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Splanchnic net balance of oxygen and metabolites in response to mesenteric vein infusion of ammonia in sheep*

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ABSTRACT

In order to simulate daily episodes of high ammonia (NH_4^+) absorption associated with the intake of diets with high content of non-protein N or rapidly rumen degradable protein, four wethers (42±3.4 kg BW), surgically fitted with indwelling catheters in the posterior aorta and splanchnic veins, were infused with 340 μmol $NH_4^+HCO_3^-$/min into the mesenteric vein for 3 h, over 7 consecutive days. On the 7th day, net mass transfers of NH_4^+, urea, glucose, lactate, ß-OH-butyrate and oxygen were measured across portal-drained viscera (PDV), liver and splanchnic tissues during the last 90 min of the NH_4^+ infusion (NH_4^+ treatment, AT). Measurements were repeated on the following day, after withdrawal of the NH_4^+ infusion (Control treatment, CT). NH_4^+ infusion increased liver NH_4^+ uptake (+396 μmol/min; sed, 72; P=0.04) and urea production (+152 μmol/min; sed, 55; P=0.14), and oxygen consumption by the liver (+151 μmol/min; sed, 6; P=0.002), the PDV (+224 μmol/min; sed, 56; P=0.03) and the splanchnic tissues (+352 μmol/min; sed, 57; P=0.009). Net mass transfers of glucose, lactate and ß-OH-butyrate across the PDV and the liver, and the acid-base status of the animals were unchanged.

KEY WORDS: ammonia, urea, glucose, liver, energy expenditure, sheep

INTRODUCTION

In ruminants, a high proportion of the dietary N is absorbed as NH_4^+. The liver removes all the NH_4^+ absorbed and then converts it to urea. NH_4^+ metabolism could alter both liver energy expenditure and glucose production, but there is a controversy about the intensity and the sign (in the latter case) of these effects. In addition, it is unknown whether liver metabolism of other energy metabolites could also be altered. The objective of the present experiment was to examine the response of hepatic,

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portal-drained viscera (PDV) and splanchnic net mass transfers of NH_4^+, glucose, urea, lactate, ß-OH-butyrate and oxygen to a 3-h NH_4^+ infusion into the mesenteric vein, administered during 7 d in order to simulate daily episodes of high NH_4^+ absorption associated with the intake of diets with high concentration of non-protein N or rapidly rumen degradable protein.

MATERIAL AND METHODS

Animals, diet and design

Four Corriedale wethers (42±3.4 kg BW), surgically prepared with indwelling catheters in the posterior aorta, portal, hepatic and mesenteric veins, were housed in individual pens (2.6 m^2) and fed 450 g/d of lucerne hay plus 360 g/d of cracked maize (8.1 MJ ME/d; 16.0 g N/d) in 4 equal portions, at 0:30, 6:30, 12:30 and 18:00 h, during 4 weeks, using automatic feeders. Water was offered *ad libitum*. Throughout the last week, at 8:30 h, a solution of 900 mM $NH_4^+HCO_3^-$ was infused into the mesenteric vein for 3 h (340 µmol NH_4^+/min; 8.1 µmol NH_4^+/kgBW/min). On the 7th day, a solution containing 0.1M Na *p*-aminohippuric acid (*p*AH), 0.05 M Na phosphate buffer (pH 7.4) and 460 UI heparin/g (APH solution) was also infused into the mesenteric vein (0.36 g/min) during the last 135 min of the $NH_4^+HCO_3^-$ infusion, in order to determine the portal and hepatic blood flow (NH_4^+ treatment, AT). On the next day, the $NH_4^+HCO_3^-$ infusion was withdrawn and only the APH solution was infused at identical times (Control treatment, CT).

Samples and analysis

In both treatments, 45 min after the start of the infusion of the APH solution, two simultaneous blood samples were continuously withdrawn from the aorta and the portal and hepatic veins over 90 min (0.17 ml/min). The collection lines were allowed to pass through ice-cold water and the blood samples were collected directly into 10 ml syringes stored in ice-cold water. Samples were carefully mixed and analysed for blood pO_2, pCO_2 and pH immediately after collection, using a Blood Gas Analyser. The packed cell volume, blood haemoglobin and *p*AH concentration were analysed as described by Milano et al. (2000). A portion of blood was centrifuged (1000 *g*; 4ºC), and the plasma was processed for determination of NH_4^+, urea and glucose (Milano et al., 2000), lactate (Lactate, Randox®) and ß-OH-butyrate (Ranbut, Randox®). Infusions and blood collections were performed with peristaltic pumps.

