Glutamine Release from Hindlimb and Uptake by Kidney in the Acutely Acidotic Rat

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The arteriovenous difference (release) for glutamine across the hindlimb increases significantly during acute HCl-induced acidosis. This additional amount released by muscle tissue can account for the extra glutamine taken up by the kidney.

It is now well established that during acidosis the mammalian kidney extracts large quantities of glutamine from the circulation and converts it into ammonia for purposes of urinary acid neutralization (Goldstein, 1976; Tannen, 1978). The response of the renal glutamine extraction and ammonia production to acidosis is rapid; it occurs within 2 h after acid-loading (Goldstein & Boylan, 1978; Hughey, 1979). Since the blood flow to the kidney is high (approx. 20% of the cardiac output), there is a considerable amount of glutamine removed from the circulation by the acidotic kidneys within a short period of time.

The source of the extra glutamine extracted by the acidotic kidneys was previously not known. Addae & Lotspeich (1968) suggested, on the basis of arteriovenous-difference measurements, that the liver was a major site of glutamine release in normal and acutely acidotic dogs. No glutamine release from the hindlimb could be detected in these studies. However, Hills *et al.* (1972) could not confirm these findings and reported that significant amounts of glutamine were released from hindlimbs of starved dogs and spider monkeys. Furthermore, studies in man (Felig *et al.*, 1973) showed that during the post-absorptive state glutamine is released by skeletal muscle and taken up by the liver. Thus the current weight of evidence favours the view that the main source of circulating glutamine is that produced and released by skeletal muscle. Nevertheless, there was no experimental evidence that glutamine release was stimulated during acidosis to supply the extra amino acid utilized by the kidneys. Lemieux *et al.* (1979) reported that there was a tendency for the arteriovenous concentration difference across hindlimbs of rats to increase during chronic acidosis, but the difference was not statistically significant. In the present study we showed that under conditions in which each animal was used as its own control glutamine release by rat

hindlimbs was significantly stimulated by acute metabolic acidosis.

Materials and Methods

Animals, treatments and surgery

Male Sprague–Dawley rats (Caesarian derived) weighing 319 ± 10 g ($n=23$) were obtained from Charles River Laboratories and maintained on Purina Rat Chow and water supplied *ad libitum*. Acute acidosis was induced by intragastric application of 10 mmol of HCl/kg body wt. in a volume of 2.5% of the body weight. Control rats received an equal volume of tap water.

The complete experiment was done in the following order. Rats were anaesthetized with Inactin (5-s-butyl-5-ethyl-2-thiobarbituric acid; Promonta-Hamburg, Henley and Co., New York, NY, U.S.A.). Body temperature was maintained at $37 \pm 1^\circ\text{C}$. The trachea was catheterized with Intramedic PE tubing (no. 7445) to facilitate breathing. The left carotid artery was catheterized with Intramedic PE tubing (no. 7410) and an arterial blood sample (A_1) drawn; 0.5 ml of plasma was removed for analysis. The remainder of the sample (plasma and erythrocytes) was reconstituted to the original volume with 0.5 ml of 0.9% NaCl and injected back into the circulation. The same procedure was performed after each blood sampling. The abdominal cavity was opened and a blood sample drawn from the iliac vein (I_1). Then HCl or tap water was administered by stomach tube, and 2 h later blood samples were drawn in the following order: arterial sample A_2 , iliac vein (I_2), renal vein (R), and arterial sample A_3 . Sampling was finished within 20 min. Plasma samples were stored in a deep-freezer at -80°C until assayed.

Glutamine assay

Plasma proteins were precipitated with an equal volume of 10% (v/v) HClO_4 . The HClO_4 super-

natants were neutralized with an equimolar amount of KOH, and KClO_4 was removed by centrifugation (12800g, 1 min). The neutralized supernatant solutions were assayed for glutamine with a bacterial glutaminase preparation (Sigma, grade V) and glutamate dehydrogenase (Sigma, type II) as described by Lund (1974). Triplicate analyses were done on each 0.5 ml plasma sample.

