The effects of chronic metabolic acidosis on liver and muscle glutamine metabolism in the dog *in vivo*

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1. Chronic HCl acidosis was induced in dogs. 2. Hepatic extraction of glutamine fell compared with normal animals. 3. Muscle glutamine production was unchanged in acidosis. 4. The results are discussed in relation to inter-organ glutamine metabolism in acidosis *in vivo*.

Increased renal ammoniogenesis from arterial glutamine represents the major renal response to chronic acidosis to allow elimination of excess acid (Pitts, 1964). As arterial glutamine levels do not fall in chronic acidosis (Pitts *et al.*, 1972) and renal glutamine extraction increases, excess glutamine must be generated by another organ to provide adequate substrate. In acute acidosis in the rat, muscle production of glutamine increases (Schrock *et al.*, 1980), whereas in the dog it remains unaltered (Fine, 1981), clearly demonstrating species variation. In acute acidosis in the dog diminished hepatic glutamine extraction occurs (Fine, 1981). In chronic acidosis in the rat a recent report described diminished hepatosplanchnic glutamine extraction, and this was presumed to be due to diminished hepatic extraction, although hepatic arterial and portal-venous blood flows were not measured separately to allow precise quantification of hepatic and gut glutamine extractions (Schrock & Goldstein, 1981). Similar studies in chronically acidotic dogs have now been performed and form the basis of this report.

Methods

Adult mongrel dogs of either sex weighing between 17 and 30 kg were given 10 mmol of 0.15 M-HCl/kg per day for 4 days via a nasogastric tube. This was divided into three equal daily doses. A similar number of dogs were studied as controls. All dogs had free access to water and received similar quantities of dog Purina chow. Food was withheld for 12 h before anaesthesia. Different groups of dogs were used for muscle and hepatosplanchnic experiments. The animals were anaesthetized and ventilated with room air with a rate- and volume-adjustable ventilator (Harvard Pump). The ventilator was adjusted to maintain the same pH as an

arterial blood sample taken within 30 s of induction of anaesthesia. A catheter was placed in the femoral artery and one of the following preparations was made via a laparotomy incision. For muscle experiments the distal aorta immediately proximal to the division into iliac arteries was cleared for placement of an electromagnetic flow probe. A catheter was placed in a femoral vein. For the hepatosplanchnic experiments the portal vein and common hepatic artery were meticulously cleared of surrounding tissue for placement of probes. The gastroduodenal and right gastric arteries, which come off the common hepatic artery distal to the area for probe placement, were ligated so that the probe measured arterial flow going only to the liver. The portal-vein probe was placed between the pancreaticoduodenal vein and the gastrosplenic vein and a sampling catheter was passed into the former and up to the portal hepatic for sampling. A catheter was passed into a hepatic vein via a jugular vein; its position was verified by palpation and its final position was 1–2 cm proximal to wedging. Statham 7518 E.M.F. handle cuff probes were placed on the cleared vessels. Blood-flow readings were obtained on Statham 2202 flowmeters and recorded on a Statham 2200 recorder. Surgical procedures were usually completed within 90 min; 45 min was then allowed before sampling. Arterial blood gases were determined on a Corning pH/blood gas machine 165.

Five simultaneous arterial and venous samples were taken. Whole blood samples were immediately deproteinized with HClO_4 and kept on ice. Whole-blood glutamine was determined in duplicate by glutamate dehydrogenase after hydrolysis of glutamine (Lund, 1974). Blanks (without glutaminase) were performed on each sample. From these values the content or amount per ml of whole blood was calculated assuming that whole blood is

80% water (Bergmeyer *et al.*, 1974). These values were then multiplied by blood flow to give the amount of glutamine produced or extracted by that organ. These methods allow precise quantification of both hepatic and gut extractions.

Calculations

Hepatic extraction = $(PVF \times PV + HAF \times A) - (HBF \times HV)$; hepatosplanchnic extraction = $(A - HV) \times HBF$; gut extraction = $(A - PV) \times PVF$; muscle production = $(FV - A) \times ABF$, where *PV*, *HV*, *A* and *FV* are the contents in portal vein, hepatic vein, artery and femoral vein respectively, *HAF*, *PVF* and *ABF* are the blood flows in hepatic artery, portal vein and lower aorta respectively, and hepatic blood flow (*HBF*) = *PVF* + *HAF*. Flows are given in ml/min per kg and extraction/production as $\mu\text{mol/min per kg}$. In the Tables results are recorded as means $\pm$ S.E.M. Statistical difference was determined by the unpaired *t* test.

