Metabolic fate of glutamate carbon in rat renal tubules

Studies with ^{13}C nuclear magnetic resonance and gas chromatography–mass spectrometry

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^{13}C -n.m.r. spectroscopy and g.c.–m.s. were used to determine the metabolic fate of glutamate carbon in rat kidney. The main purpose was to characterize the effect of chronic metabolic acidosis on the utilization of glutamate carbon. Renal tubules obtained from normal and chronically acidotic rats were incubated in Krebs buffer, pH 7.4, in the presence of 2.5 mM-[3- ^{13}C]glutamate. During the course of incubation the concentrations of total glucose and NH_3 were significantly ($P < 0.05$) higher in tissue from acidotic rats. The levels of some tricarboxylic-acid-cycle intermediates were higher ($P < 0.05$) in control tissue. In control tissue, ^{13}C -n.m.r. spectra demonstrated a significantly higher rate of ^{13}C appearance of aspartate, glutamine and [2,4- ^{13}C]glutamate. However, in acidosis the resonances of [^{13}C]glucose carbon atoms were significantly higher. In the control, approx. 15% of glutamate carbon was accounted for by [^{13}C]glucose formation as against 30% in chronic acidosis. However, in control tissue, 44% of glutamate carbon utilization was accounted for by recycling to glutamate and formation of aspartate, glutamine and GABA. In acidosis, only 11% was so recovered. Analysis of $^{15}\text{NH}_3$ formation during the course of incubation with 2.5 mM-[^{15}N]glutamate demonstrated a positive association between the appearance of [^{13}C]glucose and $^{15}\text{NH}_3$ both in the control and in acidosis. The data suggest that the control of gluconeogenesis and ammoniogenesis in acidosis is, in part, referable to a diminution in the rate of the reductive amination of α -oxoglutarate, that of the transamination reaction and that of glutamine synthesis.

INTRODUCTION

The major pathway of renal NH_3 production from glutamine is via phosphate-dependent glutaminase, in which NH_3 and glutamate are formed from glutamine [1–4]. The glutamate produced is metabolized in diverse ways: (1) oxidative deamination catalysed by glutamate dehydrogenase; (2) transamination via glutamate:oxaloacetate transaminase; (3) oxidative decarboxylation mediated by glutamate decarboxylase; and (4) conversion of glutamate into glutamine catalysed by glutamine synthetase [1–4].

An understanding of the relative importance of these pathways is necessary for a full appreciation of the mechanisms regulating renal ammoniogenesis in various acid–base states. The fate of glutamate nitrogen was detailed in our previous studies with [^{15}N]glutamate [4]. However, the extent to which the carbon of glutamate is distributed among glucose, aspartate, GABA and tricarboxylic-acid-cycle intermediates in acidosis remains uncertain.

In the present study we utilized both ^{13}C -n.m.r. spectroscopy and g.c.–m.s. to analyse both ^{13}C in glutamate metabolites and $^{15}\text{NH}_3$ in renal tubules incubated with [3- ^{13}C]glutamate or [^{15}N]glutamate. ^{13}C n.m.r. is well suited to this purpose because it enables a detailed analysis of basic metabolic processes [5–9]. We have demonstrated elsewhere the utility of g.c.–m.s. in studies of this kind [4]. Experiments were performed in tubules obtained from normal and acidotic rats. The data show that removal of NH_3 by recycling to glutamate and glutamine could play an important role in regulating

renal ammoniogenesis and gluconeogenesis in various acid states.

MATERIALS AND METHODS

Preparation and incubation of renal-cortical tubes

Male Sprague–Dawley rats weighing between 250 and 300 g were obtained from Charles River Breeding Laboratories. Induction of chronic metabolic acidosis by NH_4Cl administration and preparation of tubules were done as described previously [4]. Tubules (25–30 mg wet wt./ml) were incubated at 37 °C for the times indicated in 4 ml of Krebs–Henseleit buffer, pH 7.4, containing 2.5% albumin (dialysed bovine fraction V) in stoppered 25 ml Erlenmeyer flasks in a water bath. The buffer was pre-gassed with O_2/CO_2 (19:1). Incubations were initiated by addition of substrate to 4 ml of cell suspension. The substrates were either 2.5 mM-[3- ^{13}C]glutamate or [^{15}N]glutamate [99 atom% excess; MSD Ltd. (Montreal, Canada)]. Incubations were stopped by the addition of 400 μl of 40% (w/v) HClO_4 to each flask. After neutralization with KOH, the supernatants were utilized for determination of metabolites as well as measurement of isotopic abundance with either g.c.–m.s. or n.m.r.

