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Ovine Hepatic Metabolism. II. The Effect of Osmolarity and Ph of the Perfusion Medium on Hepatic Uptake of Ammonia and Nitrogen Excretion

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Abstract: The present investigation was carried out to assess the influence of changing perfusion medium osmolarity and pH with or without additional amino acid on the hepatic uptake of ammonia and production of urea and glutamine. To elucidate this effect, the caudal liver lobes of twenty Merino sheep (24-35 kg) were perfused using a non-reticulating perfusion model for 3-4 h using Krebs-Henseliet buffer solution containing 2.1 mM lactate, 0.3 mM pyruvate, 10 IU heparin m^{-1} at 37°C and gassed with O_2-CO_2 (95:5 v/v). An increase of perfusion osmolarity from 300 mosmol L^{-1} to 330 mosmol L^{-1} resulted in an increase in hepatic uptake of ammonia from 1200-nmol g^{-1} min^{-1} to 1900 nmol g min^{-1}, thus assisting the hepatic clearance of ammonia. Similarly, hepatic glutamine production rosed from 48 to 177 nmol g^{-1} min^{-1}. As the influent pH rose, a decline in urea and glutamine production was observed. The additions of amino acids favour glutamine hepatic uptake rather than production.

Key words: Liver, osmolarity, perfusion, pH, sheep

INTRODUCTION

The ruminant liver must balance the nitrogen requirements for the entire animal. The rate of gluconeogenesis in the ruminant liver is greatest after a meal and declines during fasting, almost the mirror image of the situation in monogastric animals[1]. In the rat liver, the ammonia is partitioned equally between urea synthesis and glutamine synthesis[2]. The zonation of N-metabolising pathways in sheep liver have been described and studied using the liver perfusion technique[3].

The hepatic metabolism in the rat may be manipulated by changing the portal blood osmolarity and pH. This may also be induced through nutrient uptake[4]. The isolated, perfused rat liver has been widely used to examine the function of the liver *in vivo*. The role of the urea or glutamine cycle in the liver of the rat and that of cellular swelling in controlling the energy metabolism within the cell has been derived using perfusion models according to Häussinger[2]. An improved technique for the perfusion of liver was developed by Ali *et al.*[5]. Both the hepatic structure and cell polarity are preserved and the technique allows the composition of the medium to remain constant. Better knowledge of how hepatic metabolism is controlled will lead to a better understanding of intermediary metabolism. Therefore, the present study was designed to investigate the effect of the pH and osmolarity of the perfusion medium with and without an additional amino acid load on the uptake of ammonia and the output of urea and glutamine using this technique.

MATERIALS AND METHODS

Animals and diet: Twenty South African Mutton Merino withers (24-35 kg live weight) were used in this experiment. They were fed a (1:1) mixed diet of lucerne (*Medicago sativa) and* tef *(Eragrostis tef)* hays, mixed 1:1, supplied in equal meals of 600 g daily at 08.00 and 15.00 h. Water was provided *ad libitum*.

Experimental procedures: The anaesthesia, surgical procedures, Canulation, infusion and canulation are outlined by Ali *et al.*[5]. A Krebs-Henseleit medium plus 2.1 mM lactate, 0.3 mM pyruvate and 10 U heparin ml^{-1} at 37°C, gassed with O_2-CO_2 (95:5, v/v) was used. Additional amino acids were infused at concentrations found in ovine portal blood[6]. The pH was adjusted by changing the carbonate: bicarbonate ratio. The osmotic pressure of the buffer was raised from 300 to 330 mOsm L^{-1} by the addition of sucrose.

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Chemical Analysis: The concentrations of ammonia, urea and glutamine in effluent and influent samples were determined using diagnostic kits (Boehringer Mannheim, Germany) based on the methods described by Bergmeyer and Beutler[7,8,9], Fawcet and Scott[8][9], . The pH of the perfusion medium was measured using pH-metre.

