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Changes in renal metabolite profile and ammoniagenesis during acute and chronic metabolic acidosis in dog and rat

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Changes in renal metabolite profile and ammoniagenesis during acute and chronic metabolic acidosis in dog and rat. Acute metabolic acidosis was induced by an i.v. administration of hydrochloric acid to dogs and rats to decrease the plasma bicarbonate concentration from 22 to 12 mM in dogs and from 26 to 10 mM in rats. Chronic metabolic acidosis was also induced in dogs by ammonium chloride feeding for 5 days. Rats also were given ammonium chloride for 24 hours. The renal metabolite profile was determined on the freeze-clamped renal tissue before and after 100 min (dogs) or 30 to 240 min (rats) of acute acidosis. Measurements on chronically acidotic dogs and rats with 24-hour acidosis were obtained also for comparison with acute acidosis. In both species, kidney glutamine, glutamate, and alpha-ketoglutarate concentrations decreased drastically following induction of acute or chronic acidosis. In the dog, or in the rat during the first 2 hours of acidosis, malate concentration was unchanged. Malate concentration fell significantly in the rat kidney only after 2 hours of acidosis without change in phosphoenolpyruvate (PEP) concentration. In chronically acidotic dogs, malate and oxaloacetate rose fivefold with no change in PEP concentration. Phosphoenolpyruvate carboxykinase (PEPCK) activity was not stimulated by chronic metabolic acidosis in the dog in contrast to the rat. Acute acidosis by hydrochloric acid increased net renal glutamine extraction in the rat but not in the dog. These data suggest that an increased metabolic flux occurs between alpha-ketoglutarate and malate in both rat and dog kidney during acute metabolic acidosis. In the rat, however, after 2 hours, PEPCK activation modifies the kidney metabolite profile. Intrarenal glutamine transport seems to be a rate-limiting factor for adaptation to acute acidosis in the dog but not in the rat kidney.

Profil métabolique rénal et ammoniogénèse au cours de l'acidose métabolique aiguë et chronique chez le chien et le rat. Une acidose métabolique aigue a été produite par l'administration intraveineuse d'acide chlorhydrique à des chiens et des rats de telle sorte que la concentration plasmatique de bicarbonates soit abaissée de 22 à 12 mM (chiens) et de 26 à 10 mM (rats). Une acidose métabolique chronique a aussi été produite par l'administration orale de chlorure d'ammonium pendant 5 jours (chiens) ou 24 heures (rats). Le profil métabolique rénal a été déterminé sur du tissu cortical prélevé en congélation instantanée avant et après 100 min (chiens) ou 30 à 240 min (rats) d'acidose aiguë. Les données ont été comparées aux valeurs retrouvées dans les reins de chiens et de rats en acidose chronique. Chez les deux espèces, la concentration rénale de glutamine, de glutamate, et d'alphacétoglutarate a considérablement diminué après l'induction de l'acidose aiguë ou chronique. Cependant, chez le chien, ou chez le rat pendant les premières 2 heures d'acidose, la concentration de malate ne s'est pas modifiée. La concentration de malate n'a diminué significativement chez le rat qu'après 2 heures d'acidose. Chez le chien en acidose chronique, la concentration de malate et d'oxaloacétate a quintuplé sans modification de la concentration phosphoénolpyruvate (PEP). L'activité de la PEP-carboxykinase (PEPCK) n'a pas été stimulée par l'acidose métabolique chronique chez le chien. L'acidose aiguë a augmenté l'extraction nette de glutamine par le rein chez le rat mais non chez le chien. Ces résultats suggèrent que la voie métabolique ammoniogénique est stimulée par l'acidose à une étape située entre l'alphacétoglutarate et le malate chez le rat comme chez le chien. Chez le rat, cependant, l'activation de la PEPCK modifie le profil métabolique rénal à partir de la deuxième heure d'acidose. Le transport intrarénal de glutamine semble être un facteur limitant de l'adaptation à l'acidose aiguë chez le chien mais non chez le rat.

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Measuring the renal metabolite profile is useful in studying the adaptation of renal ammoniagenic flux to chronic metabolic acidosis in the rat [1–4]. A marked decrement in the concentration of glutamine, glutamate, alphaketoglutarate, and malate, together with accumulation of phosphoenolpyruvate, 2-phosphoglycerate, and 3-phosphoglycerate, in kidney cortex suggests adaptation of phosphoenolpyruvate carboxykinase (PEPCK) during acidosis in this species [1–3, 5]. Such tissue changes also are demonstrable within 2 to 6 hours after inducting acute metabolic acidosis in the rat [2, 6]. Curiously, no comparable studies are available in the dog. Only incomplete data showing percentile changes in citrate, alphaketoglutarate, malate, and oxaloacetate concentrations in kidney and liver tissues during acute metabolic acidosis or alkalosis are available in the dog [7], and no data are published on the metabolite profile in the dog kidney under

normal acid-base status. Furthermore, no data on the effect of chronic metabolic acidosis in the dog are reported. Significant differences during acidosis are expected between rat (showing PEPCK adaptation [5]) and dog (lacking such adaptation) (see below). We therefore have examined the effect of acute and chronic metabolic acidosis on the kidney metabolite profile in the dog. We also have studied the early concentration changes of renal metabolites in the rat before PEPCK adaptation. Simultaneously, we have studied the renal ammoniagenesis response to an acute metabolic acidosis in the dog and rat.

Our study in the dog demonstrates that an immediate fall in renal cortical glutamine, glutamate, alphaketoglutarate, and citrate with no change in malate concentrations occurs following induction of an acute acidosis by hydrochloric acid. Chronic acidosis induced by ammonium chloride significantly elevates tissue malate and oxaloacetate concentrations in the dog and leaves unaffected renal cortical PEPCK activity, in contrast to the observations in the chronically acidotic rat [5]. A similar pattern of metabolite changes is observed, however, in both species if the metabolite profile is studied in the rat during the first 2 hours of acidosis. These findings indicate a direct and immediate effect of acidosis on the ammoniagenic pathway before the PEPCK step in both species. In the rat, however, the metabolite profile is rapidly modified, probably by the subsequent PEPCK adaptation. A significant difference between rat and dog for renal glutamine extraction in vivo also is demonstrated following acute acidosis by hydrochloric acid.

Methods

Metabolite profile in the dog. From 13 normal dogs (each weighing between 18 and 32 kg) and in 20 dogs (each weighing between 15 and 30 kg) with chronic metabolic acidosis induced by daily administration of 10 g of ammonium chloride mixed with commercial food as previously described [8], renal and hepatic tissue samples were obtained by the following procedure. After being anesthetized with sodium pentobarbital (30 mg/kg), the animals were intubated. The endotracheal tube, fitted with an inflatable balloon, was connected to a volume and rate-adjustable respirator (Harvard Apparatus Co., Millis, Massachusetts) to maintain arterial P_{CO_2} around 40 mm Hg. The femoral artery was cannulated to draw arterial blood samples for determining blood metabolites and acid-base parameters. Then, the abdominal cavity was opened, and a catheter was placed under direct visual inspection in the left renal vein after the left ovarian or spermatic vein was ligated. A fragment of cortical tissue was obtained rapidly from the right kidney and immediately freeze-clamped according to the method of Wollenberger, Ristau, and Schoffa [9]. A right nephrectomy was performed then. A fragment of liver also was obtained and freeze-clamped. After a 30-min equilibration period, the renal production of ammonia and the extraction of glutamine were measured from the left kidney by three determinations of arteriovenous differences and four collections of 10-min urine samples. We assumed the rates of glutamine extraction and ammonia production to be similar in both kidneys before the freeze-clamping procedure. We also ascertained that the metabolite profile of the remnant kidney was not modified 30 to 90 min following uninephrectomy.