Analytical methods

Amino acids, NH_3 , lactate, pyruvate, α -oxoglutarate, ATP and ADP were measured as described previously [4]. Glucose was measured by the glucose oxidase method [10]. Tricarboxylic-acid-cycle intermediates, succinate, malate, citrate, *cis*-aconitate and fumarate were measured

Abbreviation used: GABA, γ -aminobutyric acid.

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Fig. 1. A representative gas chromatogram of trimethylsilyl-oxime derivatives of organic acids from renal tubules

A detailed description of the g.c.-m.s. conditions is given in the Materials and methods section. Peaks are identified as follows: 1, lactate; 2, α -hydroxybutyrate; 3, pyruvate; 4, β -hydroxybutyrate; 5, unknown; 6, acetoacetate; 7, phosphate; 8, β -hydroxyisovaleric acid; 9, succinate; 10, fumarate; 11, malate; 12, *cis*-aconitate; 13, unknown; 14, citrate; 15, sebacic acid; I.S., internal standard.

on a Finnigan 4021 g.c.-m.s. instrument on line with the INCOS data system. To 300 μ l of neutralized extract, 100 μ l of hendecanedioic acid (2 mg/ml) as internal standard was added. The organic acids were converted into their trimethylsilyl-oxime derivatives, as described previously, after extraction (three times) into ethyl acetate [11]. A 5 μ l portion of the derivatized sample was injected on a 30 m SE-54 capillary column. The g.c. instrument was operated under the following conditions: injector temperature, 200 $^{\circ}$ C; transfer-line temperature, 250 $^{\circ}$ C; manifold temperature, 80 $^{\circ}$ C. The g.c. temperature program was as follows: initial temperature, 60 $^{\circ}$ C for 3 min and then an increase of 2 $^{\circ}$ /min until 270 $^{\circ}$ C. The carrier gas was helium at a flow rate of 2 ml/min. A typical gas chromatogram of organic acid trimethylsilyl-oxime derivatives from tissue extraction is depicted in Fig. 1. In each sample the lactic acid concentration was measured enzymically [12] and the final concentrations of the other organic acids were calculated by utilizing lactate as internal standard. Essentially no difference was found with either hendecanedioic acid or lactate as internal standard.

¹³C-n.m.r. techniques

The neutralized tissue extract from four flasks of approx. 800 mg of tubules (wet weight) per time point were pooled. This was freeze-dried and reconstituted in 2 ml of 33% ²H₂O. The tissue suspension (2 ml) was placed in a 10 mm n.m.r. tube with 10 μ l of 10% (v/v) dioxan in 33% ²H₂O as internal standard (66.5 p.p.m.). ¹³C-n.m.r. spectra were recorded at 25 $^{\circ}$ C at 90.55 MHz for ¹³C nuclei with a Bruker WH 360 spectrometer equipped with an ASPECT-2000 data system. For most analyses, 3000–5000 transients were accumulated by

using radio-frequency 60 $^{\circ}$ pulses (15 μ s) and a 20 kHz spectral window. Proton decoupling was achieved by composite pulse decoupling with Bruker's standard software package. The lock signal was obtained from approx. 33% ²H₂O contained in the sample. Chemical shifts were determined relative to dioxan (66.5 p.p.m. from tetramethylsilane, assigning a positive value for the downfield shift from tetramethylsilane) as well as by comparison of chemical shifts with those of the pure components in Krebs buffer (pH 7).