Statistical analysis: All studies were performed using at least three or more separate perfusion experiments. The data was statistically analysed using Student's *t* test or an analysis of variance (ANOVA); differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Figure 1 illustrates the effect of perfusion medium osmolarity on the hepatic uptake of ammonia. The capacity of the liver to take up ammonia appears to be saturated at 1200 nmol g^{-1} min^{-1} when the influent buffer osmolarity is 300 mOsm L^{-1}. However, at an osmolarity of 330 mOsm L^{-1}, the maximum uptake increased to 1900 nmol g^{-1} min^{-1}. The excretion of hepatic nitrogen (glutamine or urea) is related to the osmolarity of the perfusion medium (Fig. 2). The hepatic endogenous urea production was 120-nmol g^{-1} min^{-1}, while the exogenous urea production increased linearly with the addition of ammonia. The increase in buffer osmolarity resulted in the suppression of the former and the increase in the latter. No signs of urea saturation were observed.

The endogenously produced urea was 120-nmol g^{-1} min^{-1}. However, exogenous urea produced increased linearly upon the addition of ammonia, with no indication of saturation, even at the highest level of ammonia. As the buffer osmolarity increased, the endogenous urea production was suppressed and the exogenous production increased by 50% over the basal level (highest ammonia concentration (1mM) osmolarity of 300 mOsm L^{-1}). With the shift in osmolarity to 330 mOsm L^{-1}, a further increase to 370% occurred (48 to 177 nmol g^{-1} min^{-1}).

The effects of perfusion medium pH on the basal nitrogen excretion are shown in Table 1. Whether or not the presence of amino acids in the perfusion medium affects nitrogen excretion was addressed. Increasing the influent pH reduces the hepatic glutamine and urea output and upon the addition of amino acids, the trends of increase become even clear.

Table 1: Effect of pH on nitrogen excretion with or without additional amino acids

Treatment	pH	Net Glutamine	Urea
		nmole g^{-1} min^{-1}	
Minus amino acid	7	109.88	307.85
Plus amino acid	7	–5.15	1090
Minus amino acid	7.4	37.41	343.84
Plus amino acid	7.4	3.08	955.08
Minus amino acid	7.8	41.07	303.25
Plus amino acid	7.8	-136.3	1057.04

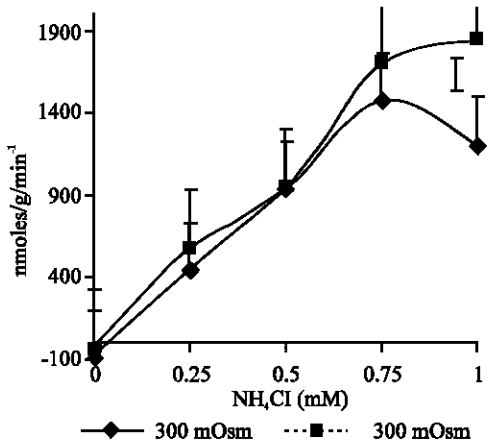

Fig. 1: Effect of Osmolarity on Hepatic Uptake of Ammonia

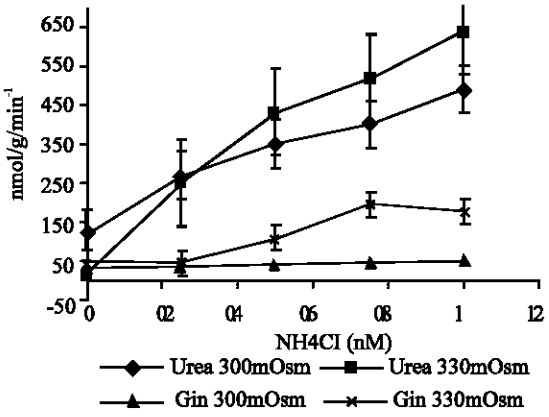

Fig. 2: Effect of Osmolarity on hepatic nitrogen excretion

Increasing the pH of the medium reduces the combined output of urea and glutamine production rates. Increasing the nitrogen 3 fold led to a further increase in production rates. The nitrogen excretion at pH 7.8 is entirely in the form of urea. The liver takes up rather than produce glutamine.

DISCUSSION

According to their habitat, mammals have evolved sophisticated behavioural and physiological responses to oppose changes in the osmolarity of their extracellular

fluid[10]. The changes that occur in the osmolarity of the portal blood during maximum absorption provide a simple, infallible signal to the liver, which may then modify the rates of uptake of nutrients and repartition the flow of excreted nitrogenous products. The current study to investigate the role of perfusate osmolarity and pH on the control of ovine hepatic metabolism follows the model described by Ali *et al.*[5].

