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Liver nitrogen movements during short-term infusion of high levels of ammonia into the mesenteric vein of sheep

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Four 40 kg wethers were used in a crossover design to quantify, by arterio–venous procedures, the mass transfer of NH_3, urea and amino acids (AAs) across the portal-drained viscera and the liver during a 31 min infusion of either 0 (C0) or 1100 (C1100) μmol NH_4HCO_3/min into the mesenteric vein. In C1100, hepatic NH_3 extraction remained stable at 1214 μmol/min (1·90 μmol/min per g wet liver weight), the capacity for hepatic NH_3 removal was exceeded by 654 μmol/min ($P < 0·05$) and the incremental (C1100–C0) urea-N release: NH_3 -N removal ratio increased progressively, from 0·52 to 0·90. The NH_4HCO_3 infusion reduced total branched-chain AA ($P < 0·05$) transfer across the portal-drained viscera and total AA-N ($P = 0·09$) and lysine ($P = 0·02$) extraction by the liver. Hepatic release of glutamate was augmented ($P = 0·03$), ornithine switched from net release to net removal ($P < 0·001$) and net splanchnic release of free essential AA (44 μmol/min (SED 9·2), $P = 0·04$) and branched-chain AA (33 μmol/min (SED 2·0), $P = 0·001$) were reduced to 0·58 of their basal rate. The study showed that conversion of excess NH_3 to urea during a short-term hepatic NH_3 overload required no additional contribution of AA-N to ureagenesis; essential AA and branched-chain AA supply to non-splanchnic tissues was, however, temporarily decreased.

Liver: Ammonia: Ureagenesis: Amino acids: Sheep

Large quantities of NH_3 are formed in the gastrointestinal tract of ruminant animals from the degradation of dietary nitrogenous compounds and the microbial hydrolysis of endogenous urea. Once absorbed across the gut wall, the NH_3 is completely removed from the splanchnic circulation by the liver (Nieto *et al.* 1996) and used for urea and, to a lesser extent, amino acid (AA) synthesis (e.g. glutamate, aspartate and glutamine; Lobley *et al.* 1996).

The role of hepatic ureagenesis in N metabolism is two-fold: prevention of portal NH_3 from reaching the peripheral circulation and disposal of the AA-N excess to body requirements. Urea synthesis requires balanced N inputs from citrulline and aspartate. Citrulline-N originates from mitochondrial NH_3 in a reaction catalysed, sequentially, by carbamoyl-phosphate synthase 1 and ornithine transcarbamylase. The aspartate-N, supplied primarily through transamination with glutamate, arises mainly from two sources: (1) the 2-amino-N of transaminating AAs; or (2) direct synthesis of glutamate from mitochondrial NH_3 and 2-oxoglutarate, via the reaction controlled by glutamate dehydrogenase (Stryer, 1988). NH_3-N can, therefore, provide both N atoms of urea, as demonstrated in cultures of sheep hepatocytes incubated with $^{15}NH_4Cl$ where, regardless of the presence of AA in the incubation medium, [$^{15}N^{15}N$]urea was the predominant labelled species synthesised (Lomax *et al.* 1995; Luo *et al.* 1995). Thus, hepatic detoxification of absorbed NH_3, which can account for up to 0·5–0·65 of dietary N intake (Parker *et al.* 1995), may not need an obligate equimolar N input from AA (Reynolds, 1992; Parker *et al.* 1995).

This view is not supported by results from short-term (2–3 h), trans-hepatic experiments *in vivo*, where the urea-N released by the liver greatly exceeded the additional NH_3 supplied to the liver (Symonds *et al.* 1981; Barej *et al.* 1987; Orzechowsky *et al.* 1988; Wilton *et al.* 1988). If transient, high inputs of NH_3 stimulate AA utilisation for ureagenesis, daily access to feedstuffs with high concentrations of non-protein-N or rapidly degradable protein-N for short periods may reduce AA availability for protein anabolism.

Abbreviations: AA, amino acid; PDV, portal-drained viscera.

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In the current experiment, the hypothesis that a large, short-term increase in portal NH_3 flow augments AA utilisation for urea synthesis was tested by increasing the supply of NH_3 beyond the maximum capacity for hepatic NH_3 by infusion of 1100 μmol NH_4HCO_3/min into the mesenteric vein of fed sheep.