Determination of isotopic enrichment

Quantitative measurements of the ¹³C content of each resonance were made by normalizing to dioxan resonance and correcting for the effect of saturation and nuclear Overhauser enhancement. Furthermore, ¹³C enrichment (%) of specific carbon atoms of metabolites was estimated by comparison with the ¹³C-n.m.r. spectra of non-enriched metabolites, in which each carbon atom contains a natural ¹³C abundance of 1.1%. For this purpose, 10 mM solutions of glucose and each amino acid and organic acid were prepared in Krebs buffer, pH 7. ¹³C-n.m.r. spectra were obtained under conditions identical with those used for obtaining tissue extracts. In most instances, measurements of ¹³C isotopic enrichment were also done by using g.c.-m.s. Enrichment of ¹³C amino acids was determined in the respective *n*-butyl-*N*-trifluoroacetyl derivatives [4]. The *m/z* 141/140, 185/184, 255/254 and 183/182 ratios for alanine, aspartate, glutamate and GABA respectively were measured with selected ion monitoring. For estimation of ¹³C enrichment in the respective trimethylsilyl-oxime derivatives of organic acids the *m/z* 247/248, 376/375, 234/233, 376/375,

Table 1. Time course of metabolite concentration in renal-tubular fragments incubated with 2.5 mM-[3- ^{13}C]glutamate

Values are means $\pm$ S.E.M. of three to four experiments. The concentration of oxaloacetate (in nmol, not $\mu\text{mol/g}$) was calculated as described by Williamson *et al.* [26], using the equation:

$$\text{Oxaloacetate} = \frac{[\text{Malate}] [\text{NAD}^+] K}{[\text{NADH}]}$$

K being the equilibrium constant of the malate dehydrogenase system. The $[\text{NAD}^+]/[\text{NADH}]$ ratio was calculated from $[\text{lactate}]/[\text{pyruvate}]$ by using $K = 1.11 \times 10^{-4}$, the equilibrium constant of the lactate dehydrogenase system [26]. * $P < 0.05$, significantly different from control.

Metabolite	Time (min) ...	Concn. ($\mu\text{mol/g}$ wet wt. of tubules)					
		Control			Acidosis		
		15	30	60	15	30	60
Glucose		7.6 $\pm$ 2.1	9.2 $\pm$ 2.4	13.1 $\pm$ 1.8	21.7 $\pm$ 6.9*	30.4 $\pm$ 3.4*	39.4 $\pm$ 5.6*
NH_3		14.2 $\pm$ 3.8	18.3 $\pm$ 2.3	26.2 $\pm$ 3.1	23.1 $\pm$ 4.3*	34.3 $\pm$ 7.2*	44.3 $\pm$ 4.7*
Lactate		1.3 $\pm$ 0.3	1.8 $\pm$ 0.4	2.7 $\pm$ 0.6	1.7 $\pm$ 0.2	1.7 $\pm$ 0.5	2.6 $\pm$ 0.7
Pyruvate		0.21 $\pm$ 0.09	0.22 $\pm$ 0.4	0.36 $\pm$ 0.08	0.21 $\pm$ 0.03	0.22 $\pm$ 0.07	0.24 $\pm$ 0.06
Citrate		0.83 $\pm$ 0.18	0.61 $\pm$ 0.21	0.73 $\pm$ 0.09	0.52 $\pm$ 0.18	0.44 $\pm$ 0.11	0.58 $\pm$ 0.21
Malate		0.67 $\pm$ 0.11	0.82 $\pm$ 0.24	0.53 $\pm$ 0.09	0.52 $\pm$ 0.17	0.21 $\pm$ 0.06*	0.24 $\pm$ 0.07*
Fumarate		1.21 $\pm$ 0.41	1.42 $\pm$ 0.28	1.11 $\pm$ 0.36	0.54 $\pm$ 0.32	0.76 $\pm$ 0.18*	0.68 $\pm$ 0.36
Succinate		0.53 $\pm$ 0.21	0.65 $\pm$ 0.22	0.43 $\pm$ 0.15	0.47 $\pm$ 0.11	0.27 $\pm$ 0.09*	0.36 $\pm$ 0.12
<i>cis</i> -Aconitate		0.32 $\pm$ 0.11	0.43 $\pm$ 0.09	0.38 $\pm$ 0.14	0.16 $\pm$ 0.07	0.28 $\pm$ 0.11	0.45 $\pm$ 0.13
α -Oxoglutarate		0.38 $\pm$ 0.09	0.46 $\pm$ 0.15	0.36 $\pm$ 0.14	0.48 $\pm$ 0.13	0.32 $\pm$ 0.09	0.22 $\pm$ 0.03
Oxaloacetate (nmol)		25.8 $\pm$ 7.3	25.1 $\pm$ 8.4	18.3 $\pm$ 5.2	7.9 $\pm$ 3.2*	7.8 $\pm$ 2.1*	6.7 $\pm$ 1.3*
ATP		2.6 $\pm$ 0.6	3.7 $\pm$ 0.8	4.3 $\pm$ 1.1	2.8 $\pm$ 0.3	3.6 $\pm$ 1.1	3.2 $\pm$ 0.7
ADP		1.1 $\pm$ 0.1	0.9 $\pm$ 0.3	1.43 $\pm$ 0.6	1.1 $\pm$ 0.4	1.2 $\pm$ 0.7	0.9 $\pm$ 0.4

