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Transport and utilization of α-ketoglutarate by the rat kidney in vivo

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Abstract. In order to establish the characteristics of net renal transport and utilization of α-ketoglutarate (α-KG) in the rat, we have precisely quantified the renal blood flow, the urinary flow and the rates of α-KG delivery, filtration, reabsorption or secretion, excretion, uptake or production by an in vivo rat kidney preparation. In normal rats, α-KG uptake was higher than α-KG reabsorption at both endogenous and elevated plasma α-KG concentrations; thus, a net peritubular transport, which was the main supplier of α-KG to the renal cells, took place. Saturation of reabsorption and peritubular transport of α-KG occurred at blood α-KG concentrations about 30 and 150 times above normal, respectively. Acute metabolic acidosis was found to have no effect on renal handling of α-KG. At endogenous plasma α-KG concentrations, alkalosis converted net renal uptake into net renal production of α-KG resulting in addition of α-KG by the renal cells both to blood and to the luminal fluid. Elevation of blood α-KG concentration restored the renal uptake of α-KG. This uptake, which was entirely accounted for by the peritubular transport of α-KG, reached a maximum which was lower than that observed in normal and acidotic rats.

Key words: Rat – Kidney – Uptake – Transport – α-Ketoglutarate – Luminal – Basolateral – Production

Introduction

It has been shown that α-ketoglutarate (α-KG), a Krebs cycle intermediate present in blood (Krebs 1939) and freely filtered by the kidney (Cohen and Wittman 1963), is a regulator of renal ammoniagenesis (Balagura-Baruch et al. 1970; Churchill et al. 1970; Welbourne et al. 1972), an important process involved in acid-base homeostasis. Thus, it is of great interest to know the characteristics of both transport and metabolism of α-KG in the kidney under different acid-base conditions.

Although rat is the species most commonly used in experiments of renal physiology and biochemistry, studies concerning the renal handling of α-KG in vivo have been performed mainly in the dog (Cohen 1960; Cohen and Wittman 1963; Vishwakarma 1963; Balagura and Pitts 1964; Selleck and Cohen 1965; Balagura and Stone 1967). Until recently, very little information was available on the renal handling of α-KG in rat in vivo (Silbernagl 1980; Sheridan et al. 1983).

Using micropuncture techniques and an ultramicromethod for measuring very low amounts of α-KG in nanoliter samples of tubular fluid, we have recently established the characteristics of net α-KG transport across the luminal membrane of different segments of the rat nephron (Ferrier et al. 1985); in this study, we have shown that, depending on the micropuncture site along the nephron as well as on the acid-base condition, α-KG was either reabsorbed or secreted.

These observations raised the following questions. (1) Does the rat kidney metabolize α-KG in vivo as it does in vitro (Balagura 1966), and, if so, is α-KG reabsorption sufficient to explain the renal consumption of this substrate? (2) What is the influence of changes in acid-base balance on renal uptake of α-KG? (3) Is the α-KG added to the tubular fluid across the luminal membrane in alkalotic rats (Ferrier et al. 1985) transported from peritubular blood or is it synthesized in the renal tubular cells?

In an attempt to answer these questions, we have carried out experiments to quantitate the renal uptake of α-KG using an in vivo rat kidney preparation. For this, renal blood flow, urine flow, glomerular filtration rate as well as transport, consumption or production, and excretion of α-KG have been precisely determined in normal and acutely made acidotic or alkalotic rats.

The data obtained demonstrate that the kidney of normal and acidotic rats utilizes at high rates the α-KG taken up across the luminal and basolateral membrane of renal cells at normal and elevated blood levels of α-KG; saturation of luminal and basolateral transport of α-KG occurs at blood α-KG concentrations respectively about 30- and 50-fold higher than normal endogenous blood concentrations. Our results also show that acute alkalosis dramatically alters the characteristics of α-KG transport and metabolism by the rat kidney.

Materials and methods

Experiments were performed on male Wistar rats ranging in weight from 200 to 260 g. Preparation of the animals was done as described previously in a paper from this laboratory (Ferrier et al. 1985). The rats were infused through the jugular vein at the rate of 0.1 ml · min^{-1} with the following solution: NaCl 150 mM, polyfructosan 4% (Laevosan, Linz, Austria), p-aminohippurate (PAH) 0.1% and mannitol 3% to get sufficient diuresis. To this solution, increasing