Materials and methods

Animals

Four Suffolk cross-bred male lambs (35–40 kg body weight), surgically prepared with indwelling silicone rubber catheters in the posterior aorta, portal, hepatic and mesenteric veins (Lobley *et al.* 1995), were placed in metabolism crates under continuous lighting conditions. The sheep were adjusted to receiving 770 g DM (grass pellets)/d (10 MJ metabolizable energy/kg DM, 22 g N/kg DM) in hourly portions throughout the experiment and for at least 10 d before the first experimental period, using automated feeders. Water was freely available.

Design

The experiment was arranged as a non-randomised, crossover design with two experimental periods, each of 161 min, separated by a 5–7 d interval. Throughout each experimental period, the sheep were infused via the mesenteric vein with a solution containing 0·1 M sodium *p*-amino hippuric acid (Sigma, St Louis, MO, USA), 0·05 M-sodium phosphate buffer (pH 7·4) and 400 IU heparin/g (Heparin (Mucous); Leo Laboratories Ltd, Princes Risborough, Bucks., UK), at a rate of 20 g/h. Between 60 and 130 min of each experimental period, the sheep were infused with physiological saline (152 mM-NaCl, 167·2 μmol NaCl/min) via the mesenteric vein. From 130 min to the end of each experimental period, the infusion of physiological saline via the mesenteric vein was either maintained (C0) or replaced by a 1 M-NH_4HCO_3, 152 mM-NaCl solution (C1100; 1100 μmol NH_4HCO_3/min plus 167·2 μmol NaCl/min). Two sheep followed the sequence C0–C1100 while the other two sheep followed the reverse sequence. All solutions were sterilised by autoclave or filtration, as appropriate, and infusions were performed with peristaltic pumps.

Samples

Three simultaneous blood samples were continuously collected (Lobley *et al.* 1995) from the aorta, portal and hepatic veins at 7 min intervals (0·3 ml/min per catheter), during the last 21 min of each experimental period, using a peristaltic pump. An additional mixed blood sample (10 ml) was collected at the end of each experimental period for determination of blood and plasma DM.

Immediately following the study (2 weeks), portal catheter patency was lost in two of the sheep. This meant they could not be used for subsequent studies and were therefore killed. As they had been maintained post-experiment on the same diet and intake, the opportunity was taken to examine liver mass. This enabled the absolute measurements obtained to be expressed per unit weight. The sheep were weighed and then killed by an intravenous overdose of thiopental, followed by exsanguination. The liver was rapidly separated from other abdominal organs and surrounding connective and adipose tissue and weighed.

Analyses

Blood samples were mixed and analysed for blood pCO_2, pH, bicarbonate and haemoglobin concentration immediately after collection using a blood gas analyser (Acid Base Laboratory Radiometer ABL3; Radiometer, Copenhagen, Denmark). The packed cell volume was determined by microhaematocrit. One portion (0·5 g) of blood was deproteinised with 5 g TCA (120 g/l) and processed for gravimetric determination of *p*-amino hippuric acid (Lobley *et al.* 1995). The remainder of the blood was centrifuged at 1000 ***g*** for 10 min at 4°C, the plasma (approximately 1·0 ml) collected and processed for determination of NH_3, by an automated procedure (Kone Autoanalyser; Kone, Espoo, Finland) according to Mondzac *et al.* (1965), and urea, by GC–mass spectrometry (Milano *et al.* 2000). The three arterial, portal and hepatic vein plasma samples from each experimental period were pooled in proportion to the plasma flow (pooled sample weight, approximately 0·5 g), and processed for AA analysis in physiological fluids with an Alpha Plus amino acid analyser (Pharmacia-LKB Biochrom Ltd, Cambridge, UK) as described by Lobley *et al.* (1995).

Calculations

Blood, plasma and whole blood water flows and NH_3, urea and AA transfers across the portal-drained viscera (PDV) and the liver were calculated as described by Milano *et al.* (2000). Hepatic NH_3 and urea-N inflow (*I*, μmol/min) and outflow (*O*, μmol/min) were calculated from:

$$I = Fp\,Cp + (Fh - Fp)Ca,$$

and

$$O = Fh\;\;Ch,$$

where *Fp* and *Fh* are the blood (for NH_3) or whole blood water (for urea) flow in the portal vein and hepatic vein (g/min), respectively and *Ca*, *Cp* and *Ch* are the concentrations of metabolites in plasma (NH_3, nmol/g) or plasma water fraction (urea, estimated as plasma urea concentration/(1−plasma DM fraction); nmol/g) in the aorta, portal vein and hepatic vein, respectively.