Calculations and statistics

Blood flow and net mass transfers of metabolites and oxygen across the liver, the PDV and the splanchnic tissues were calculated as described by Milano et al. (2000). The data were analysed by ANOVA, with animals and treatment as main factors.

RESULTS AND DISCUSSION

The results are shown in Table 1. PDV and liver data represent the average of 3 data points per treatment because the portal vein catheter lost patency in one of the animals.

Table 1. Mass transfers of NH_4^+, glucose, urea, lactate, ß-OH-butyrate and oxygen (µmol/min) across the portal-drained viscera (PDV), the liver and the splanchnic tissues, and arterial base excess (mM) and pH in response to a 3-h infusion of 340 µmol $NH_4^+HCO_3^-$/min into the mesenteric vein, administered for 7 d, in wethers

	CT	AT	Sed	P
PDV				
NH_4^+	214	650	44	0.013
glucose	178	51	41	0.11
urea	-61	63	194	NS
lactate	81	95	29	NS
ß-OH-butyrate	46	143	81	NS
oxygen	-1110	-1334	56	0.029
Liver				
NH_4^+	-260	-656	72	0.04
glucose	199	210	9	NS
urea	289	441	55	0.14
lactate	-95	-88	11	NS
ß-OH-butyrate	71	72	6	NS
oxygen	-1188	-1339	6	0.002
Splanchnic tissues				
oxygen	-2608	-2960	57	0.009
Arterial base excess	4.17	5.52	0.42	0.11
Arterial pH	7.498	7.483	0.017	NS

n=6, except for splanchnic tissues (n=8). Positive and negative values indicate net production and net uptake of the metabolite across the relevant organ, respectively

NH_4^+

In both treatments, liver NH_4^+ uptake was higher than PDV NH_4^+ absorption. The difference between treatments in liver NH_4^+ uptake (396 µmol/min; P=0.04) was similar to the mean NH_4^+ infusion rate (340 µmol/min).

Glucose

There was a trend for lower absorption or higher utilization of glucose by the PDV in AT (130 µmol/min; P=0.11). Hepatic glucose production was not significantly different between treatments. Blood BE and pH were not altered by $NH_4^+HCO_3^-$ infusion in the current experiment, suggesting that the enhanced glucose production observed in previous experiments with sheep during $NH_4^+Cl^-$ infusion into the mesenteric vein (30 µmol $NH_4^+Cl^-$/kg BW/min; Barej et al.,

1987; 6.7 μmol $NH_4^+Cl^-$/kgBW/min; Milano et al., 2001), might have been linked to a $NH_4^+Cl^-$-induced metabolic acidosis.

Urea, lactate and ß-OH-butyrate

The differences between treatments in mass transfers of urea, lactate and ß-OH-butyrate across the PDV were not significant. There was a trend for a higher hepatic urea production in AT (+303 μmol urea-N/min; P = 0.14). This increase represents a recovery of 77% of the N infused. There were no differences between treatments in liver lactate uptake or ß-OH-butyrate production.

Oxygen

PDV, liver and splanchnic oxygen consumption was significantly higher in AT (224, 151 and 352 μmol/min, respectively). Liver oxygen consumption increased by 0.39 mol/mol NH_4^+ removed or 0.49 mol/mol urea-N produced, these ratios being slightly higher than the stoichiometric ratios accepted for urea synthesis (0.33 mol/ urea-N; Stryer, 1988). Milano et al. (2000) found that the liver oxygen consumption increased by 0.69 mol/mol NH_4^+ removed or 0.61 mol/mol urea-N produced (P=0.13) during continuous infusion of 400 μmol/min of NH_4^+ HCO_3^- into the mesenteric vein of wethers for 4 days. When extrapolated to the 180 min of the NH_4^+ infusion, the increment in splanchnic oxygen consumption observed in the current experiment amounts to 63.4 mmoles or 29 KJ (0.352 mmol/min × 180 min × 460 KJ/mmol O_2), i.e. the equivalent of 1% of the daily ME intake destined to deposition (based on a daily EM intake of 8.2 MJ and 5.3 MJ ME/d of maintenance energy requirements).

CONCLUSIONS

The 3 h infusion of $NH_4^+HCO_3^-$ for 7 d, increased NH_4^+ liver uptake, with a consequently higher urea production, and oxygen consumption by the liver, the PDV and the splanchnic tissues. No changes were observed in hepatic net mass transfers of glucose, lactate and ß-OH-butyrate or in the acid-base status of the animals.

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