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amounts of α-KG (sodium salt) were added in order to increase the plasma concentration of α-KG. The infusion rates of α-KG were: 0 μmol · min^{-1} (endogenous level; groups 1 and 13); 0.5, 1, 2, 4, 8, 10 μmol · min^{-1} (groups 2–12 and 14–17). The infusion was preceeded by a priming injection of 10 ml· kg^{-1} body weight of the above solution. When the infusion rates of α-KG were higher than 4 μmol · min^{-1}, a third of α-KG was added as the acid form to prevent the development of a metabolic alkalosis. In an experimental series, acute acidosis was induced by intragastric administration of 3 ml of 0.6 M HCl 90 min before the experimental period. In another series of experiments, the rats were rendered alkalotic by infusing 0.3 M sodium bicarbonate in replacement of mannitol. After 60 min of infusion, a 30 min clearance period from the left kidney was started. An initial blood sample of approximately 0.6 ml was taken from the carotid artery and another blood sample was taken at the end of the clearance period. At the midpoint of each clearance period, renal venous blood was collected in the following way: a collecting glass pipette was pulled and sharpened with a tip of 180–200 μm, heparinized, mounted in a pipette holder and connected to a syringe by a length of polyethylene tubing. The pipette holder was fixed on a micromanipulator and a binocular lens with a magnification of 10× was used; in this way, the position of the pipette tip was continuously controlled. When the tip was within the renal vein, blood sample was aspirated slowly over a 2 min period by withdrawing the plunger of the syringe. In our hands, this procedure was never accompanied by any bleeding after withdrawal of the pipette.

Analytical procedure. Immediately after blood collection, 100 μl were deproteinized with 3 volumes of 10% chilled perchloric acid. An aliquot was used for hematocrit determination and the remainder was quickly centrifuged; then, 100 μl of plasma were deproteinized for α-KG determination and aliquots of plasma were used for PAH and polyfructosan analysis. The blood and plasma protein free extracts were neutralized with a mixture of KOH and phosphate potassium buffer just prior to α-KG analysis. Standards of α-KG were run in the same way. α-KG concentration of blood, plasma and urine was measured according to Narins and Passonneau (1974).

Some groups of animals received no α-KG or low delivery of α-KG from the perfusion; consequently, α-KG levels in plasma were lower than 150 μM, and, under these conditions, accurate α-KG analysis was done by using enzymatic cycling procedure (Chi et al. 1978) as described in a previous paper (Ferrier et al. 1985). Concentration of polyfructosan in diluted urine and deproteinized plasma was determined by the fluorimetric method of Vurek and Pegram (1966). Concentration of PAH in urine, arterial or renal venous plasma was determined by a microadaptation of the method of Bratton and Marshall (1939). In some experiments, blood pH and pCO_2 were measured at the end of the experimental period with a blood microsystem acid base analyser (model BMS3, Radiometer, Copenhagen, Denmark) and the bicarbonate content was calculated using the Henderson-Hasselbalch equation.

Calculations. The clearance of polyfructosan was taken as a measure of the glomerular filtration rate (GFR). The renal blood flow (RBF) was calculated from the arterial plasma, renal venous plasma, and urine PAH concentration, the urine flow and the arterial blood hematocrit according to the Wolf (1941) formula. Because of rapid handling of blood samples, no correction was made for diffusion of PAH from erythrocytes to plasma in renal venous specimen (Phillips et al. 1945).

Rates for α-KG handling expressed in micromoles per kg body weight per minute, were calculated as follows:

reabsorption = filtration − urinary excretion,

filtration = GFR × arterial plasma concentration,

urinary excretion = urinary concentration × urine flow,

renal extraction = renal arterial inflow − renal venous outflow,

renal arterial inflow = RBF × arterial blood concentration,

renal venous outflow = RBF × renal venous concentration,

renal uptake = renal extraction − urinary excretion.

When α-KG uptake is greater than reabsorption, this means that α-KG enters tubular cells from peritubular blood across their antiluminal membrane.

Therefore: peritubular transport into cells = uptake − reabsorption.

Results are expressed as means ± SEM. Statistical significance was evaluated by Student's *t*-test for paired or unpaired data or by U-test of Mann and Withney.

Reagents. Alpha-ketoglutarate and all enzymes and coenzymes were obtained from Boehringer (Meylan, France). All other reagents were of analytical grade.

Results

Except when stated, all the data included in the present paper were obtained during intravenous infusion of various amounts of α-KG. All our in vivo measurements were made under steady state experimental conditions since plasma levels of α-KG, PAH and polyfructosan were constant during the period of urine collection. The data are grouped according to the magnitude of the priming dose and the rate of α-KG infusion as well as according to the arterial plasma α-KG concentration.

Acid-base parameters and overall renal function in normal, acidotic and alkalotic rats

Blood pH and bicarbonate concentrations were determined in some animals of each series. In normal rats, mean blood pH was 7.39 ± 0.02 and plasma bicarbonate was 23.2 ± 1.0 mM ($n = 12$); in acidotic animals the corresponding values were 7.23 ± 0.04 and 14.0 ± 0.8 mM ($n = 14$); in alkalotic animals, mean blood pH was 7.60 ± 0.04, and plasma bicarbonate concentration was 42.1 ± 2.3 mM ($n = 9$). The mean hematocrit values were 43.6 ± 0.8% ($n = 54$), 46.0 ± 0.7% ($n = 30$) and 44.5 ± 0.9% ($n = 35$) in normal, acidotic and alkalotic animals, respectively.