Statistical analysis

The data were initially analysed by ANOVA, as a crossover design, with animals as blocks and treatment (NH_4HCO_3 administration) and period as factors. The effect of period was significant ($P < 0·05$) only for plasma urea concentration, NH_3, urea and non-NH_3 urea-N transfers across the liver and PDV release of valine. For all other data, this factor was removed and the data re-analysed by ANOVA with animals as blocks and treatment as the sole factor. Analyses were performed using the Genstat 5 (release 3.2)

statistical package (Rothamstead Experimental Station, Herts., UK).

Results

Animals, catheters and liver samples

All animals completed the experiment and the catheters remained patent (i.e. allowed both blood collection and infusion of solutions), with the exception of one arterial catheter. In this animal, an additional polyvinyl chloride catheter was placed into the right ventricle via a jugular vein and, in both experimental periods, mixed venous heart blood was used for all determinations, except blood gas analysis. Comparative analyses of arterial and mixed right ventricle blood, performed later in two other animals, showed no significant differences between sampling sites in the concentration of urea and individual or total AAs (results not shown). Under basal conditions (C0), the concentration of NH_3 in the right ventricle blood was closer to that in the hepatic vein than to that in the artery. In practice, the small contribution of the hepatic artery to the total NH_3 supply to the liver meant that the error introduced was $\leq 0{\cdot}2$ and $\leq 2{\cdot}0$ % total NH_3 supply or removal for C0 and C1100, respectively.

The fresh liver weights of the two sheep killed were 630 and 653 g, with fractional liver weights of 0·015 and 0·017 g/kg body weight, respectively.

Acid–base parameters and blood flow

Bicarbonate concentration increased in the aorta (1·2 mM, $P = 0{\cdot}03$), the hepatic (2·6 mM, $P = 0{\cdot}004$) and the portal vein (2·8 mM, $P = 0{\cdot}03$) during NH_4HCO_3 administration while pCO_2 remain unchanged in all three sampling sites (Table 1). A small elevation in blood pH (0·02 pH units, $P = 0{\cdot}055$) was recorded in the portal vein. The blood flows in the portal and hepatic veins were not altered by the NH_4HCO_3 infusion and averaged 40·6 (SEM 3·6) and 48·4 (SEM 3·5) g/kg body weight per min, respectively. The hepatic artery contributed 16 % of the total liver blood flow.

Plasma urea and ammonia concentration

Average plasma NH_3 concentration increased in the portal ($P = 0{\cdot}004$) and hepatic veins ($P = 0{\cdot}026$) following NH_4HCO_3 infusion (Table 1). In the aorta, NH_3 concentration increased progressively to 406 μM (SEM 125 μM; Fig. 1) by the end of the NH_4HCO_3 infusion. This is approximately 50 % of the value associated with severe NH_3 toxicity in ruminant animals (Symonds *et al.* 1981); but the sheep showed none of the commonly reported clinical signs of NH_3 toxicity (e.g. restlessness, respiratory distress or rapid respiratory movements; Symonds *et al.* 1981). Average plasma urea concentration was 1·0–1·2 mM higher ($P < 0{\cdot}02$) in all three sampling sites during NH_4HCO_3 infusion.

Urea and ammonia transfers

Basal (C0) NH_3 appearance across the PDV represented 0·36 of the apparent total N entering the gastrointestinal tract (i.e. N intake + urea-N uptake by the PDV, 1219 μmol N/min) and 0·57 of the total N apparently absorbed by the gastrointestinal tract (Table 2). This was not significantly

Table 1. Blood flow, pH and blood variables in aorta and splanchnic vessels of four wethers infused with 0 (C0) or 1100 (C1100) μmol NH_4HCO_3/min into the mesenteric vein for 31 min‡§