246/245 and 220/219 ratios for succinate, *cis*-aconitate, malate, citrate, fumarate and lactate respectively were utilized. The potential presence of more than one carbon atom labelled with ^{13}C in the same molecule was ascertained by determination of the $M+2/M$ or $M+3/M$ ratios. This information was compared with the splitting patterns in the ^{13}C -n.m.r. spectra as a result of spin-spin coupling between adjacent ^{13}C nuclei in the same molecule of the metabolite in question.

Analysis of $^{15}\text{NH}_3$ derived from ^{15}N]glutamate was as previously described [4].

Calculation

In each of the experiments described above, we measured ^{13}C as the product of metabolite concentration and ^{13}C either as percentage enrichment (by n.m.r. analysis) or atom % excess (by g.c.-m.s. analysis). The appearance of $^{15}\text{NH}_3$ - or ^{13}C -labelled metabolites and the rate of utilization of labelled substrate were determined as described previously [4]. Student's *t* test was used for the statistical analysis.

RESULTS

Metabolite content in renal tubules

Table 1 presents the metabolite content in renal tubules obtained from normal and chronically acidotic rats. At all time points the concentrations of ammonia and glucose were significantly higher ($P < 0.05$) in acidosis compared with control (Table 1). However, malate, fumarate, succinate and oxaloacetate are significantly higher in control- as against acidotic-rat tubules ($P < 0.05$). No significant difference was observed in the concentration of ATP and ADP or the ATP/ADP ratio

between control and acidosis (Table 1). Furthermore, the ATP/ADP ratio, which reflects the energy state of the incubation system, was unchanged during the course of incubation in both control and acidosis (Table 1). The values observed for the tricarboxylic-acid-cycle-intermediate levels are in accord with those previously observed in the kidney from control and chronically acidotic rats [13–15] and presumably reflect enhanced gluconeogenesis in acidosis [1–3].

Distribution of [3- ^{13}C]glutamate

Figs. 2 and 3 represent ^{13}C -n.m.r. spectra of renal tubules incubated with 2.5 mM-[3- ^{13}C]glutamate. In control tubules, the major resonance in the spectrum at the 15 min time point is that of malate C-3 and malate C-2 at 43.38 and 71.18 p.p.m. respectively. In contrast, in acidosis, malate C-2 and C-3 were noted at 15 min and hardly at all thereafter. Resonances corresponding to glutamate C-2 and C-4, aspartate C-2, acetoacetate C-2, GABA C-3 and succinate C-2,3 are also seen (Figs. 2 and 3). At the 60 min time point the major ^{13}C resonance in control tissue is aspartate C-2, which was less prominent in acidosis. G.c.-m.s. analysis showed the ^{13}C]aspartate enrichment to be 29.6 ± 3.4 and 10.9 ± 2.1 atom% excess ($\pm$ S.E.M., $n = 4$) for control and acidosis respectively, at the 60 min time point. Labelling of aspartate C-2 must reflect [3- ^{13}C] α -oxoglutarate formation from the labelled glutamate via glutamate dehydrogenase and subsequent mitochondrial generation of [2- ^{13}C]oxaloacetate, from which [2- ^{13}C]aspartate is derived via transamination. Another major single peak in Figs. 2 and 3 is that of GABA C-3, probably derived from glutamate C-3 by the activity of cytoplasmic glutamate decarboxylase, which is very active in the kidney [4,16]. An interesting feature in the ^{13}C -n.m.r. spectra of Figs. 2 and 3 is the lack of

Fig. 2. ^{13}C -n.m.r. spectrum of neutralized HClO_4 extract prepared from approx. 800 mg wet wt. of pooled tubules obtained from control rats