Tables 1, 2 and 3 provide background information on the overall function of the left kidney for each group of animals in the normal, acidotic and alkalotic state. The average values of RBF and GFR were similar in the different groups of the normal and the alkalotic series, and comparable with values reported by others (Arendshorst et al. 1975; Bonjour and Malvin 1969). In the groups of acidotic animals, the values of RBF were more scattered since they

Table 1. Overall function of the left kidney of normal rats at endogenous (Group 1) and elevated plasma levels of α-ketoglutarate (Groups 2–7)

[P] α-KG mM	GFR ml · kg^{-1} · min^{-1}	Urine flow ml · kg^{-1} · min^{-1}	PAH extraction %	RBF ml · kg^{-1} · min^{-1}
0.012 ± 0.001, Group 1; $n = 7$	3.43 ± 0.14	0.180 ± 0.013	82 ± 2	20.8 ± 2.6
0.053 ± 0.005, Group 2; $n = 5$	3.42 ± 0.23	0.177 ± 0.021	85 ± 2	19.6 ± 2.9
0.13 ± 0.01, Group 3; $n = 11$	3.92 ± 0.25	0.227 ± 0.019	85 ± 1	23.1 ± 1.8
0.38 ± 0.03, Group 4; $n = 9$	4.25 ± 0.11	0.261 ± 0.021	85 ± 1	21.9 ± 0.9
0.84 ± 0.15, Group 5; $n = 7$	3.85 ± 0.44	0.236 ± 0.026	80 ± 2	22.0 ± 2.2
1.80 ± 0.14, Group 6; $n = 9$	3.58 ± 0.16	0.214 ± 0.014	68 ± 4	20.8 ± 1.2
2.94 ± 0.30, Group 7; $n = 7$	4.06 ± 0.28	0.268 ± 0.029	64 ± 2	23.0 ± 2.3

Abbreviations: α-KG = α-ketoglutarate; [P]α-KG = plasma α-KG concentration; GFR = glomerular filtration rate; RBF = renal blood flow; PAH = para-aminohippurate. Values are means ± SEM; n represents the number of rats in each group

Table 2. Overall function of the left kidney of acidotic rats at eleveted plasma levels of α-ketoglutarate

[P] α-KG mM	GFR ml · kg^{-1} · min^{-1}	Urine flow ml · kg^{-1} · min^{-1}	PAH extraction %	RBF ml · kg^{-1} · min^{-1}
0.15 ± 0.02, Group 8; $n = 4$	3.14 ± 0.27	0.194 ± 0.028	84 ± 2	16.1 ± 1.6
0.33 ± 0.03, Group 9; $n = 13$	3.73 ± 0.52	0.207 ± 0.020	81 ± 1	23.9 ± 1.7
0.83 ± 0.07, Group 10; $n = 6$	3.92 ± 0.25	0.209 ± 0.022	84 ± 3	25.4 ± 2.0
1.48 ± 0.17, Group 11; $n = 3$	3.33 ± 0.09	0.257 ± 0.026	75 ± 10	24.2 ± 0.8
2.96 ± 0.42, Group 12; $n = 4$	3.46 ± 0.16	0.224 ± 0.029	66 ± 5	18.2 ± 1.4

Abbreviations: α-KG = α-ketoglutarate; [P]α-KG = plasma α-KG concentration; GFR = glomerular filtration rate; RBF = renal blood flow; PAH = para-aminohippurate. Values are means ± SEM; n represents the number of rats in each group

Table 3. Overall function of the left kidney of alkalotic rats at endogenous (Group 13) and elevated plasma levels of α-ketoglutarate (Groups 14–17)

[P] α-KG mM	GFR ml · kg^{-1} · min^{-1}	Urine flow ml · kg^{-1} · min^{-1}	PAH extraction %	RBF ml · kg^{-1} · min^{-1}
0.021 ± 0.002, Group 13; $n = 8$	3.58 ± 0.17	0.193 ± 0.015	84 ± 3	22.8 ± 2.4
0.20 ± 0.03, Group 14; $n = 6$	3.85 ± 0.20	0.263 ± 0.030	78 ± 3	22.9 ± 1.6
0.52 ± 0.03, Group 15; $n = 9$	3.65 ± 0.18	0.182 ± 0.018	79 ± 2	21.0 ± 1.3
1.25 ± 0.11, Group 16; $n = 8$	4.43 ± 0.22	0.269 ± 0.017	82 ± 4	23.4 ± 0.8
2.62 ± 0.10, Group 17; $n = 4$	3.33 ± 0.48	0.307 ± 0.071	77 ± 3	20.6 ± 1.5

Abbreviations: α-KG = α-ketoglutarate; [P]α-KG = plasma α-KG concentration; GFR = glomerular filtration rate; RBF = renal blood flow; PAH = para-aminohippurate. Values are means ± SEM; n represents the number of rats in each group

varied from 16.1 ± 1.6 to 25.9 ± 2.0 ml · kg^{-1} · min^{-1}. In this respect, it should be emphasized that, in the acidotic series, about one half of the animals have been discarded because acidosis resulted in a drop of GFR probably due to a decrease in blood pressure.