Clearance experiments in the dog. In 7 of the uninephrectomized normal dogs already described, total renal ammonia production and glutamine, alanine, aspartate, lactate, citrate, and oxygen production or extraction by the left kidney were studied for 40 min. Each animal received a constant infusion of 5% mannitol, containing appropriate amounts of para-aminohippurate (PAH) and creatinine, adjusted to a pH of 7.4 and administered at the rate of 5 ml/min. After three control arteriovenous differences as well as four 10-min urinary collections were obtained, an infusion of 0.3 N hydrochloric acid in 0.9% sodium chloride was given for 60 min at a rate appropriate to administer a total hydrogen ion load of 5 mEq/kg. The renal metabolism then was studied for 40 min by three experimental arteriovenous differences and four urine collections before specimens of the remaining left kidney and of the liver were freeze-clamped. All urine collections were obtained under mineral oil.

Metabolite profile in the rat. The renal metabolite profile was measured in 57 normal Wistar rats, each weighing 350 to 550 g, before and after induction of an acute metabolic acidosis by hydrochloric acid. After pentobarbital anesthesia (4 mg/100 g body wt) was given, the abdominal cavity was opened and the kidneys were freeze-clamped rapidly, according to a procedure already reported [10]. An arterial blood sample also was drawn for assessment of the systemic acid-base status. Three control groups of rats were studied. In a first group of 6 rats, the kidneys were freeze-clamped immediately following anesthesia. In a second group of 8 rats, the kidneys were freeze-clamped after 30 min of 0.9% sodium chloride infusion at 0.5 ml/min. A

third control group of 6 rats received the same 30-min sodium chloride infusion, but the kidneys were freeze-clamped after 240 min. In 31 rats, the kidneys were freeze-clamped following infusion of 0.2 N hydrochloric acid in 0.9% sodium chloride at 0.5 ml/min for 30 min. In a first experimental group of 7 rats, the kidneys were obtained at the end of the hydrochloric acid infusion (30 min); in the others, they were obtained 30 (8 rats), 90 (7 rats), and 210 (9 rats) min later. In a final group of 6 rats made acidotic by tube-feeding ammonium chloride (4 mmoles in two doses), the kidneys were obtained 24 hours after the metabolic acidosis was induced.

Clearance experiments in the rat. Total ammonia production and glutamine extraction were measured in 16 normal Wistar rats (each weighing 500 to 700 g) with a catheter placed both in the carotid artery and in the left renal vein, as already described [10], allowing repetitive measurements of arteriovenous differences. Ten animals were studied 20 min before and 20 min after the i.v. administration of 0.9% sodium chloride, at 0.5 ml/min for 30 min, and 6 animals received a 30-min infusion of 0.2 N hydrochloric acid in 0.9% sodium chloride. Blood samples (0.5 ml) were obtained at least twice after the sodium chloride or the hydrochloric acid infusion. The losses of blood were immediately replaced by equivalent volumes of arterial blood obtained from a normal rat (control situation) or from a rat receiving a similar hydrochloric acid load (experimental situation). Each animal received a constant isotonic infusion of half 5% mannitol and half 0.9% sodium chloride, containing appropriate amounts of ^{14}C-inulin, at 0.06 ml/min through the left jugular vein. The urine was collected under mineral oil for 10 min.

Measurements. Blood pH, P_{CO_2}, and hematocrit, and plasma concentrations of bicarbonate, sodium, potassium, chloride, and creatinine, as well as arterial and renal venous concentrations of PAH, glutamine, glutamate, aspartate, alanine, citrate, lactate, pyruvate, and ^{14}C-inulin, were measured by procedures already reported [8, 10]. Arterial and renal venous total oxygen content was determined according to Mayers and Forster [11]. Urinary pH, bicarbonate, titratable acid, ammonia, sodium, potassium, chloride, and creatinine were estimated as already reported [12]. Glomerular filtration rate and renal blood flow were estimated, respectively, by clearance of exogenous creatinine and PAH (corrected for renal extraction) in the dog and by ^{14}C-inulin clearance and renal extraction of inulin in the rat. Tissue metabolites were measured by methods previously reported [10]. The tissue inorganic phosphate concentration was measured by the procedure of Martin and Doty [13]. All assays involved in the enzymatic determination of metabolites in the blood of dogs and in the tissues of dogs and rats were spectrophotometric. Fluorometric assays were used for blood metabolite determination in the rat. Renal extraction or production of metabolites was calculated by using Wolf's method [14]. Tissue oxaloacetate concentration and free NAD^+/NADH ratios were calculated according to Williamson and Krebs [15]. The methods for calculating the mass-action ratios for aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) systems have been reported [10].

Phosphoenolpyruvate carboxykinase, glutamate dehydrogenase, and lactate dehydrogenase activities in the dog renal cortex were measured by the following procedure. A sample of kidney cortex was homogenized in ten volumes of ice-cold 0.25 M sucrose containing Tris (5 mM) at a pH of 7.40 and dithiothreitol (1 mM) by four passes of a motor-driven teflon pestle. The homogenates were fractionated according to the technique of Johnson and Lardy [16], but the "nuclear" fraction was retained for enzymatic measurements. Mitochondrial activity was "solubilized" by freezing and thawing three times [17]. Samples were stored in ice and assayed the same day. All assays of enzymatic activities were carried out at 37° C with at least two different concentrations of enzyme. PEPCK activity was measured in the back direction according to the method of Chang and Lane [18] modified by Ballard and Hanson [19] and in the forward direction according to the method of Seubert and Huth [20] modified by Pogson and Smith [21]. Measurement of lactate dehydrogenase (technique of Wroblewski and La Due [22]) and glutamate dehydrogenase (technique of Schmidt [23]) activities were carried out to estimate the crosscontamination of the cytosolic and mitochondrial fractions, respectively. The activities of PEPCK reported are corrected for this crosscontamination.

Statistical analysis. Dunnet's test [24] was used for repeated comparisons with a single control for the data of the tissue metabolite profile in rats and dogs. In the rat, the tissue metabolite profile being similar in rats before or 30 to 240 min following a 30-min infusion of sodium chloride, all comparisons were made with the values obtained after 30 min's sodium chloride infusion as control. For clearance

studies in the dog and rat, variance analyses for repeated measurements [24] were used. Results are presented as the means ± SEM.

Results

Metabolite profile in the dog. Table 1 presents the kidney metabolite profile in dogs in normal acid-base status and acute (hydrochloric acid) or chronic (ammonium chloride) metabolic acidosis. Data of blood acid-base parameters and plasma glutamine, as well as renal glutamine utilization, ammonia production, and GFR, are also provided. The left column presents values obtained in 13 normal dogs. In this situation, the plasma glutamine concentration is 0.66 mM. A constant renal utilization of glutamine of approximately 14 μmoles/min is observed, and the ammonia production is 23 μEq/min. Therefore, some of the nitrogen of the extracted glutamine does not appear as ammonia, and the ratio of ammonia production over glutamine utilization is around 1.7. The tissue concentrations of metabolites described here are close to the values observed in the rat [4] except for alpha-ketoglutarate and malate, which are lower than the values reported by Hems and Brosnan [4] or those reported in Table 5. The free NAD^+/NADH ratios calculated for the cytoplasm from the lactate dehydrogenase system (LDH) (assuming equilibrium) and for the mito-

Table 1. Tissue metabolite profile of kidneys of dogs in normal acid-base status and acute (hydrochloric acid) or chronic (ammonium chloride) metabolic acidosis[a]