	C0	C1100	SED
Blood flow (g/min)			
Hepatic artery	294	282	86·0
Portal vein	1543	1465	186·8
Hepatic vein	1837	1746	182·5
pH			
Aorta‖¶	7·45	7·45	0·004
Portal vein	7·37	7·40	0·007*
Hepatic vein	7·38	7·38	0·010
HCO_3^- (mM)			
Aorta‖¶	24·1	25·3	0·11**
Portal vein	23·9	26·7	0·31*
Hepatic vein	24·3	26·8	0·10**
NH_3 (μM)			
Aorta	83	308	79·8†
Portal vein	369	1334	119·7**
Hepatic vein	79	472	95·4*
Urea (mM)			
Aorta‖	4·56	5·58	0·138*
Portal vein‖	4·40	5·46	0·147*
Hepatic vein‖	4·68	5·91	0·158*

† $P < 0{\cdot}1$, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.
‡ For details of procedures, see p. 508.
§ Analysed by ANOVA, with 3 d.f. for the residual mean square (n 8).
‖ Only 2 d.f. for the residual mean square.
¶ n 6.

Table 2. Mass flow of NH_3 and urea to and from the liver and net mass transfer of NH_3, urea, non-NH_3 urea-N (NAUN) and free amino acid-N (μmol/min) across the splanchnic tissues in four wethers infused with 0 (C0) or 1100 (C1100) μmol NH_4HCO_3/min into the mesenteric vein for 31 min‡§‖

(Mean values with their standard errors for four wethers)

	C0	C1100	SED
Portal-drained viscera			
NH_3	435	1504	72·9***
Urea	−202	−182	28·6
Liver			
NH_3			
Inflow	579	2008	160·3**
Outflow	140	794	135·8*
Net transfer¶	−439	−1214	14·8***
Urea			
Inflow	7188	8602	586·1†
Outflow	7588	9279	636·9†
Net transfer¶	401	677	1·5***
NAUN			
Net Transfer¶	362	140	14·6**
Free amino acid-N			
Net transfer	−346	−194	93·4

† $P < 0{\cdot}1$, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
‡ For details of procedures, see p. 508.
§ For net transfers, the positive sign indicates net release, the negative, net uptake.
‖ Analysed by ANOVA, with 3 d.f. for the residual mean square (n 8).
¶ Only 2 d.f. for the residual mean square.

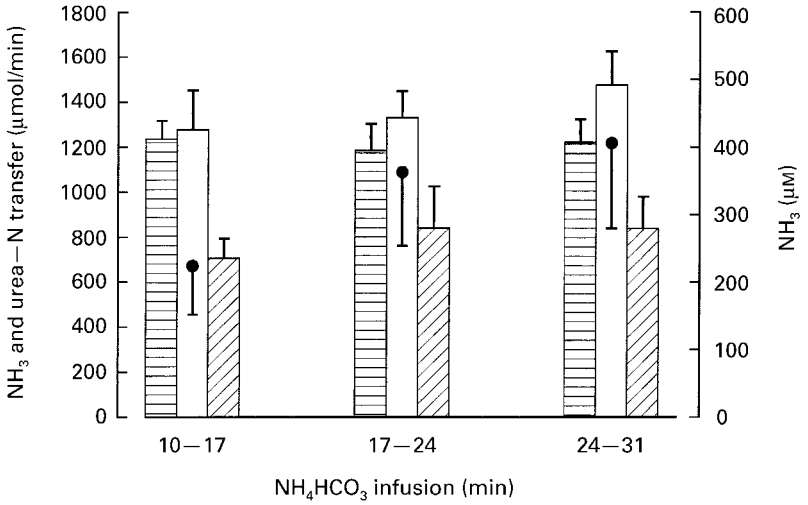

Fig. 1. Temporal changes in arterial ammonia concentration, hepatic ammonia removal, urea-N release and ammonia outflow during a short-term (31 min) infusion of 1100 μmol NH_4HCO_3/min into the mesenteric vein of four wethers. For details of procedures, see p. 508. Values are means with standard deviations shown by vertical bars. ●, Arterial ammonia; ▤, ammonia removal; □, urea-N release; ▨, ammonia outflow.