As previously observed (Ferrier et al. 1985), plasma α-KG concentration progressively increased to steady-state levels with increasing rates of α-KG infusion in normal, acidotic and alkalotic rats (Tables 1, 2 and 3).

As can be seen in Tables 1 and 2, PAH extraction values were in the range of 80–85% in the normal and acidotic rats at low and moderately increased blood concentrations of α-KG; elevation of blood α-KG concentration above 1 mM was accompanied by a significant reduction in PAH extraction ($P < 0.02$). By contrast, PAH extraction was not significantly affected by high blood levels of α-KG in alkalotic rats (Table 3).

Renal handling of α-KG in normal and acidotic rats

The data in Tables 4 and 5 show that, despite a large increase in plasma concentration and, therefore, in filtered loads of α-KG in normal (groups 2 and 3) and acidotic (groups 8 and 9) rats, fractional excretion of α-KG remained very low and close to that found in rats receiving no infusion of α-KG (group 1). However, further elevation of plasma α-KG concentration led to a large increase in fractional excretion of α-KG which reached values of 0.85–0.88 at a plasma concentration of α-KG approaching 3 mM (Tables 4 and 5).

Again, confirming previous findings (Ferrier et al. 1985), reabsorption of filtered α-KG was found to be saturated in a similar manner in both normal and acidotic rats when plasma α-KG concentration was around 0.3–0.4 mM (Tables 4 and 5). Table 4 also shows that, in normal rats and at endogenous plasma concentration of α-KG, about 20%

Table 4. Renal handling of α-KG by the left kidney of normal rats

[P]α-KG mM	Arterial delivery μmol · kg^{-1} · min^{-1}	Uptake μmol · kg^{-1} · min^{-1}	Peritubular transport μmol · kg^{-1} · min^{-1}	Reabsorption μmol · kg^{-1} · min^{-1}	Excretion μmol · kg^{-1} · min^{-1}	FE α-KG
0.012 ± 0.001, Group 1; $n = 7$	0.49 ± 0.08	0.09 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.003 ± 0.001	0.066 ± 0.130
0.053 ± 0.005, Group 2; $n = 5$	1.00 ± 0.22	0.51 ± 0.14	0.33 ± 0.13	0.18 ± 0.03	0.006 ± 0.001	0.033 ± 0.033
0.13 ± 0.01, Group 3; $n = 11$	1.73 ± 0.12	1.29 ± 0.17	0.84 ± 0.15	0.45 ± 0.03	0.019 ± 0.005	0.041 ± 0.007
0.38 ± 0.03, Group 4; $n = 9$	4.97 ± 0.37	4.30 ± 0.36	2.93 ± 0.32	1.37 ± 0.10	0.240 ± 0.079	0.127 ± 0.039
0.84 ± 0.15, Group 5; $n = 7$	10.56 ± 1.37	6.48 ± 0.86	5.32 ± 0.95	1.16 ± 0.16	1.859 ± 0.384	0.580 ± 0.066
1.80 ± 0.14, Group 6; $n = 8$	25.47 ± 1.74	9.85 ± 0.81	8.52 ± 1.11	1.33 ± 0.40	5.386 ± 0.251	0.810 ± 0.061
2.94 ± 0.30, Group 7; $n = 7$	42.84 ± 2.58	10.48 ± 0.97	8.52 ± 0.90	1.96 ± 0.33	9.649 ± 0.599	0.852 ± 0.033

Abbreviations: α-KG = α-ketoglutarate; [P]α-KG, plasma α-KG concentration; FE α-KG, fractional excretion of α-KG (taken as the ratio of α-KG clearance over polyfructosan clearance). Values are means ± SEM; n represents the number of rats in each group. Group 1: no infusion of α-KG

Table 5. Renal handling of α-KG by the left kidney of acidotic rats

[P]α-KG mM	Arterial delivery μmol · kg^{-1} · min^{-1}	Uptake μmol · kg^{-1} · min^{-1}	Peritubular transport μmol · kg^{-1} · min^{-1}	Reabsorption μmol · kg^{-1} · min^{-1}	Excretion μmol · kg^{-1} · min^{-1}	FE α-KG
0.15 ± 0.02, Group 8; $n = 4$	1.91 ± 0.02	1.32 ± 0.18	0.88 ± 0.17	0.44 ± 0.02	0.008 ± 0.004	0.021 ± 0.007
0.33 ± 0.03, Group 9; $n = 13$	5.57 ± 0.67	3.99 ± 0.51	2.77 ± 0.36	1.22 ± 0.21	0.053 ± 0.017	0.044 ± 0.013
0.83 ± 0.07, Group 10; $n = 6$	12.43 ± 0.83	8.78 ± 0.73	6.77 ± 0.89	2.01 ± 0.32	1.195 ± 0.222	0.382 ± 0.072
1.48 ± 0.17, Group 11; $n = 3$	19.52 ± 1.54	10.40 ± 0.85	9.22 ± 0.79	1.18 ± 0.39	3.903 ± 0.434	0.800 ± 0.038
2.96 ± 0.42, Group 12; $n = 4$	34.66 ± 6.23	9.49 ± 0.77	8.01 ± 1.01	1.49 ± 1.14	9.030 ± 1.669	0.875 ± 0.081

Abbreviations: α-KG = α-ketoglutarate; [P]α-KG, plasma α-KG concentration; FE α-KG, fractional excretion of α-KG (taken as the ratio of α-KG clearance over polyfructosan clearance). Values are means ± SEM; n represents the number of rats in each group.