Tubules were incubated for the times indicated with 2.5 mM-[3- ^{13}C]glutamate in Krebs buffer. The spectrum represents the accumulation of 3000 scans obtained at 90.55 MHz using composite pulse proton decoupling, 60° pulses and a 20 kHz spectral window. Resonances are defined as follows: 1, alanine C-3; 2, lactate C-3; 3, acetate C-2; 4, unknown; 5, GABA C-3; 6A, glutamine C-3; 6, glutamate C-3; 7, glutamate C-4; 8, succinate C-2,3; 9, aspartate C-3; 10, *cis*-aconitate C-4; 11, malate C-3; 13, alanine C-2; 14, acetoacetate C-2; 15, aspartate C-2; 16, glutamate C-2; 17, $\alpha\beta$ -glucose C-6; 19, dioxan; 20, lactate C-2; 21, $\alpha\beta$ -glucose C-4; 2M, malate C-2; 22, α -glucose C-3,5; 23, β -glucose C-2; 24, β -glucose C-3,5; 25, α -glucose C-1; 26, β -glucose C-1.

significant line-splittings of individual ^{13}C carbon resonances, which would reflect the interactions between adjacent ^{13}C -labelled carbon atoms in the same molecules. Despite the high level of ^{13}C incorporation, the resonances in the ^{13}C spectrum are predominantly singlet, suggesting that the ^{13}C label is introduced into specific sites with a minimum of labelling of adjacent carbon in the same molecule. Furthermore, the ^{13}C labelling most probably occurs simultaneously. The coupling patterns of C-2 and C-4 of glutamate (Figs. 2 and 3) are consequent to the neighbouring ^{13}C label on C-3 and differ from one another in control and acidotic

tissue. In the control preparation the sidebands for glutamate C-4 and C-2 resonance at 15 min have almost the same intensity as the central peak (Fig. 2). In acidotic tissue the glutamate C-2 coupling showed a similar pattern to the control, but the C-4 carbon demonstrates a centre peak that is twice as high as the sideband peaks (Fig. 3). These observations suggest more scrambling of glutamate in control as against acidotic kidney. However, the magnitude of the scrambling diminishes with increasing time of incubation with [3- ^{13}C]glutamate. At the 60 min time point, glutamate C-2 is primarily a singlet in both control and acidotic tubules (Figs. 2 and

Fig. 3. ^{13}C -n.m.r. spectrum of neutralized HClO_4 extract prepared from approx. 800 mg wet wt. of pooled tubules obtained from chronically acidotic rats incubated for times indicated with 2.5 mM- $[3-^{13}\text{C}]$ glutamate

Resonance identification is as described in the legend of Fig. 2. Peak 12, which is not seen in Fig. 2, refers to the resonance of $[2,4-^{13}\text{C}]$ citrate. Peak 18 is undefined.

3), but the coupling at C-4 is still significant at the 60 min time point. The observation that glutamate C-2 and C-4 become labelled in incubations with $[3-^{13}\text{C}]$ glutamate suggests that the glutamate pool is in free exchange with the tricarboxylic acid cycle via the glutamate dehydrogenase and glutamate aminotransferase reactions in both control and acidotic tubules. Another interesting feature in the spectrum of Fig. 2 is that, at the 60 min time point, glutamine C-3 is detectable as a shoulder on the glutamate C-3 peak. This observation supports our previous studies with ^{15}N [4] and the work of Lanctin *et al.* [17], which demonstrated appreciable activity of glutamine synthetase in normal rat kidney.

An important observation obtained from the ^{13}C -n.m.r. spectra (Figs. 2 and 3) pertains to the ^{13}C

labelling of glucose carbon atoms. The most highly enriched glucose carbon atoms are C-5 and C-2, an observation in accord with previous studies utilizing $[2-^{14}\text{C}]$ pyruvate or $[2-^{14}\text{C}]$ lactate to investigate renal gluconeogenesis [18]. Furthermore, the pattern of glucose carbon resonance indicates that both C-2 and C-3 of cytosolic oxaloacetate must be highly labelled. Presumably this occurs after formation of $[2,3-^{13}\text{C}]$ malate in the tricarboxylic acid cycle. Consequently, glucose C-5, C-2, C-6 and C-1 will be intensely labelled compared with C-3 and C-4, as demonstrated in Figs. 2 and 3. The estimated ^{13}C enrichment in C-5 of glucose is 25.2 ± 2.8 and $18.7 \pm 1.1\%$ for acidosis and control respectively. This observation implies that a large part of the glucose carbon was derived from sources other than $[3-$