Table 3. Net transfer of amino acids (μmol/min) across the splanchnic tissues of four wethers infused with 0 (C0) or 1100 (C1100) μmol NH_4HCO_3/min into the mesenteric vein for 31 min‡§‖

	Portal-drained viscera			Liver		
	C0	C1100	SED	C0	C1100	SED
Aspartic acid	3·8	1·9	2·3	−0·1	0·6	0·9
Threonine	19·5	13·3	4·6	−14·0	−9·1	3·0
Serine	20·4	18·9	6·0	−17·5	−14·7	7·5
Asparagine	36·7	23·8	5·4†	−22·1	−20·9	5·3
Glutamic acid	6·0	1·2	3·7	9·8	21·7	3·1*
Glutamine	−4·5	−8·4	12·7	−25·3	−10·5	12·6
Glycine	35·4	31·1	17·8	−43·6	−40·1	12·6
Alanine	44·8	34·0	5·9	−46·4	−32·6	15·1
Citrulline	12·3	14·4	3·3	−9·0	−0·9	3·4†
Valine¶	22·2	6·2	2·2*	−10·2	0·1	6·4
Methionine	6·2	4·3	1·0	−4·9	−2·9	0·9
Isoleucine	20·5	11·6	3·6†	−1·7	0·7	4·5
Leucine	29·0	15·7	4·8†	−3·0	−1·3	3·4
Tyrosine	14·3	8·6	2·9	−10·9	−8·0	3·0
Phenylalanine	17·5	12·0	4·3	−15·2	−14·5	2·3
Ornithine	6·2	6·0	1·9	7·1	−5·4	1·3***
Lysine	17·8	12·7	2·8	−7·7	−2·6	1·1*
1-methylhistidine	−3·6	−1·0	1·6	0·0	4·4	2·7
Histidine	5·8	4·1	1·6	−5·6	−6·0	1·7
3-methylhistidine	−0·5	−0·3	1·2	−0·5	1·5	2·0
Arginine	10·5	6·0	6·4	−12·1	1·4	5·2†
Total AA	324·5	217·3	72·6	−232·6	−145·0	65·4
Essential AA	138·5	79·9	23·8†	−62·4	−35·5	15·8
Branched-chain AA	71·7	33·5	11·4*	−14·9	−0·5	11·2
Non-essential AA	167·6	117·0	47·1	−168·3	−103·1	51·0
Glucogenic AA	96·1	75·6	34·7	−132·9	−98·0	43·3

AA, amino acids.
† $P < 0·1$, * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
‡ For details of procedures, see p. 508.
§ The positive sign indicates net release, the negative, net uptake.
‖ Analysed by ANOVA, with 3 d.f. for the residual mean square (n 8).
¶ Only 2 d.f. for the residual mean square.

different from urea-N removal by the PDV (435 *v.* 404 μmol/N min; $P > 0{\cdot}2$). PDV transfer of NH_3, but not urea, increased by 1069 μmol/min ($P < 0{\cdot}001$) during the NH_4HCO_3 infusion, similar to the average infusion rate (1180 μmol N/min), indicating that the basal rate of NH_3 appearance across the PDV was not altered by the infusion.

Liver inflow, outflow and removal of NH_3 were all increased by the NH_4HCO_3 infusion. The average NH_3 input to the liver in C1100 exceeded the maximum capacity for hepatic removal of NH_3 by 654 μmol/min, so that NH_3 exited the liver at a rate 5·7 times higher than that measured in C0. Based on an average liver weight of 642 g, the hepatic NH_3 removal during NH_4HCO_3 infusion was 1·90 μmol/min per g wet liver weight (see Table 2).

Hepatic urea-N release was 1·9 times higher than NH_3 uptake under basal (C0) conditions and increased to 1354 μmol/min in C1100 ($P < 0{\cdot}001$), sufficient to account for hepatic NH_3 removal. When the data were compared on an incremental basis (i.e. C1100−C0), urea-N release was on average only 0·71 of the NH_3 removal, resulting in an apparent net decrease in the calculated non-NH_3-N appearing as urea-N ($P = 0{\cdot}004$; Table 2). The time course of NH_3 and urea transfers during NH_4HCO_3 infusion (Fig. 1) showed, however, that while hepatic NH_3 extraction remained unaltered for the 21 min sampling period (slope −1·06 μmol/min per min (SE of the slope 4·879), $P = 0{\cdot}83$, residual mean square 9333·14), urea-N release by the liver increased progressively from 1278 to 1474 μmol/min (slope 14·06 μmol/min per min (SE of the slope 7·22), $P = 0{\cdot}07$, residual mean square 20437·6). Thus, the additional urea-N release: additional NH_3-N removal ratio increased from 0·52 to 0·90 over the same period.