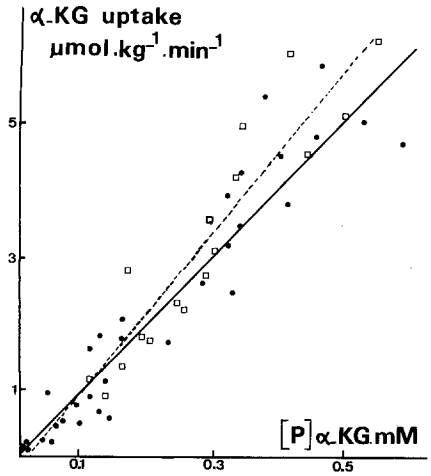

Fig. 1. α-KG uptake (y axis) rate as a function of plasma α-KG concentration (x axis). A linear relationship exists when plasma concentrations are lower than 0.6 mM in normal and acidotic rats. Each *symbol* represents one experimental value (●, ——) are for normal rats, $n = 37$, $r = 0.96$, $y = 10.4x - 0.076$ and (□, · · · ·) symbols are for acidotic rats $n = 17$, $r = 0.90$, $y = 12.3x - 0.26$

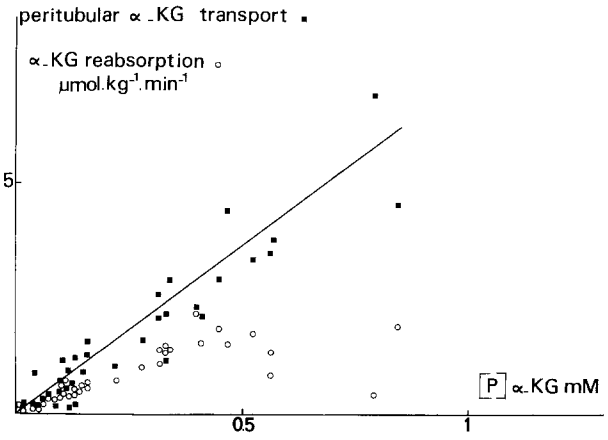

Fig. 2. Simultaneous individual observations for both α-KG peritubular transport (■) and α-KG reabsorption (○) as a function of α-KG plasma concentration in normal rats. There is a linear correlation between α-KG peritubular transport and α-KG plasma concentration ($n = 35$, $r = 0.92$, $y = 6.8x - 0.09$). The reabsorptive rate reaches a maximum at a plasma concentration value of 0.4 mM while peritubular transport still increases

of the α-KG delivered to the kidney was extracted; since, under this condition, urinary excretion of α-KG was extremely low, it is evident that renal extraction of α-KG was almost completely accounted for by renal metabolic utilization secondary to renal uptake of α-KG. This is particularly well illustrated by data from group 2, in which renal excretion of α-KG was 0.006 μmol · kg^{-1} · min^{-1} whereas renal uptake was 0.510 μmol · kg^{-1} · min^{-1}, representing about 50% of the renal arterial load of α-KG (see Table 4). As shown in Fig. 1, renal uptake of α-KG was proportional to the plasma α-KG concentration until the latter approached 0.6 mM. As expected, it was also proportional to

Table 6. Renal handling of α-KG by the left kidney of alkalotic rats

[P]α-KG mM	Arterial delivery μmol · kg^{-1} · min^{-1}	Uptake (+) Production (−) μmol · kg^{-1} · min^{-1}	Peritubular transport (+) μmol · kg^{-1} · min^{-1}	Reabsorption (+) Secretion (−) μmol · kg^{-1} · min^{-1}	Excretion μmol · kg^{-1} · min^{-1}	FE α-KG
0.021 ± 0.002, Group 13; $n = 8$	0.65 ± 0.09	−0.14 ± 0.05	−0.05 ± 0.04	−0.09 ± 0.03	0.162 ± 0.034	2.24 ± 0.59
0.20 ± 0.03, Group 14; $n = 6$	3.46 ± 0.46	−0.65 ± 0.43	+0.19 ± 0.24	−0.84 ± 0.25	1.614 ± 0.378	1.99 ± 0.19
0.52 ± 0.03, Group 15; $n = 9$	6.99 ± 0.60	2.62 ± 0.27	+2.67 ± 0.33	−0.05 ± 0.15	1.925 ± 0.145	1.05 ± 0.07
1.25 ± 0.11, Group 16; $n = 8$	18.97 ± 0.83	4.88 ± 0.57	+4.91 ± 0.58	−0.03 ± 0.24	5.277 ± 0.302	1.00 ± 0.04
2.62 ± 0.10, Group 17; $n = 4$	35.39 ± 2.88	6.46 ± 0.79	+6.32 ± 1.04	0.14 ± 0.69	8.627 ± 0.779	1.02 ± 0.07