Fig. 4. Time course of ^{13}C in glutamate C-3, glutamate C-(2+4), aspartate C-(2+3) and GABA C-3 in tubules obtained from control (●) or chronically acidotic rats (○) incubated with 2.5 mM-[3- ^{13}C]glutamate

The concentration represents the product of ^{13}C (atom% excess) obtained from g.c.-m.s. analysis and the concentration ($\mu\text{mol/g}$ wet wt. of tubules) of each metabolite. Each point is the mean $\pm$ S.E.M. for three or four experiments.

^{13}C]glutamate. Similarly, Krebs *et al.* [19] observed that the specific radioactivity of the glucose carbon was only 29% in rat kidney cortex incubated with L-[U- ^{14}C]lactate [19]. Furthermore, Lemieux *et al.* [20] performed incubation with Krebs buffer alone and found that approx. 60 and 40% of glucose production was derived from endogenous sources in renal slices obtained from control and chronically acidotic rats respectively.

Of interest also is the formation of C-2- and C-3-labelled alanine in incubations of tubules with [3- ^{13}C]glutamate. The only possible pathway for ^{13}C labelling of alanine is through pyruvate kinase. The fact that no significant difference was found in the intensity of [^{13}C]alanine peaks between control and acidotic states indicates that the activity of the glycolytic pathway is similar under both normal and acidotic conditions. Similarly, no significant difference was found between control and acidosis in the appearance of [2- ^{13}C]lactate.

The time course of [3- ^{13}C]glutamate disappearance and ^{13}C appearance in various metabolites in renal tubules obtained from control and chronically acidotic rats are shown in Figs. 4, 5 and 6. The appearance of ^{13}C in aspartate C-2 and C-3, fumarate C-2 and C-3 and lactate C-3 is linear during the course of incubation in both control and acidotic tissue (Figs. 4 and 5). In acidosis the concentration of [^{13}C]malate (C-2+C-3) is approx. 80 nmol/g wet weight of tissue at the 15 min time point and declines to approximately half this

amount at 30 min (Fig. 5). In control tissue the ^{13}C malate level is approx. 90 nmol/g wet weight of tissue at 15 min, 143 nmol/g at 30 min and 125 nmol/g wet weight at 60 min. The rate of [^{13}C]aspartate appearance (Table 2) is in excellent agreement with the rate of [^{15}N]aspartate appearance from [^{15}N]glutamate in rat renal tubules [4] and is approx. 5-fold higher in control than in acidosis.

Fig. 6 illustrates the appearance of total [5- ^{13}C]glucose and $^{15}\text{NH}_3$ in renal tubules. There is a positive association between the increase in ^{13}C contained in C-5 of glucose and in $^{15}\text{NH}_3$. The rates of $^{15}\text{NH}_3$ appearance calculated from the data of Fig. 6 are 0.037 ± 0.012 as against $0.221 \pm 0.05 \mu\text{mol/min}$ per g wet weight in control and acidotic states respectively. These values are similar to those previously reported [4]. The rate of [^{13}C]glucose appearance is approx. 3-fold higher in acidosis than in the control (Table 2).

The positive association between [^{13}C]glucose formation and $^{15}\text{NH}_3$ (Fig. 6) is in good accord with the study of Alleyne [21], who found a positive correlation between NH_3 and glucose formation in renal-cortical slices from acidotic rats. The calculated ratios between the rates of $^{15}\text{NH}_3$ and [5- ^{13}C]glucose appearance are 1.6 and 0.8 in acidosis and control respectively. These calculations showed that, in the control, the ratio is far below the expected ratio of 2, assuming that 1 mol of glucose will be formed from 2 mol of glutamate [3,22]. In control

Fig. 5. Time course of ^{13}C in tricarboxylic-acid-cycle intermediates [succinate C-(2+3), *cis*-aconitate C-4, lactate C-3, malate C-(2+3) and fumarate C-(2+3)] in tubules obtained from control (●) or chronically acidotic rats (○) incubated with 2.5 mM-[3- ^{13}C]glutamate

Concentrations are defined in the legend of Fig. 4. Each point is the mean $\pm$ S.E.M. of three or four experiments.

tissue the higher ^{13}C glucose/ $^{15}\text{NH}_3$ ratio presumably reflects the observation (Table 2) that part of glutamate N is recycled from $^{15}\text{NH}_3$ to ^{15}N glutamate via reductive amination of α -oxoglutarate and to glutamine by glutamine synthetase, but that glutamate carbon is not re-cycled as rapidly from ^{13}C glucose to ^{13}C glutamate.

