Arteriovenous Differences for Amino Acids and Lactate across Kidneys of Normal and Acidotic Rats

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1. Arteriovenous differences for amino acids across kidneys of normal and chronically acidotic rats were measured. Glutamine was the only amino acid extracted in increased amounts in acidosis. There was a considerable production of serine by kidneys from both normal and acidotic rats. 2. The arterial blood concentration of glutamine was significantly decreased in acidotic animals. 3. The glutamine extracted by kidneys of acidotic rats was largely and probably exclusively derived from the plasma. 4. The blood lactate concentration was unchanged in acidosis, as was the uptake of lactate by the kidney.

In metabolic acidosis the kidney excretes large quantities of ammonia in the urine so as to facilitate the elimination of strong acids (Pitts, 1964). That this ammonia derives from circulating glutamine has been demonstrated by experiments involving the infusion of [^{15}N]glutamine into the renal artery of dogs (Pitts *et al.*, 1965). In addition, arteriovenous differences for all the amino acids across the kidney have revealed a large uptake of glutamine in metabolically acidotic man (Owen & Robinson, 1963) and dog (Shalhoub *et al.*, 1963). Arteriovenous differences across the kidney for the entire spectrum of amino acids have not been measured in the rat, and thus it is uncertain whether other amino acids may also contribute appreciably to the presence of ammonia in the urine. Experiments *in vitro* have demonstrated ammonia production from amino acids other than glutamine in rat kidney-cortex slices (Davies & Yudkin, 1952) and in the perfused rat kidney (Pitts, 1971), and, further, such ammonia production is increased *in vitro* in preparations from chronically acidotic rats. Much work on the regulation of renal ammonia production involving mitochondria, kidney slices, tubule preparations and perfused kidneys has used the rat as the experimental animal. For the further prosecution of such studies *in vitro* it is clearly essential to establish which amino acids contribute to renal ammonia production *in vivo*. We therefore report on the renal arteriovenous differences for amino acids across kidneys of normal and acidotic rats. Since it is now appreciated that erythrocytes can play a dynamic role in inter-organ amino acid transport (Elwyn *et al.*, 1972; Aoki *et al.*, 1972; Felig *et al.*, 1973) we used whole blood for our measurements. In view of the decreased uptake of lactate by kidneys of acidotic dogs demonstrated by Leal-Pinto *et al.*

(1973), we have also measured renal arteriovenous differences for lactate in normal and acidotic rats.

Materials and Methods

Animals

Male Sprague–Dawley rats were allowed free access to food and water. Rats were rendered chronically acidotic by substituting 1.5% (w/v) NH_4Cl for their drinking water for 7–10 days. In preliminary experiments we observed that rats of less than 250 g ate poorly and lost weight when given NH_4Cl . This does not occur with larger rats. All of the experiments reported were performed on animals weighing 300–400 g.

Blood samples

Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (6.5 mg/100 g body wt.), and the abdominal cavity was exposed by a midline incision. Blood, about 1 ml per sample, was withdrawn into heparinized plastic syringes. Arterial and renal venous samples were taken from the same animal; the renal venous sample was taken first and then arterial blood was sampled from the abdominal aorta. The blood samples were immediately added to an equal volume of 6% (w/v) HClO_4 , mixed vigorously with a vortex mixer, allowed to stand on ice for 15 min, and the precipitated proteins were removed by centrifugation. The clear extracts were then adjusted to pH 2.0 with either 5 M-NaOH (if the entire range of amino acids was to be determined) or with 5 M-LiOH (if resolution of glutamine from asparagine was being effected). Plasma samples were deproteinized and adjusted to pH 2.0 exactly as described above except that the plasma samples were de-

proteinized by the addition of a one-half volume of 12% (w/v) HClO_4 to each sample.