	Normal ($N = 13$)	Acute acidosis (HCl; 100 min) ($N = 7$)	Chronic acidosis (NH_4Cl; 5 to 6 days) ($N = 20$)
Blood pH	7.35 ± 0.03	7.10 ± 0.02[b]	7.16 ± 0.02[b]
Plasma bicarbonate, *mM*	21.3 ± 1.1	11.3 ± 0.4[b]	13.6 ± 0.7[b]
Plasma glutamine, *mM*	0.663 ± 0.038	0.678 ± 0.061	0.730 ± 0.042
Renal glutamine utilization, *μmoles/min*	13.8 ± 2.4	14.9 ± 1.9	23.9 ± 2.2[b, c]
Renal ammonia production, *μmoles/min*	23.0 ± 4.5	32.2 ± 4.7	48.6 ± 3.7[b, c]
Ammonium production/glutamine utilization	1.67	2.16	2.03
GFR, *ml/min*	34 ± 5	26 ± 5[b]	30 ± 5
Tissue metabolites, *μmoles/g*			
Glutamine	1.46 ± 0.16	1.00 ± 0.13[b]	1.19 ± 0.09
Glutamate	4.63 ± 0.26	2.47 ± 0.24[b]	3.63 ± 0.13[b, c]
α-Ketoglutarate	0.098 ± 0.012	0.042 ± 0.015[b]	0.052 ± 0.005[b]
Malate	0.109 ± 0.018	0.164 ± 0.073	0.518 ± 0.075[b, c]
Oxaloacetate (calculated) (*nmoles*)	1.98	2.63	10.25
Phosphoenolpyruvate	0.080 ± 0.009	0.077 ± 0.021	0.097 ± 0.009
2-Phosphoglycerate	0.052 ± 0.005	0.052 ± 0.006	0.055 ± 0.006
3-Phosphoglycerate	0.048 ± 0.006	0.056 ± 0.014	0.054 ± 0.005
Glucose-6-phosphate	0.042 ± 0.004	0.028 ± 0.007[b]	0.038 ± 0.003
Lactate	0.661 ± 0.067	1.00 ± 0.23	0.747 ± 0.087
Pyruvate	0.048 ± 0.007	0.064 ± 0.023	0.059 ± 0.005
Lactate/pyruvate	13.8	15.6	12.7
Citrate	0.651 ± 0.046	0.260 ± 0.037[b]	0.521 ± 0.027[b]
Alanine	0.894 ± 0.036	0.985 ± 0.098	1.10 ± 0.08[b]
Aspartate	0.972 ± 0.113	0.907 ± 0.090	1.66 ± 0.17[b]
B-OH-butyrate	0.051 ± 0.008	0.033 ± 0.007	0.033 ± 0.006
Acetoacetate	0.082 ± 0.008	0.094 ± 0.002	0.136 ± 0.037
Ammonium (calculated)	0.301 ± 0.029	0.496 ± 0.099[b]	0.424 ± 0.028[b]
ATP	1.59 ± 0.07	1.22 ± 0.10	1.61 ± 0.07
ADP	0.77 ± 0.09	0.63 ± 0.13	0.63 ± 0.04
AMP	0.22 ± 0.04	0.22 ± 0.04	0.17 ± 0.02
Inorganic phosphate	3.26 ± 0.32	2.87 ± 0.57	2.70 ± 0.19
Calculated values			
NAD/NADH LDH	654	576	711
NAD/NADH HBDH	32.6	57.8	83.6
NAD/NADH GLDH	1.65	2.18	1.57
K_{GOT}	10.4	5.86	2.32
K_{GPT}	0.39	0.26	0.27

[a] Data are means ± SEM. Abbreviations are defined as NAD/NADH, redox potential calculated for the cytosol or mitochondrial compartments from the metabolites of the lactate dehydrogenase (LDH) (cytosol), hydroxybutyrate dehydrogenase (HBDH) or glutamate dehydrogenase (GLDH) (mitochondria); K_{GOT} and K_{GPT} refer to the mass-action ratio of the aspartate (GOT) or alanine (GPT) aminotransferase.

[b] $P < 0.05$, a significant difference from control.

[c] $P < 0.05$, a significant difference between acute and chronic acidosis.

chondria from the glutamate dehydrogenase system (GLDH) are close to the values observed in the rat [4, 10]. However, when the mitochondrial NAD^+/NADH ratio is calculated from the beta-hydroxybutyrate dehydrogenase system (HBDH), values significantly different from those observed with the GLDH system are observed. This probably is related to determination difficulties due to the extremely small concentration of ketone bodies found in the kidneys of normal and fed animals.

One hundred minutes after acute acidosis in 7 dogs was induced by hydrochloric acid, which diminished their blood pH to 7.10 and their plasma bicarbonate concentrations to 11.3 mM (Table 1, middle column), no significant change of renal glutamine utilization can be seen. The renal ammonia production rises slightly[1], and the ratio of ammonia production over glutamine utilization is now 2.2.[1] During chronic acidosis by ammonium chloride (20 dogs), values for blood pH and plasma bicarbonate comparable to those of acutely acidotic dogs are observed (Table 1, right column). The plasma glutamine concentration tends to rise, but the difference does not reach statistical significance. Renal glutamine utilization and ammonia production are now significantly higher than the control values, and a ratio of ammonia production over glutamine utilization near 2.0 is maintained, indicating that both nitrogens of the glutamine molecule are liberated as ammonia. Furthermore, only in this group does the glutamine utilized exceed the filtered glutamine, establishing the existence of an antiluminal uptake of glutamine, a finding not observed in acutely acidotic dogs ($P < 0.05$).

The tissue changes observed following acute or chronic metabolic acidosis are similar. A rapid fall of tissue glutamine, glutamate, and alpha-ketoglutarate concentrations is observed, the fall being more pronounced following acute than chronic acidosis. In contrast, the tissue concentration of malate tends to rise following acute acidosis, and more markedly so following chronic ammonium chloride acidosis. Calculated values of oxaloacetate follow a similar pattern. No significant changes of phosphoenolpyruvate, 2-phosphoglycerate, or 3-phosphoglycerate are seen. The glucose-6-phosphate concentration tends to fall, but statistical significance is obtained only after acute acidosis by hydrochloric acid. The lactate/pyruvate ratio shows a slight reduction following acute hydrogen ion administration that is not seen in chronically acidotic dogs. The kidney concentration of citrate is acutely and markedly depressed following hydrochloric acid administration and remains low with chronic acidosis. The tissue ammonia concentration calculated from the renal vein ammonia concentration [25] is significantly higher and comparable in acute and chronic acidosis. No significant effect of metabolic acidosis on kidney adenine nucleotide content or inorganic phosphate can be detected. It should be noted that the kidney free-NAD^+/NADH ratio for the cytosolic or mitochondrial compartments is similar in control dogs and during acute or chronic metabolic acidosis, suggesting that a redox shift does not play a major role in the control of ammoniagenesis in that species. The mass-action ratio of the aspartate aminotransferase (K_{GOT}) or alanine aminotransferase (K_{GPT}) remains relatively close to their equilibrium constants, respectively, of 6.7 and 1.5 [26].

Table 2 presents the liver metabolite profiles in these dogs. It is clear that the liver concentration for each metabolite measured is similar in normal and chronically acidotic dogs. Following acute acidosis by hydrochloric acid, however, an abrupt fall of tissue glutamine, glutamate, alphaketoglutarate, citrate, and aspartate is observed with no concomitant rise of malate concentration. Thus, the liver response to acute acidosis is similar to that of the kidney, whereas that is not the case during chronic acidosis by ammonium chloride.

