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UREA AND GLUCOSE FORMATION IN OVINE LIVER AFTER AMMONIA AND LACTATE LOADING *IN VIVO*

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Résumé

ADMINISTRATION D'AMMONIAQUE ET DE LACTATE CHEZ LE MOUTON : FORMATION D'URÉE ET DE GLUCOSE DANS LE FOIE. — Des moutons ont été perfusés pendant deux heures par la veine mésentérique avec du chlorure d'ammonium (30 $\mu\text{mole}/\text{min}/\text{kg}$ PV), du lactate (50 $\mu\text{mole}/\text{min}/\text{kg}$ PV) ou un mélange des deux composés. Des prises de sang ont été effectuées dans les veines porte, hépatique et jugulaire, avant, pendant et deux heures après la perfusion, pour déterminer les teneurs en ammoniac, urée, lactate et glucose. Le flux portal hépatique moyen a été estimé à 59 ± 35 et 89 ± 48 ml/min/kg PV, respectivement pour le chlorure d'ammonium et le lactate sans variation importante, lorsque ces substrats sont perfusés indépendamment. Le traitement avec le chlorure d'ammonium accroît la fixation hépatique d'ammoniaque et la formation d'urée. Le chlorure d'ammonium perfusé seul entraîne une hyperammoniémie périphérique, qui augmente les sources endogènes d'ammoniaque pour la production d'urée. Le lactate et l'ammoniaque provoquent une augmentation de la production de glucose hépatique. Il semble que le lactate stimulerait principalement la néoglucogénèse et que l'ammoniaque agirait sur la glycogénolyse. L'effet de l'ammoniaque sur la glycogénolyse ainsi que l'augmentation de la production nette d'urée dans le foie pourraient être dus à une hypo-insulinémie et/ou une augmentation de la sécrétion d'adrénaline.

The ability of liver to bind ammonia delivered with portal blood and the formation of urea have been studied for many years (Linzell *et al* 1971, Norton *et al* 1982). Cocimano and Leng (1967) pointed out that the body urea entry is directly related to the liver urea formation and dietary nitrogen intake.

The experiment on rat hepatocytes indicated that the efficiency of urea production from ammonia can be related to the accessibility of three or four carbon intermediates and ornithine (Briggs and Freedland 1976). Glucogenic precursors, however, interfered ureagenesis from ammonia by the increased synthesis of aspartate, which, in the fact, provides the second atom of nitrogen for urea synthesis and also it is a limiting substrate for glucose formation (Krebs *et al* 1979). Aspartate as a precursor of cytosolic oxaloacetate is transferred from mitochondria to cytosol by the aspartate shuttle in exchange with glutamate.

There is no reason for qualitative discrimination of ureagenesis and gluconeogenesis in sheep from those in rat. However, the ruminant liver is highly loaded with ammonia, volatile fatty acids and lactate from the digestive tract, and it shows the

greater ability to form urea production than it is observed in monogastric animals (Owczarczyk and Barej 1975). Linzell *et al* (1971) described the capacity of the ovine liver to bind ammonia, but the regulation of this process is not clear. The *in vivo* technique of liver perfusion (Katz and Bergman 1969) allows direct net measurement of glucose and urea formation in this organ. The present experiment on sheep with perfused liver was undertaken for estimation of the urea and glucose formation after portal loading with ammonia and/or lactate.

Materials and Methods

The experiments were carried out on 6 sheep about 12 months old (30-40 kg bw). The vessel catheters were inserted into the mesenteric, portal, and hepatic veins according to the method described by Katz and Bergman (1969). A few days after surgery, animals were subjected to the following treatments, each of which was performed one day :

- a : infusion of ammonium chloride (30 $\mu\text{mol}/\text{kg}$ bw/min) or,
- b : infusion of lactic acid (50 $\mu\text{mol}/\text{kg}$ bw/min) or,

Table 1. — The average concentration (\pm SD) of blood ammonia, urea, lactate, and glucose in portal and hepatic veins in sheep given to the mesenteric vein saline (NaCl pretreatment) and next ammonium chloride, lactate or ammonium chloride plus lactate.