Results

Blood pH fell from 7.40 ± 0.01 to 7.24 ± 0.02 ($P < 0.001$) following oral HCl and blood HCO_3^- fell from 18.9 ± 0.9 to 12.6 ± 1.15 mM ($P < 0.005$). Arterial blood glutamine was unaltered in acidosis in both groups of dogs (Tables 1 and 2). Hepatic glutamine extraction decreased markedly in acidosis, and in several dogs small quantities of glutamine were produced by the liver in acidosis. Total hepatosplanchnic glutamine extraction fell by comparable amounts, as there was no significant change in gut glutamine extraction. Hepatic and portal blood flows were unaltered (Table 1). No increase in muscle glutamine release was observed in acidosis in these dogs. Muscle blood flow fell by 35% (Table 2).

Table 1. *Effects of chronic metabolic acidosis on gut and hepatic extraction of glutamine*

Glutamine content of whole blood was calculated as described in the Methods section, and is expressed as mM; blood flows are ml/min per kg and extractions are $\mu\text{mol/min per kg}$. Results are means $\pm$ S.E.M. *Significantly different from control ($P < 0.005$).

	Control (<i>n</i> = 10)	Acidotic (<i>n</i> = 8)
Artery	0.646 ± 0.06	0.644 ± 0.04
Portal vein	0.600 ± 0.06	0.601 ± 0.04
Hepatic vein	0.535 ± 0.06	0.600 ± 0.03
Hepatic blood flow	32.96 ± 2.53	27.23 ± 2.97
Portal blood flow	20.51 ± 1.8	17.0 ± 2.01
Hepatic extraction	2.66 ± 0.51	$0.404 \pm 0.53^*$
Gut extraction	0.871 ± 0.4	0.634 ± 0.24
Hepatosplanchnic extraction	3.58 ± 0.58	$0.99 \pm 0.56^*$

Table 2. *Effects of chronic metabolic acidosis on release of glutamine by muscle*

Units are as for Table 1. Results are means $\pm$ S.E.M.

*Significantly different from control ($P < 0.005$).

	Control (<i>n</i> = 7)	Acidotic (<i>n</i> = 8)
Artery	0.717 ± 0.08	0.616 ± 0.05
Femoral vein	0.770 ± 0.07	0.686 ± 0.04
Blood flow	17.4 ± 1.59	$11.32 \pm 1.05^*$
Production	0.88 ± 0.15	0.94 ± 0.18

Discussion

This study demonstrates an important inter-organ relationship for glutamine metabolism: namely that, in order to provide additional substrate for renal ammoniogenesis in chronic acidosis the liver, which in the normal dog is the major organ for glutamine extraction, reduces this extraction. Similar results have been found in acute acidosis in the dog (Fine, 1981), although in this situation arterial glutamine rose without changes in renal glutamine extraction. Glutamine levels in arterial blood exceed those of any other amino acid, and it is produced or extracted by most of the major internal organs. It is a major carrier of waste nitrogen from muscle (Ruderman & Berger, 1974), a major energy substrate for the gut (Windmueller & Spaeth, 1980) and provides the major source of urinary ammonia (Pitts, 1964). The liver contains metabolic pathways which are operative *in vivo* for both glutamine hydrolysis and glutamine synthesis (Lueck & Miller, 1970), and previous work has provided conflicting evidence as to whether the liver removes or adds glutamine to the circulation *in vivo*. However, this report, combined with two very recent reports (Fine, 1981; Schrock & Goldstein, 1981), clearly demonstrates that the liver removes glutamine from the circulation *in vivo* in both the dog and the rat.

It is clear that there are marked species differences in organ metabolism of glutamine. In the dog the major site for glutamine extraction across the hepatosplanchnic bed is the liver, whereas in the rat the gut accounts for an estimated 75% of this extraction (Schrock & Goldstein, 1981). Schrock & Goldstein (1981) did not measure portal-vein blood flow, so that the present report allows precise quantification of these separate metabolic processes for the first time. In these experiments I have found no significant change in glutamine release by muscle in chronic acidosis in the dog, and have reported similar findings in acute acidosis in the dog (Fine, 1981), whereas in both of these conditions in the rat muscle release of glutamine increases (Schrock & Goldstein, 1981; Schrock *et al.*, 1980). Part of the species differences across muscle may be due to haemodynamic changes occurring in acidosis. In both species, arteriovenous glutamine differences

increase in acidosis, but in the dog the observed reduction in blood flow results in no change in net glutamine release, whereas in the rat muscle blood flow is unchanged. It is unclear therefore whether there is a species difference in relation to muscle metabolism of glutamine in acidosis or whether the observed differences are due to blood-flow changes.

Evidence exists for an effect of acidosis on cellular uptake and metabolism of glutamine. Murine Ehrlich ascites-tumour cells have reduced glutamine uptake at acidic pH (Heinz *et al.*, 1965). A decrease in bicarbonate leads to decreased glutamine extraction by hepatocytes (Baverel & Lund, 1979), and glutamine utilization by the perfused liver in which glutamine synthesis has been inhibited is reduced in acidosis, suggesting an effect on glutamine hydrolysis (Lueck & Miller, 1970). The precise mechanism for the observed changes in hepatic glutamine extraction *in vivo* in acidosis therefore remains unclear.

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