Renal PEPCK activity during chronic metabolic acidosis in the dog. Table 3 presents the total cortical, cytosolic, and mitochondrial PEPCK activi-

[1] Although the observed rise in ammonia production and the ammonia production over glutamine extraction ratio do not reach statistical significance in this series of acutely acidotic dogs, the increment of these parameters is in accord with a survey of values measured in our laboratory (unpublished). In a series of 22 normal dogs, the renal ammonia production averages 17.3 ± 1.0 μmoles/min (15.1 to 19.4 μmoles/min; 95% confidence limits [CL]) with a glutamine uptake of 11.3 ± 1.0 μmoles/min (9.2 to 13.4 μmoles/min; 95% CL). In 9 acutely acidotic dogs (including the seven experiments reported here), the ammonia production rises to 30.1 ± 4.5 μmoles/min (15 to 45 μmoles/min; 95% CL), a value significantly higher than that observed in control animals ($P < 0.05$), but glutamine extraction remains at 14.9 ± 2.4 μmoles/min (9.0 to 20.8 μmoles/min; 95% CL). In 59 chronically acidotic dogs, the ammonia production averages 47.5 ± 2.1 μmoles/min (43.2 to 51.7 μmoles/min; 95% CL) whereas glutamine extraction reaches 23.2 ± 1.1 μmoles/min (20.9 to 25.4 μmoles/min; 95% CL). The ratio of ammonia production over glutamine extraction averages 1.63 ± 0.1 (1.42 to 1.84; 95% CL) in the normal dog and increases significantly ($P < 0.05$) to 2.16 ± 0.06 (2.02 to 2.2, 95% CL) in acutely acidotic dogs. In chronically acidotic dogs, this value is maintained at 2.1 ± 0.15 (2.0 to 2.2; 95% CL). Only in chronically acidotic dogs is renal glutamine extraction greater than the filtered load of glutamine (comparison between chronic acidosis and control, $P < 0.05$; comparison between chronic and acute acidosis, $P < 0.05$).

ties, measured in the kidney cortex of 5 normal dogs (mean blood pH, 7.41; plasma bicarbonate, 22 mM) and 3 acidotic dogs (mean blood pH, 7.28; plasma bicarbonate, 15 mM). The assays were performed both in the "forward" (phosphoenolpyruvate generation) and the "back" (oxaloacetate generation) directions. No change in activity of either the cytosolic or the mitochondrial PEPCK enzymes was noted with chronic acidosis. The distribution of PEPCK between cytosolic and mitochondrial compartments is similar to that reported by Garthoff, Wolf, and Mehlman [27] for the normal dog. No PEPCK activity was found in the "nuclear" fraction when corrected for cross-contamination.

Renal ammoniagenesis and glutamine metabolism during acute acidosis induced by hydrochloric

Table 2. Tissue metabolite profile of liver of dogs in normal acid-base status and acute (hydrochloric acid) or chronic (ammonium chloride) metabolic acidosis[a]

	Normal (N = 13)	Acute acidosis (HCl; 100 min) (N = 7)	Chronic acidosis (NH_4Cl; 5 to 6 days) (N = 20)
Tissue metabolites, *μmoles/g*			
Glutamine	4.41 ± 0.24	2.91 ± 0.22[b]	4.77 ± 0.24
Glutamate	3.55 ± 0.33	2.08 ± 0.33[b]	3.68 ± 0.20
α-Ketoglutarate	0.200 ± 0.024	0.063 ± 0.011[b]	0.180 ± 0.013
Malate	0.880 ± 0.128	0.610 ± 0.107	0.890 ± 0.114
Oxaloacetate (calculated) (*nmoles*)	12.51	5.86	16.48
Phosphoenolpyruvate	0.089 ± 0.010	0.091 ± 0.027	0.103 ± 0.011
2-Phosphoglycerate	0.056 ± 0.007	0.061 ± 0.011	0.085 ± 0.089
3-Phosphoglycerate	0.061 ± 0.010	0.079 ± 0.090	0.089 ± 0.008
Glucose-6-phosphate	0.170 ± 0.023	0.218 ± 0.060	0.108 ± 0.013
Lactate	1.25 ± 0.16	1.93 ± 0.36	1.30 ± 0.13
Pyruvate	0.071 ± 0.008	0.074 ± 0.018	0.067 ± 0.005
Lactate/pyruvate	17.60	26.06	19.40
Citrate	0.715 ± 0.089	0.298 ± 0.05[b]	0.729 ± 0.056
Alanine	0.848 ± 0.114	1.041 ± 0.242	0.814 ± 0.055
Aspartate	1.10 ± 0.11	0.66 ± 0.130[b]	0.94 ± 0.08
B-OH-butyrate	0.101 ± 0.009	0.095 ± 0.010	0.095 ± 0.012
Acetoacetate	0.086 ± 0.013	0.108 ± 0.034	0.145 ± 0.028
Ammonium	0.565 ± 0.068	0.425 ± 0.068	0.650 ± 0.039
ATP	2.21 ± 0.08	2.21 ± 0.21	2.23 ± 0.16
ADP	0.63 ± 0.07	0.61 ± 0.13	0.69 ± 0.05
AMP	0.24 ± 0.03	0.20 ± 0.07	0.19 ± 0.02
Inorganic phosphate	3.45 ± 0.39	4.31 ± 1.0	3.21 ± 0.84
Calculated values			
NAD/NADH LDH	511	345	464
NAD/NADH HBDH	17.23	23.06	30.95
NAD/NADH GLDH	7.91	3.04	7.89
K_{GOT}	4.41	4.00	3.82
K_{CPT}	0.67	0.43	0.59

[a] Data are means ± SEM. Abbreviations are defined in Table 1.
[b] $P < 0.05$, a significant difference from control.

Table 3. PEPCK activity in the renal cortex of normal and acidotic dogs[a]

	Forward direction (generation of PEP)			Back direction (generation of OAA)		
Assays	Homogenate	Cytosol	Mitochondria	Homogenate	Cytosol	Mitochondria
Normal dogs (N = 5)	4.86 ±0.92	1.05 ±0.13 (22%)	3.70 ±0.66 (76%)	3.84 ±0.34	1.29 ±0.15 (34%)	2.78 ±0.33 (73%)
Acidotic dogs (N = 3)	6.32 ±0.27	1.27 ±0.34 (20%)	4.96 ±0.41 (78%)	3.44 ±0.75	1.36 ±0.45 (40%)	2.10 ±0.36 (61%)

[a] Values are expressed as micromoles of PEP or OAA produced per gram wet wt per min at 37° C and have been corrected for cross-contamination of the fractions by using lactate dehydrogenase and glutamate dehydrogenase as cytosolic and mitochondrial markers, respectively. The fraction of total cortical activity found in the cytosol and mitochondrial compartments are indicated between parentheses.

acid in the dog. Table 4 presents the systemic acid-base parameters and the renal production or extraction of some metabolites before and after acute acidosis. It can be seen that abrupt acidification is induced by the 60-min infusion of hydrochloric acid, imposing a hydrogen ion load of 5 mEq/kg. The urinary excretion and clearance of citrate is also immediately halved by acidosis (not shown). The GFR falls by 25% and the renal blood flow by 30% following hydrochloric acid administration.

No significant increment in glutamine extraction is seen, whereas the total ammonia production rises only slightly. An abrupt diminution of lactate extraction is observed, but no effect on aspartate or alanine production or oxygen extraction is noted. It is therefore clear that, in the dog, the induction of an acute metabolic acidosis produces no significant change in glutamine extraction but it improves the efficiency of the intrarenal ammoniagenic mechanisms, the ratio of ammonia production over glutamine extraction rising from 1.7 to 2.2. In contrast, during chronic metabolic acidosis, both glutamine extraction and ammonia production are elevated significantly (Table 1).

Renal metabolite profile in the rat. Table 5 presents determinations of the renal metabolite profile observed in the kidney of normal and acutely acidotic rats. Three control groups of rats with similar arterial pH and bicarbonate content in the normal range were used. The kidney metabolite profile, determined immediately after anesthesia, and 30 or 240 min following a short 30-min 0.9% sodium chloride infusion, is very similar in the three control situations, and close to that reported by Hems and Brosnan [4].