Treatment	blood concentration			
	ammoniac (μ mol/l)	urea (mmol/l)	lactate (mmol/l)	glucose (mmol/l)
<i>NaCl</i> (2 samplings \times 3 different conditions \times 6 sheep)				
portal vein	255 \pm 62	3.32 \pm 0.45	1.29 \pm 0.43	2.52 \pm 0.53
hepatic vein	89 \pm 22	3.29 \pm 0.44	1.19 \pm 0.46	2.93 \pm 0.44
<i>NH₄Cl</i> (2 samplings \times 6 sheep)				
portal vein	812 \pm 133	3.62 \pm 0.43	1.15 \pm 0.42	2.20 \pm 0.45
hepatic vein	296 \pm 94	4.43 \pm 0.52	1.49 \pm 0.50	2.80 \pm 0.45
<i>NH₄Cl</i> 2 h after treatment (6 sheep)				
portal vein	263 \pm 59	3.70 \pm 0.38	1.17 \pm 0.52	2.27 \pm 0.44
hepatic vein	125 \pm 37	4.87 \pm 0.70	1.50 \pm 0.59	2.87 \pm 0.37
<i>Lactate</i> (6 sheep)				
portal vein	222 \pm 57	2.83 \pm 0.56	4.80 \pm 0.70	2.66 \pm 0.55
hepatic vein	59 \pm 20	3.08 \pm 0.37	4.30 \pm 0.92	3.23 \pm 0.30
<i>Lactate</i> 2 h after treatment (6 sheep)				
portal vein	256 \pm 56	2.76 \pm 0.50	2.32 \pm 1.14	2.55 \pm 0.60
hepatic vein	83 \pm 23	2.97 \pm 0.41	2.30 \pm 0.99	3.21 \pm 0.36
<i>NH₄Cl plus lactate</i> (2 samplings \times 6 sheep)				
portal vein	778 \pm 194	3.64 \pm 0.55	4.86 \pm 1.05	2.67 \pm 0.35
hepatic vein	165 \pm 96	3.60 \pm 0.62	3.14 \pm 0.60	3.46 \pm 0.80
<i>NH₄Cl plus lactate</i> 2 h after treatment (6 sheep)				
portal vein	255 \pm 79	3.43 \pm 0.48	2.37 \pm 1.14	2.73 \pm 0.90
hepatic vein	84 \pm 35	3.52 \pm 0.76	1.90 \pm 0.87	3.36 \pm 0.60

The least significant difference ($P \leq 0.05$) is for ammonia, 45 μ mol/l, urea, 0.33 mmol/l, lactate, 0.33 mmol/l, glucose, 0.43 mmol/l

c: infusion of ammonium chloride plus lactic acid (in amounts as above).

On the day of experiment, the animals were not fed. Each treatment was preceded by saline pretreatment. All infusates were prepared in saline and buffered to pH 7.0. They were administered by a peristaltic pump (1 ml/min) to the mesenteric vein as follows: saline pretreatment for 120 min (8:00 to 10:00), and then experimental treatment for the next 120 min (10:00 to 12:00).

Blood samples were collected from the portal, hepatic, and jugular veins twice during the NaCl pretreatment (at 9:00 and 10:00), twice during experimental treatments (11:00 and 12:00), and then at 14:00, which corresponds to 120 min after infusions.

In all testing conditions, the portal and hepatic blood flow was measured by para-aminohippurate (PAH) clearance as it was described by Katz and Bergman (1969).

The following compounds were measured in the blood: ammonia (Okuda *et al* 1965), glucose by the enzymatic method using the «Blut-Zucker Test Fermo-

gnost», L-lactate (Boehringer-Test). Urea was determined in the blood plasma using the method of Okuda *et al* (1965) adopted by Kulasek (1972). The plasma insulin and glucagon concentrations were estimated in portal blood by radioimmunoassay technique; insulin-IBJ test, Swierk and glucagon with Novo-kit. Analysis of variance (split-plot), Tukey's test and standard deviation (SD) were used for evaluation of the results.

Results

The mesenteric loading with ammonia and/or lactate affected the concentration of urea and glucose in the hepatic blood in different degree (table 1).