Determination of amino acids and of lactate

Amino acids were measured on a Beckman model 121 automatic amino acid analyser. Two different types of analysis were performed: (a) the analysis of Benson & Patterson (1965) to determine the full spectrum of amino acids without resolution of glutamine from asparagine, and (b) the accelerated analysis of Benson *et al.* (1967) to permit resolution of glutamine and asparagine. The following adaptations of the published methods were used. In the analysis of acidic and neutral amino acids, a 53 cm $\times$ 0.9 cm column was used, the sodium citrate buffers were changed at 185 min from pH 3.16 (0.20 M in Na^+) to pH 4.25 (0.20 M in Na^+), the temperature-change time was 140 min and the total analysis time was 400 min. In the analysis of basic amino acids the first sodium citrate buffer was pH 4.25 (0.38 M in Na^+), the second buffer was pH 5.36 (0.35 M in Na^+), the initial temperature was 30°C and the total analysis time was 300 min. In the accelerated method for glutamine and asparagine, HP-AN-90 resin (Hamilton Co., Whittier, CA, U.S.A.) was used in a 36 cm $\times$ 0.90 cm column and the total analysis time was only 95 min, because 10 min after the elution of glutamine the run was ended. The column was then regenerated with 0.3 M-LiOH, and equilibrated with the starting buffer. By

using these methods it was possible to measure the amino acids without interference from glutathione. Lactate was measured in deproteinized neutralized blood samples by the method of Lowry & Passonneau (1972).

Presentation and analysis of data

Differences between populations were tested for statistical significance by Student's *t* test. A paired *t* test was used for the paired data. *P* values of 0.05 or less were taken to indicate a significant difference between populations.

Results and Discussion

Arterial whole-blood amino acid concentrations in normal rats and acidotic rats are shown in Table 1. The concentrations of serine, threonine and lysine in blood were all significantly increased in the acidotic rats. The reason for this is unknown. There was a substantial decrease in the concentration of circulating glutamine in acidosis. Although this decrease has not been observed previously in acidotic rats (Welbourne, 1974), we found it to be a highly reproducible and statistically significant event. A similar decrease in plasma glutamine has also been observed in acidotic man (Owen & Robinson, 1963) and sheep (Heitmann & Bergman, 1975). Such a decrease in circulating glutamine concentration may be due to the increased

Table 1. Arterial amino acid concentrations and renal arteriovenous differences in normal and acidotic rats

Results are expressed as $\mu\text{mol/ml}$ of blood $\pm$ S.D.; $n = 6$, except where noted. A positive difference implies an uptake by the kidney. NS, not significant.

Amino acid	Normal			Acidotic		
	Arterial	Arteriovenous difference	<i>P</i> value (arteriovenous difference different from zero)	Arterial	Arteriovenous difference	<i>P</i> value (arteriovenous difference different from zero)
Glycine	0.39 ± 0.13	$+0.05 \pm 0.13$	NS	0.47 ± 0.08	$+0.04 \pm 0.06$	NS
Alanine	0.46 ± 0.15	0.00 ± 0.08	NS	0.46 ± 0.03	$+0.01 \pm 0.02$ (4)	NS
Serine	0.20 ± 0.05	-0.06 ± 0.03	<0.025	0.33 ± 0.07	-0.05 ± 0.02	<0.005
Threonine	0.25 ± 0.05	-0.03 ± 0.04	NS	0.43 ± 0.08	-0.01 ± 0.01 (5)	NS
Leucine	0.12 ± 0.02	-0.01 ± 0.02	NS	0.11 ± 0.02	0.00 ± 0.01	NS
Isoleucine	0.07 ± 0.01	-0.01 ± 0.01	NS	0.06 ± 0.02	0.00 ± 0.01	NS
Valine	0.17 ± 0.03	-0.02 ± 0.03	NS	0.16 ± 0.03	0.00 ± 0.01 (5)	NS
Proline	0.22 ± 0.09	$+0.02 \pm 0.06$	NS	0.22 ± 0.04	-0.01 ± 0.02	NS
Tyrosine	0.08 ± 0.02	-0.01 ± 0.01	NS	0.10 ± 0.02	-0.01 ± 0.01	NS
Phenylalanine	0.05 ± 0.01	0.00 ± 0.01	NS	0.05 ± 0.01	$+0.01 \pm 0.02$	NS
Methionine	0.03 ± 0.01	-0.01 ± 0.01 (5)	NS	0.02 ± 0.01	-0.01 ± 0.01	NS
Aspartic acid	0.04 ± 0.02	-0.01 ± 0.02	NS	0.02 ± 0.02	-0.01 ± 0.02 (4)	NS
Glutamic acid	0.29 ± 0.07	0.00 ± 0.09	NS	0.20 ± 0.05	-0.02 ± 0.05 (5)	NS
Asparagine+glutamine	0.48 ± 0.08	$+0.03 \pm 0.05$	NS	0.36 ± 0.05	$+0.11 \pm 0.05$	<0.005
Lysine	0.45 ± 0.10	0.00 ± 0.04	NS	0.62 ± 0.08	$+0.04 \pm 0.13$	NS
Ornithine	0.06 ± 0.02	0.00 ± 0.01	NS	0.05 ± 0.01	0.00 ± 0.01	NS
Histidine	0.05 ± 0.00	-0.01 ± 0.00	<0.005	0.06 ± 0.01	$+0.01 \pm 0.01$	NS
Arginine	0.23 ± 0.04	-0.02 ± 0.01	<0.05	0.32 ± 0.05	$+0.01 \pm 0.07$	NS