Abbreviations: α-KG = α-ketoglutarate; [P]α-KG, plasma α-KG concentration; FE α-KG, fractional excretion of α-KG (taken as the ratio of α-KG clearance over polyfructosan clearance). Values are means ± SEM; n represents the number of rats in each group. Group 13: no α-KG was infused. A negative peritubular transport means α-KG transport from the cells to blood

the renal arterial delivery of α-KG up to a value of 10 μmol · kg^{-1} · min^{-1} (see Table 4); this increase reached a plateau with further elevation of renal arterial load of α-KG as shown in Table 4 where α-KG uptake in group 7 was not statistically different from that in group 6 despite a 68% elevation of the renal arterial delivery of α-KG.

In all groups of normal rats (Table 4), the rate of α-KG uptake exceeded the rate of reabsorption. This observation clearly indicates that transport of α-KG from peritubular blood into tubular cells occurred. As can be seen in Table 4, peritubular transport of α-KG was always much larger than the luminal transport of α-KG. This peritubular transport was linearly correlated to the plasma α-KG concentration until this concentration reached about 0.8 mM (Fig. 2). It also increased in proportion to the renal arterial α-KG delivery until it reached a plateau as shown in Table 4; saturation of antiluminal transport of α-KG is clearly demonstrated in Table 4 where this transport in group 7 is not statistically different from that in group 6 whereas renal arterial delivery of α-KG increased by 68%.

Table 5 shows that the pattern of α-KG handling by the kidney of acidotic rats is quantitatively and qualitatively very similar to that of normal rats; uptake, reabsorption and peritubular transport of α-KG increased and reached maximum values on elevation of plasma concentration and renal arterial delivery of α-KG. In all groups of acidotic rats, peritubular transport of α-KG was also higher than luminal transport of α-KG (see Table 5). As has been observed in normal rats, renal α-KG uptake was also proportional to plasma α-KG concentration at values up to 0.5 mM (Fig. 1).

Renal handling of α-KG in alkalotic rats

Table 6 shows that acute alkalosis greatly modified the renal handling of α-KG. At endogenous plasma concentration of α-KG (group 13), the renal venous output of α-KG (0.63 ± 0.03 μmol · kg^{-1} · min^{-1}) was not statistically different from the renal arterial delivery of α-KG (0.65 ± 0.09 μmol · kg^{-1} · min^{-1}), which means that no α-KG was extracted by the kidney. On the contrary, a small renal production of α-KG occurred since the sum of the renal venous output and the urinary excretion of α-KG was significantly greater than the renal arterial input of α-KG ($P < 0.02$); the renal cells were found to add α-KG both to peritubular blood, as reflected by a negative peritubular transport of α-KG, and to the tubular fluid resulting in a net secretion of α-KG (see Table 6, group 13).

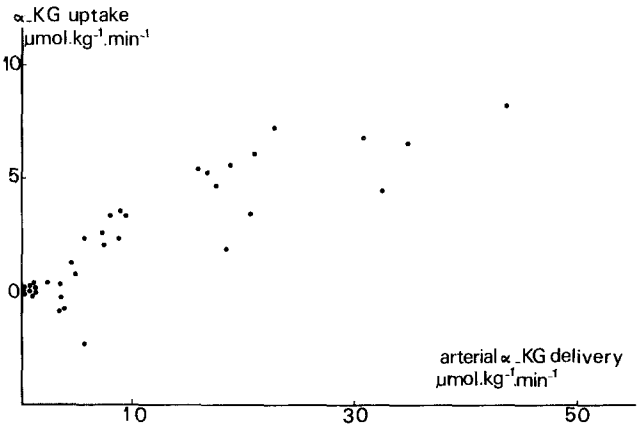

Fig. 3. α-KG uptake as a function of arterial α-KG delivery in alkalotic animals. α-KG uptake is observed with α-KG delivery values higher than 4 μmol · kg^{-1} · min^{-1} and reaches a plateau with α-KG delivery values higher than 15 μmol · kg^{-1} · min^{-1}. Each symbol represents one experimental value

At a mean plasma α-KG concentration of 0.2 mM (group 14), a significant renal extraction of α-KG occurred since the renal venous output of α-KG was 28% lower than the renal arterial input; this extraction of α-KG was explained not only by the glomerular filtration but also by a small peritubular transport of α-KG. In this group of alkalotic rats, an increased mean renal production of α-KG as compared to that in group 13 resulted in an increased mean secretion of α-KG.