Plasma amino acid concentrations

Arterial, portal and hepatic vein plasma concentrations of free total AA (aorta 2625 μM (SEM 99·5)), essential AA (aorta 986 μM (SEM 53·0)), non-essential AA (aorta 1351 μM (SEM 42·6)), branched-chain AA (aorta 566 μM (SEM 36·5)) and glucogenic AA (aorta 927 μM (SEM 34·8)) were not altered by the NH_4HCO_3 infusion. Glutamine concentration was higher in the aorta (222 *v.* 308 μM (SED 18·6), $P = 0{\cdot}02$), the portal (221 *v.* 302 μM (SED 11·8), $P = 0{\cdot}01$) and the hepatic (199 *v.* 292 μM (SED 17·3), $P = 0{\cdot}02$) veins and a similar, though smaller, response was apparent for aspartate (aorta 7 *v.* 10 μM (SED 0·8), $P < 0{\cdot}05$). In contrast, ornithine concentration was consistently decreased at all sites (aorta 102 *v.* 70 μM (SED 7·3), $P = 0{\cdot}02$; portal vein 108 *v.* 76 μM (SED 8·0), $P = 0{\cdot}03$; hepatic vein 112 *v.* 71 μM (SED 8·6), $P = 0{\cdot}02$) in C1100. Lower concentrations were also observed for alanine (200 *v.* 173 μM (SED 7·4), $P = 0{\cdot}04$), tyrosine (104 *v.* 98 μM (SED 2·0), $P = 0{\cdot}05$) and phenylalanine in the portal vein (86 *v.* 74 μM (SED 3·1), $P = 0{\cdot}03$), with similar trends for threonine in both the aorta (86 *v.* 74 μM (SED 3·1), $P = 0{\cdot}08$) and the portal vein (194 *v.* 168 μM (SED 9·3), $P = 0{\cdot}07$) and alanine in the aorta (158 *v.* 140 μM (SED 7·9), $P = 0{\cdot}1$).

Amino acid transfers

With the exception of glutamine, which was removed by the gastrointestinal tissues, a net absorption of all other AAs was measured across the PDV in C0, with essential AA representing 0·43 of total AA transfers. The net release of free total AA, non-essential AA and glucogenic AA across the PDV was not modified by NH_4HCO_3 infusion. The reduction in net PDV release of valine ($P = 0{\cdot}018$) and the trend for decreased net PDV release of isoleucine ($P = 0{\cdot}09$) and leucine ($P = 0{\cdot}07$), resulted in significantly reduced total branched-chain AA transfers ($P = 0{\cdot}04$) and in a trend for lower free essential AA transfers across the PDV ($P = 0{\cdot}09$) in C1100 (see Table 3).

Under basal conditions, the liver removed 0·71, 0·45, 0·21 and all of the free total AA, essential AA, branched-chain AA and non-essential AA respectively, absorbed across the PDV. The free glucogenic AA were extracted at a rate 1·4 times greater than the PDV appearance, while net hepatic release was observed for glutamate and ornithine. The NH_4HCO_3 infusion reduced total AA-N extraction by 152 μmol/min, a decrease similar to the average reduction in non-NH_3 urea-N release (222 μmol/min (SED 63), $P = 0{\cdot}59$; Table 2). Glutamate release ($P = 0{\cdot}03$) was significantly augmented, ornithine switched from net release to net removal ($P < 0{\cdot}001$) and there was a reduction in lysine extraction ($P = 0{\cdot}02$; Table 3).

Splanchnic bed release of valine (12 *v.* 6 μmol/min (SED 22·4), $P = 0{\cdot}09$), isoleucine (19 *v.* 12 μmol/min (SED 2·3), $P = 0{\cdot}07$), leucine (26 *v.* 14 μmol/min (SED 1·4), $P < 0{\cdot}01$), and tyrosine (4 *v.* 1 μmol/min (SED 0·7), $P = 0{\cdot}03$) were reduced during NH_4HCO_3 infusion. This meant that the net appearance of free branched-chain AA (57 *v.* 33 μmol/min (SED 2·0), $P = 0{\cdot}001$) and essential AA (76 *v.* 44 μmol/min (SED 9·2), $P = 0{\cdot}04$) across the splanchnic tissues during C1100 dropped to 0·58 of that under basal conditions. In addition, the splanchnic tissues released more citrulline (3 *v.* 14 μmol/min (SED 1·7), $P = 0{\cdot}01$) and arginine (−2 *v.* 7 μmol/min (SED 2·4), $P = 0{\cdot}04$), but almost no ornithine (13 *v.* 1 μmol/min (SED 1·3), $P < 0{\cdot}01$), to the peripheral circulation.