Glutamine is the most abundant free amino acid in the body fluids. It is a conditionally essential amino acid acting as nitrogen shuttle between organs and is a major product of ammonia trapping in the liver. Under normal conditions, the liver is rather a glutamine consuming than glutamine-releasing tissue. The liver shows glutamine output in metabolic acidosis, prolonged starvation and animals bearing tumour, but net glutamine uptake in the postoperative state, on consuming high protein diets and in uncontrolled sepsis or diabetes[11].

Häussinger *et al.*[12] indicated that hypertonic perfusion media in rat liver switched the glutamine balance in the liver from net release to net uptake. The conversion of excess NH_3 to urea during a short-term hepatic NH_3 overload requires no additional contribution of AA-N to ureagenesis. Essential AA and branched-chain AA supply to non-splanchnic tissue was, however, temporarily decreased[6].

At an osmolarity of 300 mOsm L^{-1}, the capacity of the liver to take up ammonia is similar to but somewhat more than, that reported *in vivo* in sheep (700 to 1000 nmoles min^{-1} g^{-1};[13]. However, when the osmolarity of the buffer was increased to 330 mOsm L^{-1}, then the maximum rate increased significantly to almost 1900 nmol g^{-1} min^{-1}. The raised osmolarity of portal blood results from the absorption of nutrients at peak fermentation, precisely when spillover of ammonia from the rumen may occur. This implies that the raised osmolarity at this time will assist the liver in clearing the ammonia from the circulation. The formation of glutamate from ammonia may serve as a scavenger for an excess amount of ammonia as it is toxic[3].

This study indicated greater synthesis and release of urea using a perfusate containing an amino acid mixture when compared with a perfusate containing only glutamine and ammonia. When no ammonia was added to the buffer, significant amounts were detected in the effluent from the perfused lobe. Lowering of the osmolarity of the influent in rat liver perfusate from 305 to 225 mmol L^{-1} led to 60% reduction in urea synthesis[4].

In this study the hepatic production of urea reached 120-nmol g^{-1} min^{-1}, even when no exogenous ammonia is added to the buffer. The rate increases linearly with the addition of ammonia to the influent buffer. Raising the osmolarity of the buffer suppresses the endogenous production of urea, but then increases the exogenous urea production thereafter, with no signs of saturation. While the rate of glutamine production is considerably lower than that of urea, it followed a similar pattern to that of ammonia as indicated earlier[14,5]. At normal osmolarity (300 mOsm L^{-1}), the rate increased 50% above the basal rate of 34 nmol g^{-1} min^{-1} at the highest ammonia concentration tested (1 mM). However, when the osmolarity increased to 330 Osm/l, then the glutamine production rate increased more dramatically, rising from 48 to 177 nmol g^{-1} min^{-1}, showing once again the effect of peak-absorptive osmolarity changes on the ability of the liver to handle the extra portal ammonia.

Factors affecting the hepatic metabolism in the rat include changes in blood pH, as well as the osmotic pressure of portal blood induced by the uptake of nutrients from the gastrointestinal tract[4]. As the influent pH rises the output of hepatic glutamine and urea decreases, largely due to a decline in the glutamine production rate. This trend becomes even clearer when additional nitrogen is provided in the form of amino acids (3x basal values), where the excretion at pH 7.8 is entirely in the form of urea and the liver takes up (rather than producing) additional glutamine. This perfusion model can be used to identify amino acids that may be anabolic signals, as not all amino acids contribute to urea synthesis equally. At pH of 7, the ratio of urea: glutamine production was 4:1. In rat liver, however, the ratio was 2.1 and 0.9 during antegrade and retrograde perfusion, respectively[15].

Although previous data using the sheep hepatic perfusion model[3,5] failed to find a direct link between pH and the control of partitioning between urea and glutamine as demonstrated in the rat, the current study suggest that both glutamine and urea synthesis rates are inhibited by an increase in pH.

The portal osmolality provides a simple, infallible signal to the liver. Previous reports have indicated clearly that osmolarity affects the hepatic volume (16, 1991; 11). The cell volume is expected to play a vital role in the regulation of the nitrogen metabolism in sheep. Therefore, the fundamental (but yet unresolved) question in sheep that needs to be fully explored is the measurement of cell volume in relation to the effect of osmolarity and pH.

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