Table 2. Plasma and whole-blood concentrations and arteriovenous differences for glutamine, glutamate and asparagine across kidneys of acidotic rats

Results are expressed as $\mu\text{mol/ml} \pm \text{s.d.}$; $n = 5$ in all cases. NS, not significant.

Amino acid		Blood	Plasma
Glutamine	Renal vein	0.37 ± 0.07	0.33 ± 0.11
	Artery	0.46 ± 0.03	0.50 ± 0.08
	<i>P</i> for arteriovenous difference different from zero	<0.025	<0.005
Glutamate	Renal vein	0.15 ± 0.01	0.06 ± 0.02
	Artery	0.14 ± 0.02	0.08 ± 0.01
	<i>P</i> for arteriovenous difference different from zero	NS	<0.005
Asparagine	Renal vein	0.05 ± 0.01	0.05 ± 0.01
	Artery	0.05 ± 0.01	0.05 ± 0.01
	<i>P</i> for arteriovenous difference different from zero	NS	NS

renal utilization of this amino acid in metabolic acidosis. The lower concentrations may well serve as a stimulus for increased glutamine synthesis in the tissues responsible for glutamine production and may also lessen glutamine utilization by extrarenal tissues.

The measurement of the arteriovenous differences across the kidneys of normal rats (Table 1) revealed statistically significant but extremely small outputs of histidine and arginine as well as a substantial output of serine. No amino acids were taken up in significant quantities. There was also a significant production of serine by the kidney of the acidotic rat and a large and significant uptake of glutamine plus asparagine. No other amino acids were removed or produced in significant quantities. Amino acids were also measured in 24 h urine samples collected from normal and acidotic rats. The quantities of all amino acids measured were extremely small. In particular, the quantity of glutamine excreted could in no way account for the large amounts removed by the acidotic kidney.

Renal output of serine has been observed in acidotic and alkalotic dogs (Shaloub *et al.*, 1963; Pitts *et al.*, 1963), in normal and acidotic men (Owen & Robinson, 1963) and in normal and starved rats (Aikawa *et al.*, 1973; Yamamoto *et al.*, 1974). That such renal serine production is seen in acidosis and in alkalosis as well as in fed and starved animals indicates that its production is related neither to the acid-base status of the animal nor to the provision of gluconeogenic amino acids for hepatic glucose production. This serine may be derived from circulating glycine, although Pitts *et al.* (1970) point out that the extraction of glycine does not invariably parallel serine output. In our own studies (see Table 1) the arterial glycine concentrations may have been higher than those in the renal venous blood by an amount that corresponds to the renal output of serine. However,

the arteriovenous differences for glycine uptake were not statistically significant.