Thirty minutes after the infusion of hydrochloric acid and reduction of plasma pH to 7.00 and plasma bicarbonate to 10.0 mM, the tissue concentrations of glutamine, glutamate, and alpha-ketoglutarate are significantly lowered with no significant change in the tissue malate concentration. The calculated oxaloacetate and the phosphoenolpyruvate concentrations also are unchanged. Thirty minutes later, similar or slightly more marked changes in glutamine, glutamate, and alpha-ketoglutarate concentrations are observed, and the citrate concentration now is depressed significantly but the renal aspartate content rises. A slight fall (24%) in malate concentration is observed (Table 5), but this change does not reach statistical significance.

However, 90 to 120 min following the 30-min hydrochloric acid infusion, the malate and oxaloacetate concentrations fall. Therefore, an early fall in concentration of glutamine, glutamate, and alpha-ketoglutarate is observed in the rat kidney with no significant change of malate when the renal metabolite profile is studied in the first hour following the induction of acute metabolic acidosis. Thereafter, however, a concomitant fall of malate concentration is observed.

After 24 hours of ammonium chloride acidosis, the malate and oxaloacetate concentrations are even lower, whereas the phosphoenolpyruvate, 2-phosphoglycerate, and 3-phosphoglycerate concentrations are significantly higher than the control values and comparable to data published for chronic acidosis [1–3, 5].

Renal ammoniagenesis and glutamine metabolism during acute acidosis induced by hydrochloric acid in the rat. Table 6 shows the total renal ammonia production measured in the rat with repetitive renal arteriovenous differences obtained during at least 1 hour. In control animals, constant GFR and renal blood flow are observed. No significant

Table 4. Effect of acute metabolic acidosis (hydrochloric acid) on renal metabolism in the dog[a]

Time *min*	Blood pH	Plasma bicarbonate *mM*	Plasma glutamine *mM*	Glutamine extraction *μmoles/min*	Total NH_4 production *μmoles/min*	Lactate extraction *μmoles/min*	Citrate extraction *μmoles/min*	Aspartate production *μmoles/min*	Alanine production *μmoles/min*	O_2 extraction *μmoles/min*
0	7.32	19	0.66	13.8	23.0	70	6.4	1.1	10.6	246
	±0.01	±1	±0.05	±8.4	±4.5	±38	± 2.1	±0.9	± 2.7	±27
20	7.31	19	0.66	12.1	22.1	72	9.1	−0.5	10.6	275
	±0.01	±1	±0.05	±8.0	±3.9	±33	± 3.0	±0.5	± 4.0	±58
40	7.30	18	0.64	15.7	25.2	70	9.2	−0.8	8.7	294
	±0.02	±1	±0.05	±8.0	±5.1	±39	± 2.1	±0.4	± 2.0	±51
	Began 0.3 N hydrochloric acid in 0.9% sodium chloride (5 mEq/kg, 60-min infusion)									
100	7.10	10	0.67	14.4	32.2	39	8	0.3	15.2	313
	±0.02[b]	±0[b]	±0.06	±3.2	±6.7	±17[b]	± 2.3	±0.8	± 4.0	±61
120	7.11	12	0.70	15.9	30.7	35	6.2	0.9	9.7	336
	±0.02[b]	±0[b]	±0.06	±2.7	±6.2	±11[b]	± 1.8	±0.7	± 5.7	±76
140	7.10	11	0.68	14.9	32.3	33	5.5	0.7	18.0	265
	±0.02[b]	±0[b]	±0.06	±1.9	±8.7	±10[b]	± 1.9	±0.6	± 4.6	±57

[a] Values are means ± SEM ($N = 7$). Extraction and production were calculated using Wolf's correction [14].
[b] $P < 0.05$, significantly different from controls.

changes in ammonia production or glutamine extraction can be shown to occur following infusion of 0.9% sodium chloride for 30 min, and the results of these experiments are therefore not reported in detail. In normal rats, the renal glutamine extraction varies considerably from rat to rat, and this is reflected by the high SEM values calculated. Indeed, in some rats, no net glutamine extraction or small

Table 5. Tissue metabolite profile of kidneys of rats in normal acid-base status and acute (hydrochloric acid) or prolonged (ammonium chloride) metabolic acidosis[a]

	Control			Acidosis				
		NaCl infusion		HCl infusion				Oral NH_4Cl
	At 0 time	At 30 min	At 240 min	At 30 min	At 60 min	At 120 min	At 240 min	At 1440 min
N	6	8	6	7	8	7	9	6
Weight of rats, *g*	438 ± 15	395 ± 9	381 ± 11	418 ± 6	468 ± 12	481 ± 9	497 ± 18	458 ± 7
Arterial pH	7.41 ± 0.02	7.45 ± 0.01	7.42 ± 0.02	7.00 ± 0.01[b]	7.13 ± 0.01[b]	7.24 ± 0.02[b]	7.32 ± 0.02[b]	7.30 ± 0.02[b]
Plasma bicarbonate	26.7 ± 1.3	27.2 ± 0.7	25.6 ± 1.0	10.0 ± 0.4[b]	9.8 ± 0.4[b]	9.3 ± 0.3[b]	7.38 ± 0.34[b]	19.4 ± 0.4[b]
Tissue metabolites, *μmoles/g*								
Glutamine	1.49 ± 0.08	1.49 ± 0.04	1.42 ± 0.03	1.22 ± 0.04[b]	1.07 ± 0.05[b]	0.97 ± 0.04[b]	0.98 ± 0.05[b]	1.29 ± 0.05[b]
Glutamate	2.38 ± 0.17	2.73 ± 0.10	2.83 ± 0.18	1.72 ± 0.10[b]	1.63 ± 0.08[b]	2.0 ± 0.1[b]	2.06 ± 0.09[b]	2.26 ± 0.15[b]
α-Ketoglutarate	0.33 ± 0.02	0.20 ± 0.02	0.19 ± 0.03	0.12 ± 0.01[b]	0.099 ± 0.008[b]	0.077 ± 0.007[b]	0.08 ± 0.01[b]	0.092 ± 0.007[b]
Malate	0.370 ± 0.041	0.483 ± 0.012	0.46 ± 0.02	0.43 ± 0.06	0.37 ± 0.02	0.19 ± 0.02[b]	0.15 ± 0.01[b]	0.10 ± 0.03[b]
Oxaloacetate (calculated) (*nmoles*)	7.51	11.54	8.15	10.7	14.2	4.6	2.71	1.60
Phosphoenolpyruvate	0.172 ± 0.007	0.197 ± 0.007	0.120 ± 0.009	0.164 ± 0.004	0.159 ± 0.007	0.166 ± 0.005	0.183 ± 0.012	0.630 ± 0.085[b]
2-Phosphoglycerate	0.175 ± 0.011	0.145 ± 0.009	0.19 ± 0.06	0.130 ± 0.005	0.232 ± 0.022	0.227 ± 0.006	0.185 ± 0.024	0.269 ± 0.034[b]
3-Phosphoglycerate	0.111 ± 0.005	0.083 ± 0.004	0.114 ± 0.028	0.072 ± 0.01	0.145 ± 0.008	0.152 ± 0.008	0.110 ± 0.009	0.244 ± 0.069[b]
Glucose-6-P	0.08 ± 0.01	0.051 ± 0.005	0.053 ± 0.004	0.035 ± 0.004	0.031 ± 0.009	0.042 ± 0.005	0.052 ± 0.005	0.054 ± 0.007
Lactate	0.36 ± 0.02	0.37 ± 0.04	0.42 ± 0.03	0.30 ± 0.03	0.30 ± 0.03	0.43 ± 0.04	0.51 ± 0.06	0.73 ± 0.09
Pyruvate	0.029 ± 0.006	0.035 ± 0.014	0.03 ± 0.06	0.030 ± 0.004	0.047 ± 0.004	0.043 ± 0.005	0.038 ± 0.005	0.048 ± 0.005
Lactate/pyruvate	12.3	10.5	14.1	10.0	6.5	10.1	13.4	15.2
Citrate	0.82 ± 0.02	0.68 ± 0.05	0.96 ± 0.02	0.60 ± 0.04	0.49 ± 0.03[b]	0.41 ± 0.03[b]	0.40 ± 0.03[b]	0.42 ± 0.03[b]
Alanine	0.63 ± 0.04	0.55 ± 0.03	0.50 ± 0.02	0.69 ± 0.03	0.65 ± 0.05	0.70 ± 0.04	0.71 ± 0.04	0.73 ± 0.02
Aspartate	0.78 ± 0.03	0.80 ± 0.04	0.81 ± 0.05	0.86 ± 0.02	1.03 ± 0.06[b]	0.93 ± 0.04	1.24 ± 0.04[b]	1.36 ± 0.06[b]
B-OH-butyrate	0.07 ± 0.01	0.02 ± 0.00	0.06 ± 0.03	0.02 ± 0.00	0.02 ± 0.01	0.07 ± 0.04	0.02 ± 0.00	0.07 ± 0.01
Acetoacetate	0.16 ± 0.02	0.108 ± 0.008	0.305 ± 0.050	0.127 ± 0.004	0.079 ± 0.015	0.10 ± 0.01	0.18 ± 0.02	0.27 ± 0.02
ATP	1.42 ± 0.09	1.69 ± 0.08	1.41 ± 0.02	1.55 ± 0.08	1.46 ± 0.06	1.29 ± 0.09	1.27 ± 0.06	1.66 ± 0.07
ADP	0.84 ± 0.05	0.76 ± 0.07	0.76 ± 0.06	0.79 ± 0.05	0.81 ± 0.03	0.741 ± 0.040	0.83 ± 0.08	1.02 ± 0.10
AMP	0.20 ± 0.03	0.22 ± 0.02	0.21 ± 0.06	0.26 ± 0.01	0.21 ± 0.02	0.25 ± 0.04	0.15 ± 0.02	0.21 ± 0.03
Inorganic phosphate	5.52 ± 0.55	4.98 ± 0.66	5.45 ± 0.48	4.14 ± 0.13	4.26 ± 0.23	3.39 ± 0.35	4.75 ± 0.31	4.54 ± 0.26
NAD/NADH LDH	730	859	637	900	1393	892	671	592
NAD/NADH HBDH	45.5	109.5	103.5	128.8	80.1	29.8	179.5	78.8
NAD/NADH GLDH	10.31	6.99	5.00	3.89	3.39	2.12	2.19	3.61
K_{GOT}	14.45	5.08	6.63	5.57	4.4	7.78	17.99	34.60
K_{GPT}	2.99	1.15	1.12	1.59	0.85	0.63	0.73	0.61