At higher plasma α-KG concentrations, renal α-KG uptake was again observed; this uptake (Table 6), which was lower than that in normal and acidotic rats for similar renal arterial delivery of α-KG (Tables 4, 5), was linearly correlated to the renal arterial delivery of α-KG and reached a maximum value (Fig. 3). Saturation of α-KG uptake was confirmed using the U-test of Mann and Whitney to compare the values of renal α-KG uptake in groups 16 and 17; these values were not statistically different. Table 6 also shows that the renal uptake of α-KG, when it occurred, was entirely accounted for by peritubular transport of α-KG; under this condition, renal reabsorption of α-KG was absent or negligible when compared to the peritubular transport of α-KG.

Discussion

This study demonstrates for the first time that the intact functioning kidney of normal and acutely made acidotic rats avidly takes up α-KG at both endogenous and elevated blood α-KG levels (Tables 4 and 5). Similar observations have been made in normal and acidotic dogs but only at high plasma concentrations of α-KG (Cohen 1963; Balagura and Pitts 1964). Saturation of renal α-KG uptake in normal and acidotic rats occurred at a plasma α-KG concentration at least 100 times the endogenous level (Tables 4 and 5) indicating that, in vivo, the rat kidney has a very large capacity to take up, and presumably metabolize, this organic compound.

Our study also establishes that, in normal and acidotic rats, α-KG uptake occurs not only from the lumen of the nephron but also across the basolateral membrane of the renal cells. Under all the conditions studied in normal and acidotic rats the antiluminal transport of α-KG was the predominant supplier of α-KG to the kidney; this was especially true at high plasma α-KG concentrations when the T_m for α-KG reabsorption was reached; under these conditions peritubular transport of α-KG was found to be up to 7-fold greater than the luminal transport of α-KG. It should be underlined that, in the present study, the rates of reabsorption and antiluminal uptake of α-KG are net and not unidirectional rates across the luminal and basolateral membranes, respectively; thus they represent minimal values. In this respect, it should be stressed that microperfused rat nephrons have been shown to transport ^{14}C-α-KG from the lumen to peritubular blood (Silbernagl 1980; Sheridan et al. 1983); therefore, it is evident that, in our study, part of the α-KG unidirectionally reabsorbed across the luminal membrane was not metabolized by the renal cells but rather transported from the cell to the interstitium across the basolateral membrane and then into the blood capillaries. This means that, the contribution of the unidirectional antiluminal transport of α-KG to the provision of α-KG to the renal cells is greater than the values (which represent net rates) given in the present paper. Similarly, the present study cannot rule out the possibility that a transcellular transport of α-KG occurred from peritubular blood to the luminal fluid despite the absence of a net secretion of α-KG even at very high plasma α-KG concentration in both normal and acidotic rats (see Tables 4 and 5). To our knowledge, such a unidirectional transport of α-KG has never been reported in the literature.

Beside confirming our previous finding (Ferrier et al. 1985) that α-KG reabsorption in the kidney of normal and acidotic rats is limited by a T_m, our results demonstrate that the peritubular transport of α-KG is also saturable but at a much higher plasma α-KG concentration than the luminal transport (Tables 4, 5). This is suggestive of a carrier-mediated basolateral transport of α-KG into the renal cells. In this respect, it should be stressed that the existence of a basolateral dicarboxylate transport system has been demonstrated in the rat kidney (Burckhardt 1984). Although the characteristic of α-KG transport by this system have not been studied, it has been demonstrated that α-KG competitively inhibits the transport of dicarboxylic acids such as methylsuccinate (Burckhardt 1984; Ullrich et al. 1984); thus it is very likely that the basolateral dicarboxylate carrier is also involved in the basolateral α-KG transport observed in the present study. The information available in the literature indicates that, in normal rats and at endogenous plasma α-KG concentration, the renal tissue concentration of α-KG is about 0.1 – 0.3 mM (Boyd and Golstein 1979), a value higher than the plasma concentration found in normal rats of groups 1, 2 and 3 (see Table 4). Thus under these conditions, peritubular transport of α-KG occurred against a chemical gradient for α-KG which suggests that this transport was active. In this respect, no conclusion can be drawn about the peritubular transport of α-KG in other groups of normal and acidotic rats because we have not measured the renal concentration of α-KG and no data on this subject are available in the literature at elevated blood concentrations of α-KG. It should also be pointed out that saturation of net antiluminal uptake of α-KG might also be secondary to saturation of the metabolic utilization of α-KG; studies performed in vitro with dog kidney cortex slices by Cohen et al. (1969) or with rat kidney cortex slices by Balagura (1966) are in favour of such a possibility. Whether this is also the case in the rat kidney in vivo remains to be shown by studying the kinetic characteristics of the unidirectional antiluminal transport of α-KG.