There was no output of alanine by either the normal or acidotic kidney. Renal alanine output has been found in dogs (Shalhoub *et al.*, 1963; Pitts *et al.*, 1963), rats (Aikawa *et al.*, 1973; Yamamoto *et al.*, 1974) and man (Owen & Robinson, 1963). However, the output reported in the rat was quite modest, and Cahill *et al.* (1975) reported a small uptake of alanine in man. Thus it appears that alanine release is not an inevitable result of renal metabolism.

The only amino acid extracted in statistically significant quantities by the acidotic rat kidney was glutamine. Thus, although other amino acids may be utilized by the kidney in some preparations *in vitro*, it is apparent that glutamine is the major source of ammonia in the urine of the acidotic rat. It is noteworthy that no amino acid is released by the acidotic kidney other than serine. Thus both nitrogen atoms of glutamine are probably used for ammonia production.

The results summarized in Table 2 examine the relative roles of the plasma and erythrocyte pools of glutamine in donating this amino acid to the acidotic kidney. In these experiments, arterial blood and plasma samples, and renal venous blood and plasma samples, were obtained from the same animal. We also measured the haematocrit of arterial blood, and obtained values of 43.6 ± 2.4 (4) for normal rats and 43.7 ± 3.9 (6) for acidotic rats. Thus, since plasma occupies 56.3% of the blood volume of these acidotic rats, we may calculate that for every millilitre of blood that perfuses these kidneys *in vivo* $0.096 \mu\text{mol}$ of glutamine (0.563×0.170) (kidney arterio-renal-venous difference for plasma glutamine is $0.170 \mu\text{mol/ml}$; see Table 2) is, on average, extracted from the plasma. Similarly, for every millilitre of blood that perfuses these kidneys *in vivo* a total of $0.095 \mu\text{mol}$ of

glutamine (1×0.095) (kidney arteriovenous difference for blood glutamine is $0.095 \mu\text{mol/ml}$; see Table 2) is extracted from the blood. The remarkable agreement between these two values indicates that the glutamine taken up by the acidotic rat kidney is derived predominantly and probably exclusively from the plasma. That erythrocyte glutamine is not removed is undoubtedly due to a slow rate of movement of glutamine across the cell membrane of erythrocytes. Pitts *et al.* (1965, 1972) have also demonstrated in the dog that the kidneys remove glutamine primarily from plasma.

Although there was no significant uptake or output of glutamate by the acidotic kidney, the concentration of glutamate in arterial plasma was significantly higher than in the renal venous plasma. Hence a movement of glutamate from plasma to erythrocytes occurred as the blood passed through the kidney. This result is reminiscent of the experiments of Aoki *et al.* (1972) on the role of erythrocytes in the uptake and release of glutamate by muscle. There was no significant change in the concentrations of asparagine, either in blood or plasma, after passage through the kidney.

Studies by Leal-Pinto *et al.* (1973) have shown that kidneys of alkalotic dogs extract and oxidize more lactate than do kidneys of acidotic dogs. These workers have suggested that, in acidotic dogs, glutamine, which is extracted in increased amounts, is oxidized to CO_2 , and hence less lactate oxidation is needed for energy production. In addition, blood lactate in alkalotic dogs was considerably higher than in acidotic dogs. Therefore we measured renal arteriovenous differences for lactate in normal and acidotic rats. There was no significant difference in arterial lactate concentrations between normal [$1.65 \pm 0.46 \mu\text{mol/ml}$ (6)] and acidotic rats [$1.63 \pm 0.38 \mu\text{mol/ml}$ (9)]. In addition, normal and acidotic rat kidneys extracted similar and significant amounts of lactate (normal arteriovenous difference = $+0.31 \pm 0.08 \mu\text{mol/ml}$; acidotic arteriovenous difference = $+0.32 \pm 0.22 \mu\text{mol/ml}$). Thus metabolic acidosis has no effect on the removal of lactate by the rat kidney.

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