for the nitrogen shuttle from skeletal muscle to the splanchnic bed, and together with alanine accounts for more than 50% of the amino acids released from the skeletal muscle [8, 9]. Recent observations have suggested that the intravenous administration of ornithine–α-KG, solely or as an adjunct to amino acid solutions used for total parenteral nutrition, improved the nitrogen balance in catabolic states [10, 11] and increased muscle intracellular glutamine levels. This led us to the hypothesis that the nitrogen-sparing effect attributed to ornithine–α-KG may be exerted via an improved supply

of intracellular α-KG, which in turn is destined for glutamine synthesis. Glutamine is unstable in aqueous solutions and is therefore not contained in commercially available solutions used for parenteral nutrition. Recent studies have confirmed that of the investigated glutamine analogues, alanylglutamine and glycylglutamine, but not acetylglutamine, are possible sources of glutamine for parenteral nutrition [12].

The purpose of this study was to investigate the impact of infusion of high amounts of α-KG on the organ-specific metabolism of α-KG, glutamate and glutamine in a catabolic dog model.

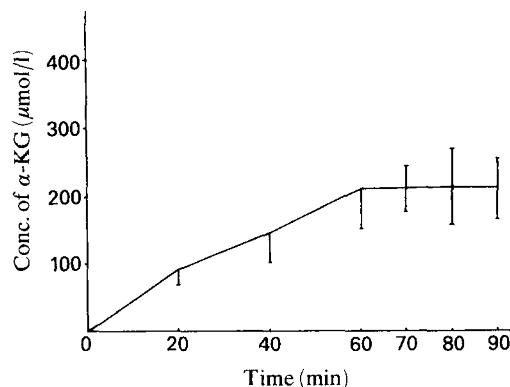

Fig. 1. Arterial whole-blood concentration of α-KG during the infusion of α-KG at 3 μmol min^{-1} kg^{-1} in five dogs.

METHODS

Procedures

Experiments were carried out in male Beagle dogs aged about 2 years and weighing 10–15 kg. The dogs were fasted overnight (12 h). On the morning of the study all dogs were subjected to general anaesthesia using nitrous oxide, oxygen and halothane in the proportion of 1:1:0.5. Each animal was subjected to laparotomy through a midline abdominal incision for placement of silastic catheters (1 mm inner diameter; 2.2 mm outer diameter; Dow Corning, Midland, MI, U.S.A.). The catheters were implanted into the abdominal aorta, femoral artery, portal vein, hepatic vein, renal vein and femoral vein as described previously [13, 14]. Another catheter was placed in the internal jugular vein and was used for the infusion of α-KG or saline [0.9 (w/w) NaCl]. Flow probes of appropriate inner and outer diameters (Hellige GmbH, Freiburg, Germany) were placed around the vena cava as well as the portal and renal veins.

The operation and the implantation of the catheters took about 1 h. The experiment was started 30 min after the end of these procedures.

Experimental design

Each experiment started with the infusion of saline for at least 30 min (basal period). In five dogs this was followed by the infusion of α-KG at 10 μmol min^{-1} kg^{-1} for 60 min followed by the infusion of α-KG at 20 μmol min^{-1} kg^{-1} for an additional 60 min. This group of dogs was also used for the determination of muscular α-KG, glutamine and glutamate contents. Muscle biopsies were performed in the anaesthetized dogs before catheterization and immediately after the end of the second infusion period. In another five dogs we infused α-KG at 3 μmol min^{-1} kg^{-1} for 90 min to measure α-KG, glutamine and glutamate balances under steady-state conditions. We compared the α-KG, glutamine and glutamate concentrations and organ balances in the basal period with those of a group of five dogs receiving only saline for 3 h by the same study design.

The α-KG solution was dissolved in distilled water; the solution was rendered isotonic by addition of NaCl. All solutions were sterilized by filtration through 0.22 μm filters (Millipore, Milford, MA, U.S.A.). Blood sampling in the basal period was performed at 0, 15 and 30 min and during the escalating infusion of α-KG at 10 and 20 μmol min^{-1} kg^{-1} (each for 60 min) samples were taken at +20, +40, +60, +80, +100 and +120 min. During the infusion of α-KG at 3 μmol min^{-1} kg^{-1}, samples were taken at +20, +40, +60, +70, +80 and +90 min after start of the α-KG infusion.