Amino acid analyses

Plasma was deproteinized with sulphosalicylic acid, and the supernatant was adjusted with LiOH to pH 2.2 and analysed with a Dionex D-556 automatic amino acid analyser modified as described by Lee (1974).

Blood flow to the hindlegs

This was monitored with an electromagnetic flow meter (flow meter SWF-4; Zepeda Instruments, Seattle, WA, U.S.A.). The electromagnetic cuff was placed around the abdominal aorta just before it split into two branches leading to the hindquarters. Student's *t* test was used to test the level of significance for differences between means, and to test whether arteriovenous differences were significantly different from zero.

Results and Discussion

There was a significant increase in the arteriovenous difference for glutamine across the hindleg after 2 h of HCl-induced acidosis (Table 1). Blood flow to the hindlegs was 3.1 ± 0.5 ml/min per 100 g body wt. before and 2.4 ± 0.2 ml/min per 100 g after 2 h acidosis ($n = 6$, $P < 0.05$). Despite a 20% decrease in blood flow, total glutamine output from the hindlegs was increased 100–200% during acute acidosis. Ruderman *et al.* (1971) showed that muscle tissue and fat account for 95% of the O_2 consumption by the rat hindquarter and make up 78% of the total weight. Since skeletal muscle is an active site of glutamine synthesis (Garber *et al.*, 1976), it is reasonable to assume that the extra glutamine released into the circulation by the hindlimb during acute acidosis came mainly from

the skeletal muscle. Thus skeletal muscle is an important site of glutamine supply for the kidneys during acute acidosis.

Kidney glutamine uptake was stimulated significantly after acute administration of HCl (Table 1), which is consistent with similar findings of Hughey (1979). There was a small but significant uptake of glutamine by the kidneys of the control group ($P < 0.01$). This is not consistent with the findings of Squires *et al.* (1976), in which no significant arteriovenous differences for glutamine across the kidneys of control rats could be established. Our renal measurements were made in rats which had undergone anaesthesia and surgery at least 2.5 h before renal blood samples were taken. Thus, slight respiratory acidosis might have been present and stimulated renal glutamine extraction. The blood pH at zero time and 2 h after administration of tap water was 7.39 ± 0.02 and 7.38 ± 0.02 for control animals, and 7.35 ± 0.05 and 7.21 ± 0.02 before and after HCl administration.

Arterial plasma amino acid concentrations before and after acute HCl-induced acidosis in the same animals are shown in Table 2. Arterial plasma concentrations of threonine, serine, asparagine, glutamine, glycine, citrulline, valine, cysteine, leucine, lysine and histidine increased significantly during the experimental period (cf. samples A_1 and A_3 , $P < 0.05$). The rise in glutamine concentration indicates that production in muscle tissue increased more than kidney extraction. However, the same effect could have been brought about by a decrease in glutamine uptake in the gut, which is known to be the main organ for extraction of glutamine under normal conditions (Windmueller & Spaeth, 1974). This rise in plasma glutamine concentration in the rat with acute HCl-induced acidosis is in contrast with the chronic acidotic rat, in which glutamine concentration is lower than normal (Squires *et al.*, 1976). There was a 6-fold increase in taurine concentration, but this increase is not statistically significant. Plasma tryptophan concentration decreased significantly.

Total amino acid arteriovenous differences were measured across the hindleg before and 2 h after

Table 1. Arterial (A) glutamine concentrations and arteriovenous differences across the hindleg (A–I) and the kidney (A–R) in controls and in rats with acute HCl-induced acidosis

Results are means $\pm$ S.E.M. ($n = 9$); values are expressed in $\mu\text{mol/ml}$ of plasma. A positive difference implies an uptake by the organ, negative a release. For details see the Materials and Methods section. *Significantly different from control ($P < 0.02$); †significantly different from value at $t = 0$ ($P < 0.005$).