Calculations from a graph published by Fonteles et al. (1983) reveal that, in the isolated rat kidney pefused with a medium containing between 1 and 5 mM α-KG as sole substrate, consumption of α-KG was about 5 μmol · g^{-1} · min^{-1}; this value is about twice that calculated from our own data given in Tables 4 and 5. The fact that our in vivo rat kidney preparation used much less α-KG than the isolated perfused rat kidney may be due in our study to the competition with α-KG of other circulating substrates for renal uptake and metabolism. In view of the demonstration by Burckhardt (1984) and Ullrich et al. (1984, 1987) that the dicarboxylic anion and the PAH transport systems have overlapping specificities, the lowest uptake of α-KG in our in vivo preparation than in the isolated perfused rat kidney might also be due to an inhibition of antiluminal α-KG transport by the PAH used in our study to measure renal blood flow. This possibility has been ruled out by performing two experimental series in which PAH was omitted and polyfructosan extraction used to determine renal blood flow; at an endogenous plasma α-KG concentration of 13.4 ± 1.1 μM ($n = 5$) and at a plasma α-KG concentration of 124.4 ± 4.4 μM ($n = 4$), extraction of α-KG was not enhanced in the absence of PAH and did not significantly differ from that in the presence of PAH (results not shown).

It should be emphasized that high plasma concentrations of α-KG did not affect renal hemodynamics and, therefore, did not alter the renal arterial supply or filtered loads of α-KG. The only effect of high plasma concentrations of α-KG was a slight inhibition of renal PAH extraction in normal and acidotic rats (Tables 1 and 2). In this respect it is of interest to note that, in our study, inhibition by α-KG (2 – 3 mM) of the renal extraction of circulating PAH (about 0.1 mM) was much smaller than the inhibition by α-KG (1 and 10 mM) of the antiluminal transport of PAH (0.1 mM) reported by Ullrich et al. (1987); this is probably due to differences in transport and/or metabolic properties between our intact rat kidney preparation and the rat kidney preparation used by Ullrich et al. (1987) in which the renal artery has to be clampped.

The precise nephron segment responsible for α-KG uptake and metabolism cannot be determined from the results of the present study. However, previous demonstrations that α-KG reabsorption occurs along the proximal convoluted

tubule (Silbernagl 1980; Sheridan et al. 1983; Ferrier et al. 1985) as well as between the late proximal and the distal tubule (Ferrier et al. 1985) make it very likely that α-KG consumption occurred in the cells of the proximal convoluted tubule as well as in those of the pars recta and/or of the Henle loop. Since no net reabsorption of α-KG occurred beyond the distal tubule (Ferrier et al. 1985), provision of α-KG to the collecting duct cells cannot occur through their apical membrane. In view of the existence of dicarboxylate carrier in the rat proximal tubule (Ullrich et al. 1984), it is also very likely that a large fraction of the antiluminal uptake of α-KG measured in this study occurred in this nephron segment. However, considering that α-ketoglutarate dehydrogenase, the main enzyme responsible for α-KG metabolism, is present in all nephron segments studied (Le Hir and Dubach 1982) and the observation by Klein et al. (1981) that $^{14}CO_2$ is produced from ^{14}C-α-KG in all nephron segments isolated from the proximal tubule and the medullary thick ascending limb of Henle, it cannot be excluded that uptake of α-KG also occurred through the basolateral membrane of cells localized in the loop of Henle, distal tubule and collecting duct.

Confirming previous findings (Ferrier et al. 1985), the present study shows that, at both endogenous and moderately elevated plasma α-KG concentrations (see Table 6, groups 13 and 14), a net secretion of α-KG clearly occurred along the nephron of alkalotic rats; net α-KG secretion has been shown to occur in the early proximal convoluted tubule as well as in the pars recta and/or the loop of Henle of alkalotic rats (Ferrier et al. 1985). A novel finding is that alkalosis suppressed the net renal antiluminal transport of α-KG from the blood into the renal cells at endogenous plasma α-KG concentration (Table 6, group 13); therefore, this means that under this condition, the α-KG secreted into the tubular lumen was synthesized by the renal cells and not transported trancellularly from the blood. Since, in group 13, no net extraction of α-KG was observed between the renal artery and the renal vein, an amount of α-KG equivalent to that filtered at the glomerulus must have been added to the peritubular blood; since no net reabsorption of α-KG occurred under this condition, it is clear that the α-KG transported from the renal cells into the blood was also synthesized by these cells. Thus, alkalosis which converts the net renal uptake into a net renal production of α-KG (see Table 6 and the text in the Results section) leads to an efflux of α-KG through both the luminal and the antiluminal membrane of the renal cells. Complete suppression of α-KG reabsorption and antiluminal uptake observed in alkalotic rats with endogenous α-KG concentration (group 13) may be due to the increase in the tissue concentration of α-KG [known to occur under such conditions (Boyd and Goldstein 1979)] secondary to inhibition of renal α-KG degradation caused by alkalosis; alkalosis may also have caused changes in blood concentration of other renal substrates competing with α-KG for renal transport and metabolism.

The reason why elevation of plasma α-KG concentration reverses the alkalosis-induced inhibition of antiluminal uptake but not of luminal uptake of α-KG is difficult to understand; this suggests that the factor or the factors involved in this inhibition are not the same or do not exert their inhibitory effect with the same potency at the level of the carrier systems reponsible for α-KG uptake across the luminal and basolateral membranes of the renal cells. Clearly, further studies are needed to identify such factors as well as their mechanism of interactions with the renal α-KG carriers as well as with α-KG renal metabolism.

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