Analysis

Blood samples for the determination of plasma amino acids (2 ml) were drawn in heparinized syringes. Samples used for the determination of α-KG were placed in ice-cold 1 mol $HClO_4$. For the determination of amino acids, 1 ml of plasma was deproteinized with 0.1 ml of 3% (w/v) sulphosalicylic acid containing β-thienylalanine (1 mmol/l) as the internal standard. After centrifugation, one part of the sample was diluted with 99 parts of water containing 2 mmol of NaN_3. The amino acids were analysed using an automatic precolumn derivation with ophthalaldehyde and h.p.l.c. separation as described previously [14].

For determination of free amino acids in skeletal muscle we dissected a muscle specimen of about 50 mg from the hindlimb. All visible fat and connective tissue were removed from the muscle specimen. The muscle tissue was cut into small portions. Two specimens were weighed immediately on a Cahn 25 electrobalance (Ventron Corp., Cerriots, CA, U.S.A.) and homogenized in a prechilled homogenizer with 0.5 ml of a 40 g/l solution of sulphosalicylic acid, containing 100 μmol/l β-thienylalanine added as the internal standard. After centrifugation, the pH of the supernatant was adjusted to 2.2 with 0.2 mol/l LiOH and stored at −80°C until further analysis. One portion of the muscle specimen was used for evaluation of the water content, by weighing before and after drying, and the chloride content, by electrometric titration [5, 15].

For determination of α-KG, blood samples were deproteinized with 1 mol $HClO_4$. The α-KG was enzymically analysed with glutamate dehydrogenase as described

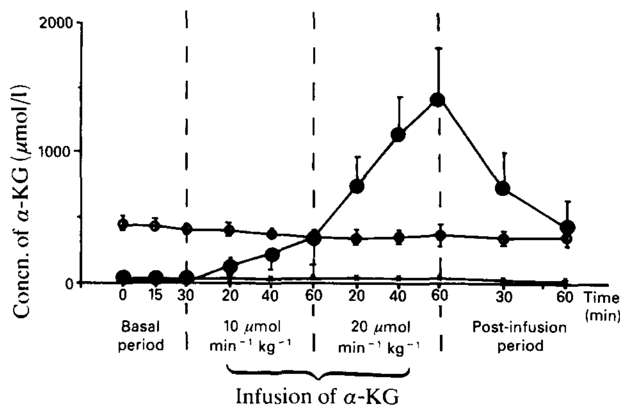

Fig. 2. Arterial whole-blood concentrations of α-KG (●) and plasma concentrations of glutamine (○) and glutamate (★) before, during and after an infusion of α-KG. Values are means ± SEM.

elsewhere [16]. Blood α-KG concentrations were corrected with the packed cell volume to obtain plasma α-KG concentrations. For the determination of α-KG in the muscle, two muscle specimens were placed in liquid nitrogen, weighed and homogenized in 1 mol/l $HClO_4$.

The blood flow in the respective vessels (vena cava, portal and renal veins) was measured electromagnetically [17] and was corrected with the corresponding packed cell volume to obtain plasma flow values. The hindlimb was assumed to be 25% of the total skeletal muscle; therefore, plasma (blood) flow in the hindlimb was multiplied by 4 to calculate plasma (blood) flow of the total skeletal muscle. The hepatic, renal and muscle plasma flows determined in the present experiment were similar to those that we and others had reported previously [12–14, 18].

Calculation and statistics

The net release or removal of amino acids or α-KG by the respective organs was calculated by multiplying the arteriovenous plasma concentration differences by the rate of plasma flow. In the case of the liver there was a dual supply of substrates, one via the portal vein and the other via the hepatic artery. It was assumed that 72% of the hepatic blood flow was via the portal vein and 28% via the hepatic artery [19]. These plasma flow data were multiplied by the respective concentrations in the portal vein and artery to calculate the total amount of substrate inflow into the liver. Balances across the hindlimbs were assumed to be representative of balances across skeletal muscle. We chose to refer to the substrate balance across the extrahepatic splanchnic tissue as that of the 'gut'.

Analysis of variance with repeated measures was used to determine whether there was a difference among the values obtained during either the control or the test period. Analysis of statistical differences was performed by using Wilcoxon's matched-pairs signed rank test, and (exact permutational) P-values were derived because of the small sample size. All data are expressed as means ± SEM.