	$t = 0$		$t = 2 \text{ h}$		$t = 2.3 \text{ h}$	
	A_1	A–I	A_2	A–I	A_3	A–R
Control (tap water)	0.57 ± 0.01	-0.08 ± 0.01	0.68 ± 0.01	-0.10 ± 0.01	0.62 ± 0.02	0.05 ± 0.01
Acidosis (10 mmol of HCl/kg)	0.62 ± 0.02	-0.05 ± 0.02	0.75 ± 0.03	$-0.19 \pm 0.03^{*†}$	0.78 ± 0.04	$0.18 \pm 0.05^{*}$

Table 2. Arterial amino acid concentrations, iliac and renal arteriovenous differences before and after 2 h acidosis. Results are expressed as $\mu\text{mol/litre}$ of plasma, $\pm$ S.E.M. ($n = 5$). *Significantly different from zero ($P < 0.02$); †significantly different from value at $t = 0$ ($P < 0.02$). Abbreviations: Tau, taurine; Cit, citrulline.

	$t = 0$		2 h acidosis		2.3 h acidosis	
	A ₁	A-I	A ₂	A-I	A ₃	A-R
Tau	68 $\pm$ 15	-4 $\pm$ 4	204 $\pm$ 54	-41 $\pm$ 28	329 $\pm$ 97	38 $\pm$ 23
Asp	10 $\pm$ 1	-25 $\pm$ 73	10 $\pm$ 1	-1 $\pm$ 1	11 $\pm$ 1	3 $\pm$ 1*
Hyp	74 $\pm$ 8	0 $\pm$ 5	79 $\pm$ 8	-11 $\pm$ 3	79 $\pm$ 6	9 $\pm$ 3
Thr	257 $\pm$ 18	2 $\pm$ 5	333 $\pm$ 30	-5 $\pm$ 14	336 $\pm$ 32	-16 $\pm$ 8
Ser	238 $\pm$ 16	7 $\pm$ 8	344 $\pm$ 40	19 $\pm$ 19	338 $\pm$ 35	-163 $\pm$ 18*
Asn	51 $\pm$ 3	2 $\pm$ 2	76 $\pm$ 9	0 $\pm$ 5	74 $\pm$ 9	-10 $\pm$ 2*
Glu	111 $\pm$ 18	15 $\pm$ 14	75 $\pm$ 14	18 $\pm$ 10	64 $\pm$ 7	-3 $\pm$ 3
Gln	541 $\pm$ 33	-22 $\pm$ 29	657 $\pm$ 57	-180 $\pm$ 38*†	669 $\pm$ 38	250 $\pm$ 28*
Pro	213 $\pm$ 25	12 $\pm$ 7	189 $\pm$ 30	-8 $\pm$ 7	173 $\pm$ 22	5 $\pm$ 4
Gly	324 $\pm$ 24	-18 $\pm$ 6	448 $\pm$ 42	-81 $\pm$ 13*†	490 $\pm$ 31	59 $\pm$ 11*
Ala	583 $\pm$ 54	29 $\pm$ 23	553 $\pm$ 88	-32 $\pm$ 47	505 $\pm$ 58	-6 $\pm$ 11
Cit	63 $\pm$ 5	5 $\pm$ 1*	85 $\pm$ 5	-5 $\pm$ 1*†	96 $\pm$ 4	44 $\pm$ 6*
Val	189 $\pm$ 11	19 $\pm$ 6	260 $\pm$ 16	15 $\pm$ 9	256 $\pm$ 19	3 $\pm$ 9
Cys	34 $\pm$ 3	8 $\pm$ 3	54 $\pm$ 2	10 $\pm$ 3	55 $\pm$ 4	-15 $\pm$ 5
Met	46 $\pm$ 3	3 $\pm$ 2	54 $\pm$ 6	2 $\pm$ 2	52 $\pm$ 6	-3 $\pm$ 2
Ile	72 $\pm$ 5	8 $\pm$ 4	90 $\pm$ 8	12 $\pm$ 4	84 $\pm$ 8	-6 $\pm$ 3
Leu	114 $\pm$ 8	16 $\pm$ 7	190 $\pm$ 17	24 $\pm$ 8	176 $\pm$ 13	-12 $\pm$ 5
Tyr	57 $\pm$ 8	1 $\pm$ 2	69 $\pm$ 9	0 $\pm$ 3	71 $\pm$ 9	-13 $\pm$ 6
Phe	53 $\pm$ 2	2 $\pm$ 3	58 $\pm$ 2	4 $\pm$ 3	61 $\pm$ 6	7 $\pm$ 3
Trp	74 $\pm$ 2	7 $\pm$ 1*	48 $\pm$ 5	0 $\pm$ 3	51 $\pm$ 8	1 $\pm$ 3
Orn	63 $\pm$ 4	8 $\pm$ 1*	87 $\pm$ 8	5 $\pm$ 5	74 $\pm$ 5	-18 $\pm$ 4*
Lys	357 $\pm$ 16	8 $\pm$ 9	502 $\pm$ 47	-42 $\pm$ 26	500 $\pm$ 55	-21 $\pm$ 9
His	57 $\pm$ 4	1 $\pm$ 2	111 $\pm$ 9	-7 $\pm$ 5	119 $\pm$ 9	-5 $\pm$ 4
Arg	148 $\pm$ 7	5 $\pm$ 6	146 $\pm$ 32	-4 $\pm$ 11	126 $\pm$ 24	-52 $\pm$ 9*