[a] Values are means ± SEM. Abbreviations are defined in Table 1.

[b] $P < 0.05$, significantly different from control. Dunnett *t* test for multiple comparisons with a single control (30-min infusion of sodium chloride).

glutamine production was measured together with a definite renal ammonia production as reported by others [28]. In control animals, and during the control period of the rats submitted to acute acidosis, glutamine extraction itself cannot totally explain the ammonia production, implying that the kidney of normal rats uses substrates for ammoniagenesis other than glutamine. Following infusion of hydrochloric acid, an acute acidosis with reduction of blood pH from 7.41 to 7.14 and plasma bicarbonate from 19.9 to 8.8 mM is observed. Blood glutamine rises significantly above control values following this maneuver. Glutamine extraction increases, and the ratio of ammonia production over glutamine extraction tends to reach 2.0, implying that glutamine is now the main substrate for renal ammonia production.

Discussion

The present study demonstrates that, in the dog, acute or chronic metabolic acidosis significantly decreases the total renal cortical concentration of glutamine, glutamate, alpha-ketoglutarate, and citrate. These changes are more marked following acute rather than chronic acidosis, whereas malate concentration does not change (acute metabolic acidosis) or increases significantly (chronic metabolic acidosis). Phosphoenolpyruvate (PEP), 2-phosphoglycerate (2PGA), or 3-phosphoglycerate (3PGA) concentrations are not modified, but glucose-6-phosphate (G6P) concentration is lowered slightly. When the ratio of the tissue concentration in acidotic kidneys over the concentration in normal kidneys is calculated and presented as a crossover plot (Fig. 1) in the manner described by Chance et al [29] and previously used to describe metabolite concentration changes during acidosis in the rat [6, 30], it is evident that a first crossover occurs between alpha-ketoglutarate and malate, and possibly a second one, between 3-PGA and G-6-P, during acute or chronic acidosis. This is generally interpretated as evidence for an effect of metabolic acidosis on metabolic step(s) situated between alpha-ketoglutarate and malate and between 3-PGA and G-6-P.

This pattern of kidney tissue changes is in accord with the data obtained in the dog following acute metabolic acidosis [7] but differs from the pattern observed in the rat (Fig. 2) following acute (2 to 6 hours) or chronic metabolic acidosis as described by Alleyne [1, 2], Goldstein [3], Hems and Brosnan [4], Narins and Relman [6], and Boyd and Goldstein [31]. Indeed, in the rat, malate and oxaloacetate concentrations are decreased acutely during metabolic acidosis [2, 6], but intermediates of the glycolytic chain such as PEP, 2-PGA, and 3-PGA rise [6] (Fig. 2). Therefore, a different tissue response to metabolic acidosis is observed in the rat and dog. In rat kidney, the low malate and oxaloacetate and high PEP concentrations have been taken as evidence of increased flux through the adapted PEPCK step and increased gluconeogenesis [2, 5, 32]. The data are also compatible with increased oxidation of glutamine, because according to classical pathways, both oxidation and glucose production from glutamine are PEPCK-dependent. In the dog, where no PEPCK adaptation occurs during chronic metabolic acidosis, an acceleration of the metabolic flux between alpha-ketoglutarate and malate must be postulated to explain the observed tissue changes.

Table 6. Effect of acute metabolic acidosis on total ammonia production and glutamine extraction by the rat kidney in vivo (left kidney)[a]

Time *min*	Blood pH	Plasma bicarbonate *mM*	Blood glutamine *mM*	Urine volume *ml/min*	$U_{NH_4}V$ *μmoles/min*	Renal vein NH_4 release *μmoles/min*	Total NH_4 production *μmoles/min*	NH_4 production / GFR	Glutamine extraction *μmoles/min*	GFR *ml/min*	RBF *ml/min*
0 to 10	7.41	19.9	0.519	0.015	0.211	0.518	0.730	0.915	0.153	0.798	4.61
	±0.06	± 0.6	±0.026	±0.010	±0.028	±0.109	±0.100	±0.134	±0.191	±0.107	±0.58
10 to 20			0.513	0.011	0.209	0.513	0.722	0.908	0.122	0.795	3.66
			±0.047	±0.001	±0.012	±0.107	±0.156	±0.217	±0.181	±0.064	±0.33
20 to 50	Infusion of 0.2 N hydrochloric acid in 0.9% sodium chloride (0.5 ml/min) for 30 min through left jugular vein										
50 to 60			0.712	0.012	0.260	0.989	1.250	2.09	0.303	0.598	3.38
			±0.112	±0.003	±0.044	±0.310	±0.344	±0.313	±0.129	±0.143	±0.75
60 to 70	7.14[b]	8.8[b]	0.923[b]	0.012	0.269	0.851	1.119	2.73[b]	0.643[b]	0.410[b]	2.49[b]
	±0.04	± 1.2	±0.170	±0.003	±0.060	±0.147	±0.175	±0.32	±0.177	±0.098	±0.54

[a] Values are means ± SEM ($N = 6$). Extraction and production were calculated by Wolf's correction [14]. All animals received a constant infusion of 5% mannitol and 0.9% sodium chloride (50:50) at 0.08 ml/min through the right jugular vein.