RESULTS

α-KG concentration and balance

In the basal period during the infusion of NaCl, the arterial whole-blood concentration of α-KG was 8.8 ± 1.2 μmol/l. A steady state in arterial blood concentration of α-KG was attained when the infusion rate was 3 μmol min^{-1} kg^{-1} (Fig. 1), but not when the infusion rate was either 10 or 20 μmol min^{-1} kg^{-1}. No significant differences were found when measuring blood concentrations and organ balances of α-KG, glutamine and glutamate 30 min (basal period) or 180 min (control dogs) after the end of the operation.

The infusion of α-KG at 3 μmol min^{-1} kg^{-1} raised the arterial whole-blood concentration to a maximum of 208 ± 41 μmol/l. The infusion of α-KG at 10 μmol min^{-1} kg^{-1} for 60 min increased the arterial whole-blood concentration of α-KG to 344 ± 61 μmol/l and a sequential 60 min infusion of α-KG at 20 μmol min^{-1} kg^{-1} increased the blood concentration to 1418 ± 315 μmol/l (Fig. 2).

In the basal period the flow rates in the hindlimbs, liver, kidneys and gut were 7.7 ± 0.5, 15.2 ± 1.3, 9.6 ± 0.9 and 10.8 ± 1.1 ml min^{-1} kg^{-1}, respectively. α-KG infusions did not alter the blood flow in any of the tissue beds examined, muscular, hepatic or renal. α-KG balances were statistially ($P < 0.05$) different from zero in all organs studied, indicating uptake of α-KG by the organs (Table 1). The infusion of α-KG evoked positive α-KG balance across each organ. α-KG uptake was highest in the muscle, followed by kidneys, liver and gut. The percentage of α-KG uptake by the organs was about the same during the infusion of α-KG at 3 or 10 μmol min^{-1} kg^{-1}.

Glutamine and glutamate concentrations and balances

During the basal period, plasma glutamine concentrations were lower in the artery (434 ± 34 μmol/l) and in the renal veins (384 ± 51 μmol/l) than in the vena cava (545 ± 67 μmol/l); the concentrations were higher in the portal vein (360 ± 56 μmol) than in the hepatic vein (305 ± 43 μmol/l). These results indicate that in the basal state the skeletal muscle produced glutamine at an average net rate of 3.4 ± 0.4 μmol min^{-1} kg^{-1}, whereas the liver, gut and kidneys consumed glutamine at average net rates of 1.4 ± 0.5, 0.54 ± 0.21 and 0.5 ± 0.1 μmol min^{-1} kg^{-1}, respectively (Figs. 3 and 4).

Figs. 3–5 compare the organ balances of glutamine and glutamate during the infusion of α-KG at 3, 10 and 20 μmol min^{-1} kg^{-1} with values obtained in the basal period. Infusion of α-KG had no effect on glutamine concentrations in the artery (Fig. 2), vena cava, portal and hepatic veins, but increased glutamine concentrations in the renal veins. Therefore, α-KG infusions had no effect on glutamine balances across muscle, liver and gut (Fig. 3).

Table 1. α-KG balance across skeletal muscle, liver, kidneys and gut during the infusion of saline (basal period) or α-KG at 3 or 10 μmol min^{-1} kg^{-1}

The values for the basal period and those for the infusion of α-KG at 3 μmol min^{-1} kg^{-1} are the averages of three determinations (basal period: blood samples taken at 0, 15 and 30 min; α-KG infusion, blood samples taken as 70, 80, 90 min after the start of infusion). During the infusion of α-KG at 10 μmol min^{-1} kg^{-1} a steady state was not reached, therefore the Table shows the balance data obtained after 20, 40 and 60 min of α-KG infusion. Results are means ± SEM.

	Flux (μmol min^{-1} kg^{-1})				
	Basal period	α-KG infusion			
		3 μmol min^{-1} kg^{-1}	10 μmol min^{-1} kg^{-1}		
			20 min	40 min	60 min
Skeletal muscle	+0.18±0.03	+0.32±0.30	+2.83±1.31	+3.75±1.12	+4.20±1.61
Liver	+0.04±0.02	+0.40±0.12	+0.70±0.11	+0.76±0.23	+0.90±0.25
Kidneys	+0.09±0.02	+0.72±0.13	+1.66±0.55	+1.33±0.09	+1.83±0.11
Gut	+0.04±0.01	+0.40±0.10	+0.66±0.08	+0.55±0.21	+0.66±0.16

However, α-KG infusions significantly reduced the renal uptake of glutamine, leading to a balance not significantly different from zero (Fig. 4).