DISCUSSION

The present study was undertaken to determine more precisely the renal metabolic fate of glutamate carbon in various acid-base states. Many details regarding glutamate carbon metabolism are unknown [1,2,22,23], and we have found both ^{13}C n.m.r. and g.c.-m.s. to be potent analytical techniques providing a comprehensive view of the metabolic adaptations chronic acidosis evokes. By utilizing ^{13}C n.m.r. we could observe directly the flow of carbon from ^{13}C -labelled glutamate to glucose, tricarboxylic-acid-cycle intermediates and amino acids. The studies with ^{15}N glutamate further defined the relationship between glutamate N and C disposal in renal tubules obtained from control and acidotic rats.

In Scheme 1 are outlined the major pathways of renal [3- ^{13}C]glutamate metabolism. These pathways are suggested by the ^{13}C -labelling patterns observed with n.m.r.

and g.c.-m.s. (Figs. 2, 3, 4 and 5). In control tissue the transamination, reductive-amination and glutamine synthase pathways (reactions 1, 2 and 3) predominated relative to the pattern noted in acidotic tubules, in which the major route of glutamate metabolism was oxidative deamination and glutamine deamidation (reaction 4). The α -oxoglutarate produced in acidotic tissue presumably was converted in large measure into glucose. Calculation of ^{13}C metabolites appearance as against [3- ^{13}C]glutamate disappearance rates (Table 2 and Figs. 4 and 5) show that, in control tubules, approx. 15, 13, 21 and 10% of [3- ^{13}C]glutamate utilization was recovered in [5- ^{13}C]glucose, [2,3- ^{13}C]aspartate, [2,4- ^{13}C]glutamate and glutamine and [3- ^{13}C]GABA (sum of 6+1+2+4+5 respectively in Scheme 1). Similarly, in acidosis, 30, 2, 4 and 5% of glutamate carbon was recovered in glucose, aspartate, glutamate and GABA. In both control and acidotic kidney a small fraction of ^{13}C (approx. 10%) was found in lactate, malate, acetate, succinate, fumarate and alanine, as depicted in Figs. 2 and 3. The unrecovered ^{13}C , approx. 30% in control and 50% in acidosis, was probably oxidized to CO_2 , as noted previously [24].

Both in control and in acidotic tissue the fraction of glutamate carbon converted into glucose is somewhat lower than the values reported by Watford *et al.* [24]. The

Fig. 6. Time course of [^{13}C]glucose as C-5 moieties and $^{15}\text{NH}_4^+$ concentration in tubules from control (●) or chronically acidotic rats (○) incubated with either 2.5 mM-[3- ^{13}C]glutamate or [^{15}N]glutamate

Each point is the mean $\pm$ S.E.M. for three or four experiments.

Table 2. (a) Rate of [3- ^{13}C]glutamate disappearance and (b) [^{13}C]metabolite production in renal-cortical tubules isolated from chronically acidotic rats and rats in normal acid-base balance

The initial rate of [3- ^{13}C]glutamate disappearance was determined by fitting the data points in Fig. 4 to $I_t = I_0 e^{-kt}$, where I_t and I_0 are the concentrations of ^{13}C -labelled substrate at time t and time 0 respectively. The rates of ^{13}C metabolite production from [3- ^{13}C]glutamate were calculated by linear-regression analysis of the curves in Figs. 4 and 5. Curve-fitting was done on a Hewlett-Packard HP-85 computer. Results are means $\pm$ S.E.M. for three to four experiments. Significance: * $P > 0.05$ (not significant compared with control); † $P < 0.02$ (compared with control); ‡ $P < 0.05$ (compared with control).