[b] $P < 0.05$, significantly different from control.

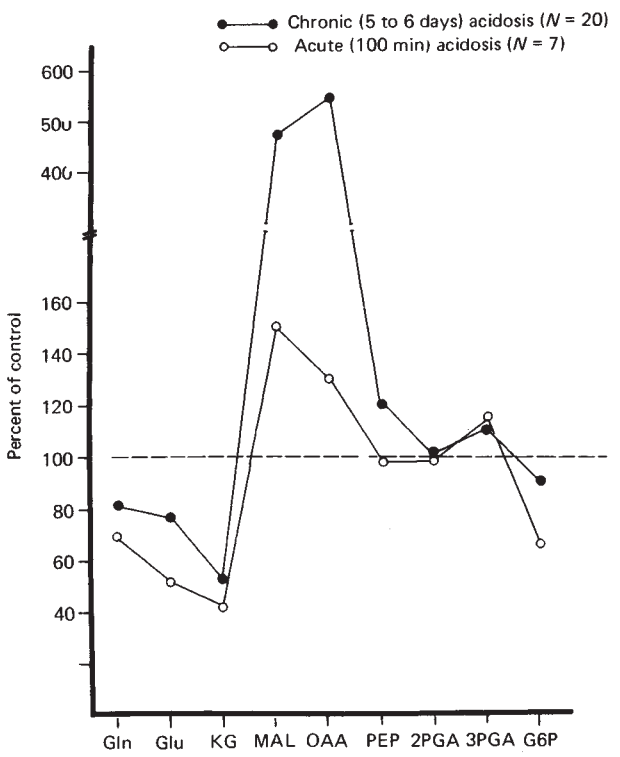

Fig. 1. *Effect of acute (hydrochloric acid) and chronic (ammonium chloride) metabolic acidosis on the renal metabolite profile of the dog.* Concentrations of metabolic intermediates in renal cortex of acidotic dog are shown as a percentage of levels in control dogs. Gln is glutamine; Glu, glutamate; Kg, alpha-ketoglutarate; MAL, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; 2PGA, 2-phosphoglycerate; 3PGA, 3-phosphoglycerate; G6P, glucose-6-phosphate.

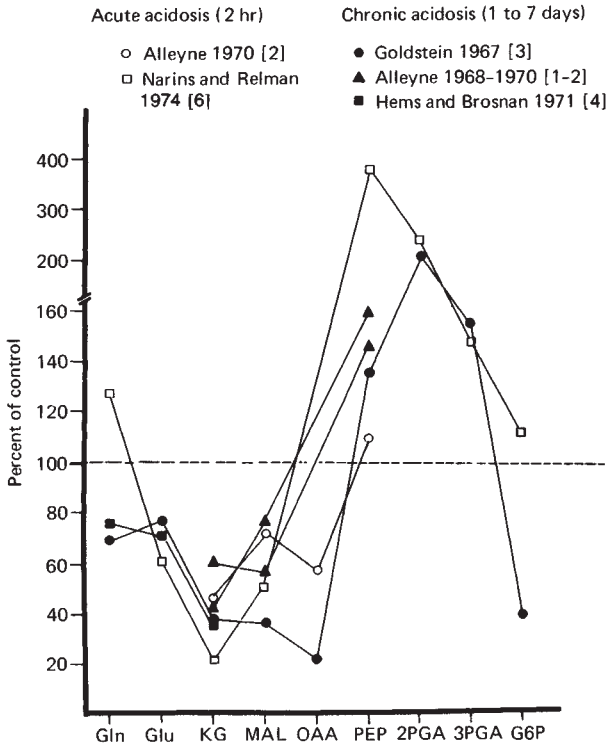

Fig. 2. *Effect of acute (2 hours) and chronic (1 to 7 days) metabolic acidosis on the renal metabolite profile of the rat.* See Fig. 1 for complete legend.

It is of interest that in the dog, the concentrations of glutamine, glutamate, and alpha-ketoglutarate fall in a more pronounced fashion, whereas malate accumulation is smaller during acute rather than chronic metabolic acidosis (Fig. 1). The difference between these two situations is possibly related to the fact that, in our study, as in that of Fine, Bennett, and Alleyne [33], net glutamine utilization by the dog kidney is not enhanced by acute metabolic acidosis, even if, as already shown by Fine et al [33], the utilization of glutamine per milliliter of GFR is somewhat increased. Greater intracellular utilization of glutamine, glutamate, and alpha-ketoglutarate without increased net cellular uptake of glutamine would indeed lead to lower tissue concentrations of these intermediates. By the same token, tissue malate accumulation will be limited. In chronic metabolic acidosis, the increment in cellular glutamine uptake would elevate the tissue concentrations of glutamine and glutamate, which remain below normal values, and allow the observed fivefold accumulation of malate and oxaloacetate to occur in the kidney tissue. The efficiency of the ammoniagenic mechanism seems to rise similarly in both acute and chronic acidosis, because a ratio of ammonia produced over glutamine extracted close to 2.0 is obtained.

These observations in the dog suggest that acute acidosis produces intracellular changes similar to those of chronic acidosis but that the ammoniagenic mechanism is limited by a delayed adaptation in glutamine transport. Because glutamine transport at the antiluminal site of proximal tubular cell is limited by the filtered load of glutamine, and because an antiluminal adaptation of glutamine transport is demonstrable during chronic metabolic acidosis in the dog [34, 35], it is tempting to propose that the antiluminal glutamine transport fails to adapt during acute metabolic acidosis in the dog. Our data indeed show that filtered glutamine is roughly equal to extracted glutamine following induction of acute metabolic acidosis. By constrast, in chronic acidosis, glutamine extraction exceeds filtration of glutamine, implying adaptation of an antiluminal transport system [34]. According to our hypothesis, the rate-limiting step for ammoniagenesis in the dog could be the transport of glutamine into the renal tubular cells and not the mitochondrial uptake of

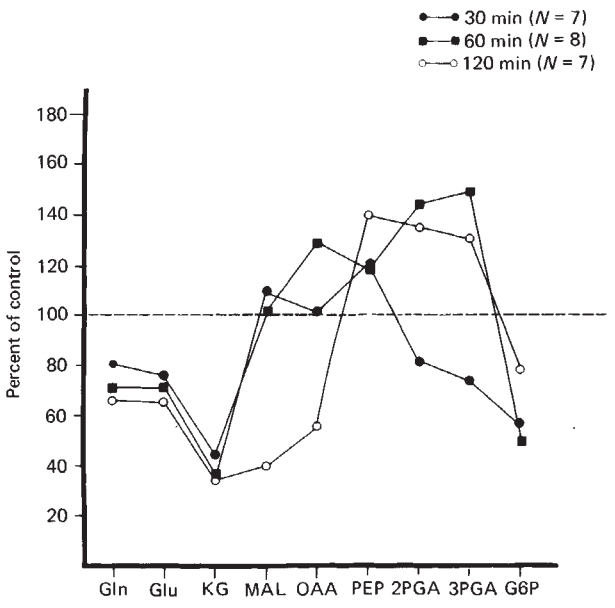

Fig. 3. *Early effect of acute metabolic acidosis (30, 60, and 120 min) on the renal metabolite profile of the rat.* See Fig. 1 for complete legend.

glutamine or the mitochondrial efflux of glutamine carbon skeletons as malate or aspartate.

Our results also suggest that PEPCK reaction is probably a limiting step for oxaloacetate metabolism through PEP formation in dog kidney tissue. The magnitude of malate accumulation in chronic metabolic acidosis, the total absence of PEPCK adaptation and of PEP accumulation, the very slight increment in gluconeogenesis observed in acidotic dog renal tissue in vitro [36], together with the significant utilization of the carbon skeleton of glutamine for total oxidation found in vivo in the dog [37], could also suggest the existence of a non-PEPCK-dependent pathway for oxaloacetate/malate oxidation. We have shown already that in some situations malic enzyme might play a significant role for glutamine oxidation in the dog [38] and the rat kidney [39].