Compared with glutamine concentrations, plasma glutamate concentrations were substantially lower in the basal period. The plasma glutamate concentrations were higher in the artery (26±7 μmol/l) than in the vena cava (24.8±6.5 μmol/l) and portal vein (21.2±5.2 μmol/l), and lower than those in the hepatic (30.3±2.7 μmol/l) and renal (33.3±4.5 μmol/l) veins. Net basal glutamate balances across the muscle and liver beds were neutral, indicating no net exchange (Fig. 5). However, glutamate was released by the kidneys (−0.07±0.007; $P<0.05$) and gut (−0.1±0.02; $P<0.05$), as shown in Figs. 4 and 5. During both α-KG infusion periods, the glutamate levels remained unaltered in the vena cava and in the renal vein, and increased slightly in the artery (from 26±3.5 to 32±2 and 36±2 μmol/l, respectively) and in the portal and hepatic veins ($P<0.05$). During the α-KG infusion glutamate balances in muscle, gut and liver remained unchanged (Fig. 5), but glutamate release across the kidneys was significantly increased ($P<0.01$; Fig. 4). The infusion of α-KG at 20 μmol min^{-1} kg^{-1} caused release of glutamate from skeletal muscle (Fig. 5).

Muscular contents of α-KG, glutamine and glutamate

α-KG infusion increased the α-KG content of the skeletal muscle about threefold from a basal level of 49±5 nmol/g of dry substance to a post-infusion value of 142±15 nmol/g of dry substance, but did not influence the glutamate (10±2 versus 8±1.7 mmol/l intracellular water) and glutamine (14±2 versus 13.5±2.6 mmol/l intracellular water) contents.

DISCUSSION

In this study we have investigated the organ-specific interdependence of α-KG, glutamate and glutamine in protein catabolic conditions. In a previous study we have shown that the nitrogen flux from skeletal muscle via glutamine and alanine is higher in anaesthetized dogs than in conscious dogs, in whom catheters were implanted 16–20 days before the study [12, 14]. The glutamine and alanine release from skeletal muscle as measured in this study was comparable with that found in septic dogs on the second post-operative day [20], indicating that anaesthesia, laparotomy and catheter implantation evoke a protein catabolic situation. In the basal state before the infusion of α-KG, organ balances of α-KG were slightly positive across all organs studied, indicating a minor net uptake of α-KG by muscle, liver, gut and kidneys. We could not find an α-KG-exporting organ; however, it is possible that α-KG is produced by and exported from the lung.

During the infusion of α-KG at 3 μmol min^{-1} kg^{-1}, the α-KG uptake was highest in the skeletal muscle followed by the kidneys, liver and gut. This is in contrast to previously published reports indicating net uptake of infused α-KG by kidneys and liver, but not by the lower extremities, intestine or heart [2]. In this study, the measurements of arteriovenous differences across the leg and intestine were only performed in a single dog. Moreover, the authors compared the disappearance rate of α-KG during ligation of the femoral and renal arteries, and based on these data concluded that the kidneys and the liver were the organs mainly responsible for α-KG disposal. Infusion of α-[U-^{14}C]KG in rats revealed uptake of α-KG by the kidneys, liver, intestines and muscle tissue [21]. The radioactivity per g of tissue in skeletal muscle was 20% of that in the kidneys. In rats, the total muscle mass represents approximately 48% of the total body weight and thus the total muscle-associated radioactivity was certainly the highest of all tissue types. These and other observations in the rat [22] support our balance data, which show α-KG extraction by all organs studied, including skeletal muscle.