Discussion

Short-term adaptation of glutamate dehydrogenase activity v. amino acid degradation: how to balance citrulline synthesis in the ornithine cycle

Experiments with $^{15}NH_4Cl$ *in vitro* (Lomax *et al.* 1995; Luo *et al.* 1995) and *in vivo* (Milano *et al.* 1995) have offered unequivocal evidence that NH_3-N can provide both N atoms of urea during hepatic ureagenesis in sheep. Moreover, in recent studies in sheep with chronic (4 d) infusion of NH_4HCO_3 into the mesenteric vein, all additional NH_3-N removed by the liver was converted into urea without the need for substantial inputs of AA-N (i.e. the additional urea-N released: additional NH_3-N removed ratio by the liver ranged from 0·9 to 1·1; Lobley *et al.* 1996; Milano *et al.* 2000). Overall, these observations indicate that under conditions of sustained, high portal NH_3 flows, hepatic glutamate dehydrogenase activity and related pathways that supply 2-oxoglutarate and NADH can adjust to allow partition of the extra portal NH_3 removed equally between glutamate dehydrogenase and carbamoyl-phosphate

synthase 1–ornithine transcarbamylase routes, without compromising amino acid-N to balance N inputs to the urea cycle.

In earlier studies, however, where ammonium salts were infused for 2 or 3 h into the mesenteric vein of sheep (Barej *et al.* 1987; Orzechowsky *et al.* 1988) and cattle (Symonds *et al.* 1981; Wilton *et al.* 1988) the additional urea-N released by the liver exceeded the additional NH_3 removed by more than two-fold. Thus, in the short term, stimulation of glutamate production from NH_3 and oxo-glutarate through glutamate dehydrogenase appeared to be limited, and additional aspartate-N from catabolic deamination of AAs was needed to balance NH_3 flow through the carbamoyl-phosphate synthase 1–ornithine transcarbamylase route.

The current observations challenge this view. Although the maximum hepatic capacity for NH_3 removal was exceeded by over 650 μmol/min during the NH_4HCO_3 infusion, with hyperammonaemia established within 10 min, there was no apparent need for additional AA-N inputs to maintain NH_3-stimulated ureagenesis. Moreover, rather than the over-production of urea-N relative to NH_3 extraction reported by others, here the average under-conversion of 222 μmol N/min to urea ($P = 0{\cdot}004$) and the reduction in lysine removal ($-5{\cdot}1$ μmol/min, $P = 0{\cdot}02$) provide support for the concept that short-term inhibition of AA oxidation may occur during NH_3 overload. Interestingly, this is contrary to the proposal that NH_3 detoxification leads to elevated catabolism of AAs (Reynolds, 1992; Parker *et al.* 1995). The reduction in non-NH_3 urea-N release by the liver could also indicate that the unaccounted N may be used for synthesis of AAs. Studies *in vitro* and *in vivo* have shown that rapid and substantial transfer of ^{15}N from $^{15}NH_4^+$ to AAs can occur within the liver (Geissler *et al.* 1992; Luo *et al.* 1995). In addition, incubation of hepatocytes or liver perfusion with NH_4Cl resulted in intracellular accumulation of citrulline, aspartate, glutamate, glycine, serine and alanine (Demigne *et al.* 1991; Nissim *et al.* 1992) or increased release of glutamate, citrulline and alanine (Nissim *et al.* 1992; Luo *et al.* 1995; Brosnan *et al.* 1996). The elevated net release of glutamate and lowered net removal of citrulline across the liver in the current study provide confirmatory evidence *in vivo* of the potential importance of these mechanisms in reponse to acute NH_3 administration.