Labelled metabolite	(a) Disappearance rate ($\mu\text{mol}/\text{min}$ per g wet wt. of tissue)	
	Control	Acidosis
[3- ^{13}C]Glutamate	0.34 ± 0.09	0.56 ± 0.14^a
Labelled metabolite	(b) Metabolite production rates (nmol/min per g wet wt. of tissue)	
	Control	Acidosis
[5- ^{13}C]Glucose	48.4 ± 7.3	$145.7 \pm 12.8^\dagger$
[2,3- ^{13}C]Aspartate	44.7 ± 9.5	$9.1 \pm 2.4^\ddagger$
[2,4- ^{13}C]Glutamate	61.3 ± 8.5	$23.4 \pm 4.8^\ddagger$
[3- ^{13}C]GABA	36.4 ± 5.2	$28.4 \pm 6.4^*$
[2- ^{13}C]Lactate	1.9 ± 0.4	$2.3 \pm 0.4^*$
cis-[3- ^{13}C]Aconitate	1.1 ± 0.2	$0.7 \pm 0.1^*$
[2,3- ^{13}C]Fumarate	2.4 ± 0.6	$1.2 \pm 0.2^*$

difference between the results of the current study and those of Watford *et al.* [24] could be explained by the fact that the other investigators utilized 5 mM-L-[U- ^{14}C]glutamine to study renal gluconeogenesis. We utilized 2.5 mM-[3- ^{13}C]glutamate as a precursor, and it is possible that gluconeogenesis was stimulated by the higher glutamine concentration. Furthermore, the permeability barriers, transport phenomena and metabolic compartmentation which may exist between glutamine and glutamate [1] could explain the differences between the results obtained in our work and previous observations.

As shown in Scheme 1, metabolism of glutamate carbon through the tricarboxylic acid cycle toward glucose formation is higher in acidosis than in the control. Presumably the initial renal response to lowered pH is augmented ammoniagenesis due to stimulated mitochondrial glutaminase I and glutamate dehydrogenase activity [1,2,4]. There then probably occurs an enhancement of the mitochondrial and extramitochondrial pathways for the conversion of α -oxoglutarate into glucose. Indeed, augmented gluconeogenesis is intrinsic to the renal adaptation to acidosis, as suggested by Goodman *et al.* [25], who proposed that increased glucose formation in acidosis provides an outlet for the disposition of the increased levels of α -oxoglutarate derived from glutamate and glutamine. In control tubules approx. 30% of the α -oxoglutarate formed by the activity of glutamate dehydrogenase was re-cycled back to glutamate by either reductive amination or transamination. The formation of both glutamate and glutamine would involve consumption of tricarboxylic-acid-cycle intermediates. Thus the relatively low rate of gluconeogenesis in control tubules probably is due to

Scheme 1. Representation of the relationship between glutamate metabolism, NH_3 formation and the tricarboxylic acid cycle in renal tubules obtained from control or chronically acidotic rats

The bold lines indicate more prominent pathways of glutamate metabolism. Ringed numbers refer to the following pathways: ①, glutamate:oxaloacetate transaminase; ②, glutamate dehydrogenase; ③, glutamine synthetase; ④, phosphate-dependent glutaminase; ⑤, glutamate decarboxylase; ⑥, malate dehydrogenase: phosphoenolpyruvate carboxykinase.

higher utilization of tricarboxylic-acid-cycle intermediates toward glutamate and glutamine formation (see Scheme 1). A similar suggestion has been advanced by Ross and co-workers [17]. The current data are consistent with roles for glutamine synthetase and reductive amination of α -oxoglutarate in the characteristic augmentation of renal ammoniogenesis in response to chronic acidosis. Lanctin *et al.* [17] also concluded that recycling of N through glutamine could be important in the control of NH_3 excretion by the kidney. Conversely, in chronic metabolic acidosis, diminished removal of NH_3 and α -oxoglutarate through glutamate and glutamine formation would result in a high rate of gluconeogenesis and ammoniogenesis from glutamate, as depicted in Figs. 2 and 3.

In summary, the present study provides evidence that decreased pH may increase ammoniogenesis and gluconeogenesis by decreasing glutamate recycling and glutamine synthesis. Thus regulation of renal ammoniogenesis is a complex process involving many biochemical reactions and transport mechanisms. ^{13}C -n.m.r. methodology appears to be uniquely suited for the study of carbon metabolism in kidney tubules. We used the technique to trace glutamate carbon flux. The method clearly is adaptable to the investigation of many different metabolic substrates.

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