Infusions of high amounts of α-KG, as in this study, had no effect on arterial or intracellular muscle glutamine

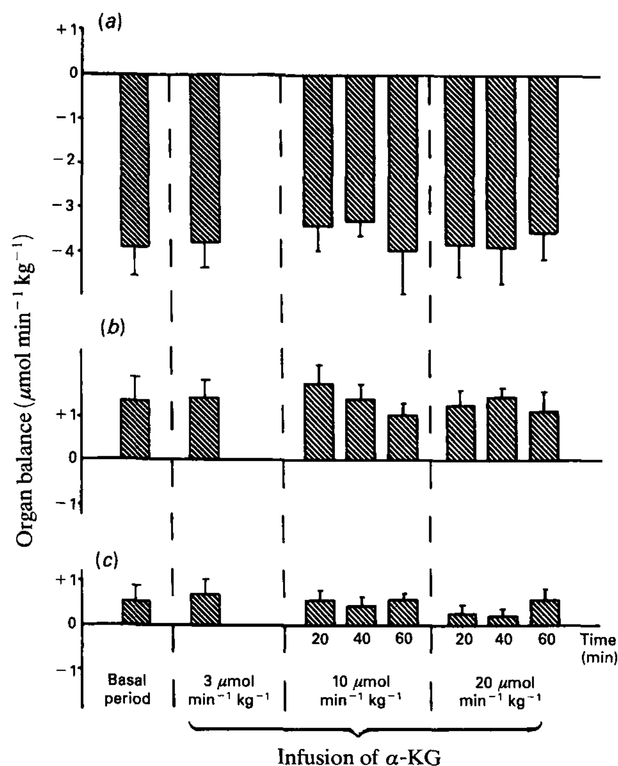

Fig. 3. Glutamine balance in skeletal muscle (*a*), liver (*b*) and gut (*c*) during the basal period (saline infusion) and during the infusion of α-KG at 10 μ min^{-1} kg^{-1} (60 min) followed by an infusion of α-KG at 20 μmol min^{-1} kg^{-1} (60 min) in anaesthetized, post-operative dogs. A plus sign (+) denotes uptake and a minus sign (−) denotes release.

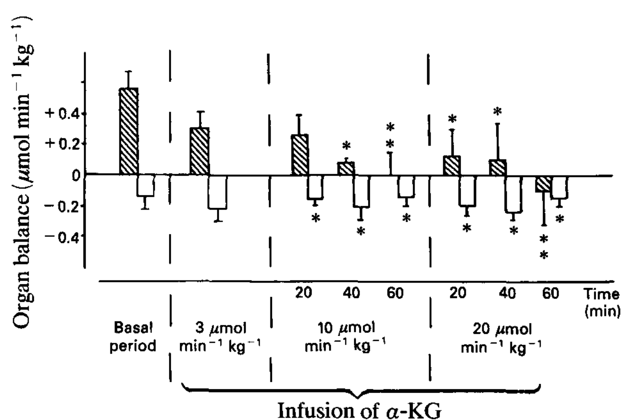

Fig. 4. Glutamine (▧) and glutamate (□) balances across the kidneys during the basal period (saline infusion) and during the infusion of α-KG at 10 μmol min^{-1} kg^{-1} (60 min) followed by the infusion of α-KG at 20 μmol min^{-1} kg^{-1} (60 min) in anaesthetized, post-operative dogs. A plus sign (+) denotes uptake and a minus sign (−) denotes release. Statistical significance: $^{*}P<0.05$, $^{**}P<0.01$.

levels nor on the glutamine and glutamate balances across the muscle, liver and gut in anaesthetized dogs. This is in contrast to the recently published observation that the addition of α-KG to total parenteral nutrition spares intracellular free glutamine in skeletal muscle [23]. However, this study performed in post-operative patients differs from our experiments in several aspects: the duration of the infusion was about 72 h and α-KG was given in addition to an amio acid solution. We have recently shown that infusion of glutamine plus alanine or the dipeptide alanylglutamine reduces nitrogen release from the skeletal muscle in dogs in the catabolic state [14, 20]. These metabolic data were confirmed by clinical trials showing that the infusion of glutamine or alanylglutamine increased the intracellular glutamine level in skeletal muscle and improved the nitrogen balance [24, 25]. The exact mechanism of the change in glutamine balance across the hindlimb in response to glutamine infusion was not apparent from the former study. The equimolar infusion of α-KG had absolutely no effect on the glutamine balance across the skeletal muscle. A twofold rise in arterial glutamine concentrations occurred during the infusions of glutamine, but not during the infusion of α-KG. Therefore we assume that the decreased glutamine efflux during glutamine infusion must have been related, in an as yet unknown way, to the observed rise in arterial glutamine concentrations. The infusion of α-KG at 20, but not at 10 or 3, μmol min^{-1} kg^{-1} caused release of glutamate from skeletal muscle. These data probably indicate that the infusion of a high amount of α-KG converts the muscle tissue from a glutamate consumer to a glutamate producer, possibly via transamination of accumulated intracellular α-KG. In the basal state, glutamine was assimilated from the liver and gut. These findings concur with the consistent observation that the gut extracts glutamine from the circulation [26, 27]. Glutamine serves as a major fuel source for both enterocytes and colonocytes and has a trophic effect on the intestinal mucosa [28]. Glutamine uptake from the gut was not affected by increased arterial levels of α-KG. α-KG uptake during the infusion of α-KG at 10 μmol min^{-1} kg^{-1} was similar to that of glutamine. From these data we cannot conclude that infused α-KG is a valuable energetic source for the gut. However, we can assume that α-KG, acting via the same oxidative pathway as glutamine, could also be used as an energetic source during α-KG infusion.