Care was taken in the present study to avoid stimulation of AA catabolism associated with acidosis, as encountered with use of NH_4Cl (Reaich *et al.* 1992; Lobley *et al.* 1995); this precaution may account for the differences observed between the current study and earlier studies conducted by Barej *et al.* (1987) and Orzechowsky *et al.* (1988).

Time course events

The stability of hepatic NH_3 utilisation together with the progressive increase in urea-N synthesis observed during maximal stimulation with NH_3 have two important consequences. First, the convergence of incremental NH_3 removal and urea-N release towards the end of the infusion minimised the apparent reduction in non-NH_3 urea-N release, suggesting that any conservation of AAs is only a transient response. Second, any factors that initially limited N flux through the ornithine cycle must have been subjected to short-term control.

The activity of carbamoyl-phosphate synthase 1, the rate-limiting enzyme of the cycle and known to be insensitive to product inhibition by carbamoyl phosphate (Meijer *et al.* 1990), was probably not altered, because that would have increased hepatic NH_3 removal over the infusion period. Under the experimental conditions created by the infusion (i.e. steady NH_3 removal and increase in average hepatic glutamate release), however, stimulation of carbamoyl-phosphate synthase 1 activity may have occurred through a progressive transfer of NH_3-N from glutamate to carbamoyl phosphate synthesis.

Ornithine availability has been shown to limit rates of urea synthesis in isolated hepatocytes (Lund & Wiggins, 1986). In the present experiment, the marked shift from release to uptake recorded for hepatic ornithine transfers ($7{\cdot}1$ *v.* $-5{\cdot}4$ μmol/min, $P < 0{\cdot}001$), and the depletion of ornithine plasma concentrations ($P < 0{\cdot}05$) indicate that the liver demand for this metabolite increased substantially during NH_4HCO_3 infusion. Whether hepatic ornithine concentration rose in parallel to these transfers is unclear, as there was also reduced net uptake of two products, citrulline and arginine.

Amino acid metabolism

Time course analysis highlighted the importance of hepatic NH_3 removal for maintaining NH_3 homeostasis in ruminant animals. The two major mechanisms for NH_3 detoxication, ureagenesis and glutamine synthesis, are highly compartmentalised in the liver acinus, the ornithine cycle enzymes being expressed in periportal hepatocytes and glutamine synthetase in perivenous cells (Haussinger *et al.* 1992; Rossouw *et al.* 1999). Thus, any overflow of NH_3 that escapes periportal ureagenesis stimulates perivenous glutamine synthesis. In the current study, however, although ureagenic capacity was exceeded, the liver remained a net consumer (albeit at a lesser rate) of glutamine during NH_3 overload. Nonetheless, plasma concentrations of glutamine were substantially increased during NH_3 infusion, suggesting that glutamine synthesis may be stimulated in other tissues. Indeed, Leweling *et al.* (1996) reported an increase in plasma and muscle glutamine concentrations during hyperammonaemia in rats. This was accompanied by a decline in glutamate, alanine and branched-chain AA concentrations in both compartments. The authors hypothesised that enhanced branched-chain AA degradation occurred to restore the intramuscular glutamate pools, depleted during the synthesis of glutamine induced by hyperammonaemia. Although arterial plasma alanine and glutamate concentrations tended to decrease during the infusion of NH_4HCO_3 in the current study, there were no changes in arterial branched-chain AA concentrations. Instead, branched-chain AA utilisation by the PDV was enhanced, suggesting mechanisms in addition to muscle degradation of branched-chain AA to provide glutamate C or N may contribute to lower arterial branched-chain AA concentrations during hepatic NH_3 overload.

Concluding remarks

The results of this experiment indicate that it is unlikely that, in the short term, maximal rates of liver NH_3 removal will stimulate AA utilisation to balance NH_3 incorporation to urea synthesis. Moreover, maximal rates of NH_3 removal were reached at least 20 min earlier than approximately equivalent rates of urea synthesis, with a significant increase in hepatic removal of ornithine and decrease in plasma ornithine concentration. Thus, in sheep, short-term adaptation of hepatic urea synthesis to NH_3 overload *in vivo* might be controlled by ornithine availability to liver cells. NH_3 supply in excess to liver removal resulted in higher arterial concentrations of NH_3 and glutamine but did not alter glutamine release by the liver. Instead, hepatic release of glutamate increased and there was a marked reduction in liver removal of lysine and splanchnic release of tyrosine and branched-chain AA.

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