It is of interest that acute metabolic acidosis leads to somewhat similar changes in the liver of the dog, because hepatic glutamine, glutamate, alpha-ketoglutarate, and citrate concentrations are significantly diminished following acute acidosis by hydrochloric acid, in accord with the data of Relman [7]. It is not possible, however, to give a precise interpretation of these changes without knowing the net flux through the glutamine utilization and synthesis pathways and cellular glutamine transport during these conditions. During chronic acidosis by ammonium chloride, however, none of these changes occur. It is also known that similar changes in tissue concentration occur in the distal tubule of the rat kidney [40], which lacks PEPCK enzyme [41]. In distal structures, glutamate concentration remains lower than it does in the proximal tubules [40] presumably because of lack of distal adaptation for glutamine transport and utilization [40]. These tissue changes may therefore reflect a general response of kidney and liver tissue to acute acidosis.

In the rat, the kidney metabolite profiles measured during acute (2 to 6 hours) or chronic acidosis are similar: a fall in glutamine, glutamate, alpha-ketoglutarate, and malate is seen together with an increment in phosphoenolpyruvate, 2-PGA, and 3-PGA concentrations (Fig. 2). Only Narins and Relman [6] report that renal tissue glutamine increases following acute acidosis, but this has never been reported by others and was not seen in our study. Therefore, the crossover plots in Fig. 2 indicates an acceleration of the metabolic flux between malate/oxaloacetate and phosphoenolpyruvate occurring as soon as 2 hours following acute metabolic acidosis and being sustained during chronic acidosis. Simultaneously, an adaptation of the renal PEPCK activity is detectable as soon as 4 to 6 hours of acidosis [5].

Our observations on the kidney tissue metabolite profile of rats 2 hours or longer following induction of metabolic acidosis (Fig. 3) are in good agreement with the data already reported (Fig. 2). However, if the concentration of glutamine, glutamate, and alpha-ketoglutarate are measured in the kidney tissue after 30 to 60 min of acidosis, an immediate fall of these intermediates is observed with no change (30 min) or no significant fall (60 min) in malate concentration (Fig. 3). Boyd and Goldstein also have reported an acute fall of alpha-ketoglutarate with no significant changes in malate concentration after 1 hour of acidosis in rats receiving orally 0.5 M ammonium chloride (1% body wt) [31]. They report, however, that with a load of 2 M ammonium chloride (1% body wt), rats experience a fall of both alpha-ketoglutarate and malate after 1 hour of acidosis. The fact that the acid load was administered by these authors as ammonium chloride (with transient secondary hyperammoniemia) and not as hydrochloric acid as in the present study does not explain the difference with the results reported here because ammonium load does not modify the malate concentration in the kidney of fasted rats per se [10]. Our data also show some decrement (24%) of malate after 1 hour of acidosis, but this fall does not reach statistical significance before 2 hours. Despite

some time-course discrepancy between the present study and that of Boyd and Goldstein [31], it is clear that an acute fall in alpha-ketoglutarate concentration can be observed at a time where no change in malate concentration could be detected in both studies.

It was proposed that, in the rat, the modification of the kidney metabolite profile was secondary to PEPCK adaptation [2, 5, 32] with faster oxaloacetate (and malate) removal, increasing in some fashion the rate of alpha-ketoglutarate utilization. This in turn would lead to faster glutamine transport [42] and utilization by lowering the mitochondrial glutamate concentration and deinhibiting the glutaminase I enzyme [3, 43]. This explanation, however, fails to account for the changes of tissue alpha-ketoglutarate concentration observed in the present study (1 hour) with unchanged malate.

Furthermore, the link between oxaloacetate and alpha-ketoglutarate concentrations in the kidney tissue is not clear because two irreversible reactions (alpha-ketoglutarate dehydrogenase and succinate thiokinase) take place between alpha-ketoglutarate and oxaloacetate. It is, therefore, unlikely that an increased flux would occur in such a system following faster removal of products through oxaloacetate utilization or transport into the extramitochondrial compartment (as malate or aspartate). Therefore, it is plausible that a direct effect of metabolic acidosis on one or both of these irreversible reactions occurs. The present findings in both rats and dogs are perfectly compatible with a primary activation of alpha-ketoglutarate dehydrogenase activity by metabolic acidosis with or without concomitant activation of succinate thiokinase activity [44, 45]. This would explain a faster alpha-ketoglutarate, glutamate, and glutamine utilization with no change or accumulation of oxaloacetate and malate. Such a mechanism could participate in the increased production of malate from glutamine observed by Kopyt et al [46] with isolated mitochondria from rat kidney cortex (in the absence of PEPCK activity) and the increased gluconeogenesis from glutamine, glutamate, or alpha-ketoglutarate observed when cortical tissue of rats is incubated in acid pH in vitro [44].

In our data, after 120 and 240 min of acidosis, as in the data of Boyd and Goldstein (60 min) [31] and Alleyne (6 hours) [2], the significant fall in tissue malate concentration is not accompanied by a proportional increment in phosphoenolpyruvate. Even if no measureable increment of PEPCK activity can be demonstrated before 6 hours of acidosis [2], it is possible that malate is converted to phosphoenolpyruvate at an increased rate following this maneuver without elevating the tissue concentration of phosphoenolpyruvate if the rate of utilization of the latter remains equal (or greater) than its rate of production. This concept has already been suggested by Boyd and Goldstein [31] and is in good agreement with our data.

Simpson [47] has attributed the acute lowering of intracellular concentration of various dicarboxylic acids in both species to a nonspecific redistribution of metabolites between the intra- and extra-mitochondrial compartments induced by acidosis. Such a mechanism would abruptly lower the cytosolic concentrations of citrate, alpha-ketoglutarate, and malate. The intramitochondrial concentrations, however, would not be expected to be lowered. If mitochondrial alpha-ketoglutarate concentration is important for glutamine transport [42] and if the rate of intramitochondrial alpha-ketoglutarate removal is important for glutamate metabolism and glutaminase I activation [3, 43], such a mechanism would not explain accelerated flux through the ammoniagenic pathway but only the changes in total tissue concentration of these metabolites. Furthermore, our observation of a fivefold increment of malate concentration in the renal cortex of chronically acidotic dogs does not support the general theory of Simpson. Indeed, it would be surprising if in the dog a significant mitochondrial uptake of malate had occurred following acute acidosis without lowering the total kidney malate concentration while similar uptake of citrate, alpha-ketoglutarate, glutamate, and glutamine is accompanied by significant reduction of the total kidney cortex concentration of these intermediates at the same time.

It is clear that in the rat, in contrast to the dog, net glutamine extraction rises acutely in early acidosis. The total ammonia production is not significantly enhanced, perhaps because of hemodynamics changes [34], but is now derived mainly from glutamine, because the ratio of ammonia production over glutamine extraction decreases from 4.8 to 1.7. The blood glutamine concentration also rises in a significant fashion in the rat following acute acidosis, whereas no significant change is observed in the dog. Therefore, the rat kidney is able to modify its antiluminal uptake of glutamine transport in a more rapid fashion than the dog kidney. Alternatively, acidosis may have produced some modification in the cytosolic glutamine synthetase activity in the rat [48]. It must be pointed out that no significant increment in plasma glutamine concentration

has been found in the chronically acidotic rat [49, 50, 51] or dog [35, 52, 53] in other studies. Fine, Bennett, and Alleyne [32], however, have recently described a significant rise in plasma glutamine in dogs with acute acidosis induced by hydrochloric acid (from 0.44 to 0.71 mM). The reasons for these divergent results are not clear.

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