HCl-induced acidosis and across the kidneys after 2.3 h acidosis. All three measurements were made in the same animal. In normal fed rats, little uptake or release of amino acids other than glutamine was observed across the hindlimb. There was a small but significant ($P < 0.02$) uptake of citrulline, tryptophan and ornithine. The only amino acid with significant release was glutamine ($P < 0.02$; Table 1). The amino-N balance across hindlimbs, calculated from arteriovenous differences, which were significantly different from zero ($P < 0.02$), was $-66 \mu\text{mol}$ of N/litre of plasma. After 2 h acidosis the balance changed to $-457 \mu\text{mol}$ of N/litre of plasma. Glutamine made up 80% of total N release. This suggests that HCl stimulated protein breakdown in muscle tissue and that amino acid N and probably some carbon skeletons were channeled into glutamine. It can be calculated that the increase in N output could be derived from the breakdown of 3.2 mg of protein/h per 100 g body wt.

The carbon skeleton of glutamine released by muscle may have been derived partly from amino acids (from proteins) and partly from glucose taken up by muscle from the circulation. Kidney takes up increased quantities of glutamine and releases more glucose under acidosis (Goodman *et al.*, 1966; Kida *et al.*, 1978). These findings suggest that a cycling of glucose and glutamine carbon might occur between

muscles and kidneys during acute acidosis. In this cycle muscle would release glutamine, which could be taken up by the kidney for ammonia production. The carbon skeleton of glutamine would be converted into glucose and released into the circulation; glucose taken up by muscle tissue would be converted into glutamine. The net result of the cycle would be a transport of amino N from muscle to kidney.

In the acutely acidotic kidney there was a significant uptake of glutamine, glycine, citrulline and aspartate, and release of serine, asparagine, ornithine and arginine ($P < 0.02$). The amino-N balance was positive ($+269 \mu\text{mol}$ of N/litre of plasma), which was expected because of an increased ammonia production under these conditions. The high extraction ratio (50%) for citrulline is notable.

Arteriovenous differences were measured in plasma and might be an underestimation of the true differences, since erythrocytes are reported to participate in the transport of some amino acids in man (Felig *et al.*, 1973). However, this complication probably does not apply to arteriovenous glutamine differences in rat blood, since Squires *et al.* (1976) reported that arteriovenous glutamine differences across rat kidney were nearly identical when measured in plasma or whole blood.

Blood flow in total rat muscle (7.3 ml/min per 100 g body wt.) and in kidney (3.6 ml/min per 100 g; Goldstein & Boylan, 1978) can be used to estimate total glutamine output and uptake. The extra amount released by muscle was $0.53 \mu\text{mol/min}$ per 100 g and the amount taken up by the kidneys was $0.36 \mu\text{mol/min}$ per 100 g. Thus it appears that muscle supplies the extra glutamine that is taken up by the kidney during acute acidosis.

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