During the entire study period, the glutamine balance across the liver was positive and was not influenced by increased arterial or portal α-KG concentrations. It is well-known that the liver exerts a regulatory effect on glutamine metabolism. The liver is a net glutamine consumer during food intake and in the postprandial period, and is a net glutamine producer during prolonged fasting and acidosis [29, 30]. The metabolic heterogeneity of the hepatocytes is responsible for this regulatory capacity of the liver [31]. The enzymes of the urea cycle and glutaminase are present in periportal hepatocytes,

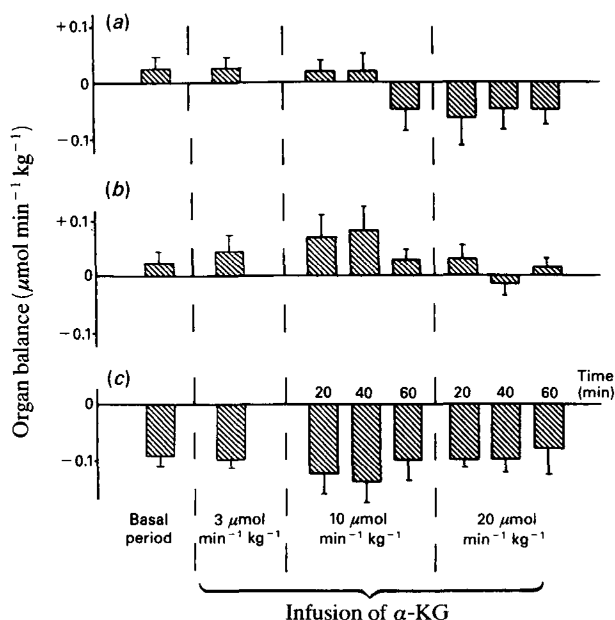

Fig. 5. Glutamate balance in skeletal muscle (a), liver (b) and gut (c) during the basal period (saline infusion) and during the infusion of α-KG at 10 μmol min^{-1} kg^{-1} (60 min) followed by the infusion of α-KG at 20 μmol min^{-1} kg^{-1} (60 min) in anaesthetized, post-operative dogs. A plus sign (+) denotes uptake and a minus sign (−) denotes release.

whereas glutamine synthetase is only found in perivenous hepatocytes. Administration of α-KG did not influence glutamate or glutamine concentrations in the hepatic vein, indicating that α-KG was not employed as a net substrate for transamination.

Infusion of α-KG reduced renal glutamine extraction and enhanced renal glutamine synthesis. This effect of exogenous α-KG supply was described earlier in dogs with chronic metabolic acidosis [1, 3]. In this study, the arterial α-KG levels, as well as the α-KG and glutamate balances across the kidneys, were similar to those obtained in this experiment. The effect of α-KG on the decreased ammonia and glutamine extraction seen with acidosis was explained by inhibition of the activity of glutaminase I or by reduced permeability of the mitochondrial membranes to glutamine [3].

In summary, this study shows that exogenously infused α-KG is extracted by muscle, kidneys, liver and gut. Even when infused in high doses, α-KG does not affect inter-organ flow of glutamine or glutamate, with the exception of the kidneys. Therefore the nitrogen-saving effect (as described for ornithine-α-KG) cannot be explained by the effect of α-KG on glutamine flow when α-KG is the only substrate infused. Further studies will be required to determine whether infusion of α-KG in combination with ornithine or with an L-amino acid solution is able to reduce nitrogen transport from the skeletal muscle to the splanchnic bed.

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