The blood ammonia concentration in the portal vein during NaCl pretreatment was 255 \pm 62 μ mol/l and it increased more than three times during NH_4Cl or NH_4Cl plus lactate infusions. The hepatic blood concentration of ammonia in all

Table 2 – The utilization of ammonia and production of urea in the liver

	ammonia utilization ($\mu\text{mol}/\text{min}/\text{kg bw}$)	urea production ($\mu\text{mol}/\text{min}/\text{kg bw}$)
	(mean \pm SD)	
<i>Control</i> (2 samplings \times 3 different conditions \times 6 sheep)		
	9.1 \pm 2.2 a	17.6 \pm 14.2 a
<i>Treatment</i> (2 samplings \times 6 sheep)		
NH ₄ Cl	25.2 \pm 5.7 b	56.4 \pm 43.2 b
Lactate	9.0 \pm 2.8 a	26.5 \pm 19.1 a
NH ₄ Cl plus lactate	30.1 \pm 11.1 b	26.1 \pm 13.4 a
<i>2 h after treatment</i> (6 sheep)		
NH ₄ Cl	7.2 \pm 1.8 a	87.4 \pm 44.4 c
lactate	7.8 \pm 1.7 a	35.4 \pm 27.0 b
NH ₄ Cl plus lactate	9.5 \pm 3.3 a	33.5 \pm 14.4 b

Means in the same column with different letter are different ($P \leq 0.05$)

cases was significantly lower than in portal blood and it was $89 \pm 22 \mu\text{mol}/\text{l}$ during NaCl treatment and $296 \pm 94 \mu\text{mol}/\text{l}$ during ammonia treatment. The treatment with lactate decreased the ammonia concentration in the blood. The concentration of plasma urea in hepatic vein increased significantly during and after ammonium chloride treatment, while ammonium chloride plus lactate caused only a slight increase in the blood urea concentration. The infusion of lactate alone slightly decreased the plasma urea concentration

The concentrations of lactate in the blood increased during lactate treatment, however the infusion of lactate plus ammonium chloride caused a smaller elevation in the lactate concentration.

The concentration of glucose in portal blood during NaCl pretreatment was $2.52 \pm 0.53 \text{ mmol}/\text{l}$. It was slightly higher in hepatic blood during NaCl and NH₄Cl treatments, and significantly higher during and after treatment with lactate alone or ammonium chloride plus lactate.

The concentrations of ammonia, urea, lactate, and glucose in jugular blood were similar to those in hepatic blood. They were used for calculation of arterial entering of these metabolites into the liver. The average portal and hepatic blood flow measured by PAH technique was $59 \pm 35 \text{ ml}/\text{min}/\text{kg bw}$ and $89 \pm 48 \text{ ml}/\text{min}/\text{kg bw}$ respectively in all experimental conditions. There was not any significant difference in the hepatic blood flow in animals treated with ammonia or lactate alone.

The uptake of ammonia and the production of urea in the liver are presented in table 2. The hepatic uptake of ammonia increased significantly during NH₄Cl or NH₄Cl plus lactate treatment. The urea production in the liver raised during and after

the same treatment: the significant increase, however was observed only when NH₄Cl alone was given and also in two hours after the end of any treatment. The treatment with ammonium chloride and lactate decreased the net urea production by comparison to the value obtained after ammonium chloride infusion.

The average utilization of lactate in the liver in control condition was $10.5 \pm 9.0 \mu\text{mol}/\text{min}/\text{kg bw}$ (table 3). It increased when lactate or ammonia plus lactate were infused. However, when only ammonia was administered no net uptake was observed. The significant increase in the hepatic glucose output appeared when ammonia with lactate was given. There was not any simple relationship between utilization of lactate and glucose production.

Table 4 presents the data of portal insulin, and glucagon concentrations. The treatment with NH₄Cl caused a decrease in insulin concentration while lactate administration, an increase. Glucagon concentrations were changed slightly in all cases.

Discussion

Ureagenesis and gluconeogenesis form two main metabolic pathways in the liver. There are many enzymes which are responsible for both processes (Walser 1983, Brosnan 1982). The extent of ureagenesis and gluconeogenesis is not dependent on the enzymes' activity in such a degree as it depends on the accessibility of precursors and some regulatory mechanisms.

Ammonia in portal blood provides the liver with nitrogen for urea production (table 2). However, there is no simple dependence of urea production on given ammonia.

The results in table 2 suggest that the infusion of ammonia alone delivered in the body additional ammonia from endogenous sources for urea formation. This comes from the fact that only 22 and 4 % of urea was produced from the net ammonium uptake during ammonia treatment and two hours after ammonia treatment, respectively, while similar calculations prepared from the values of net hepatic uptake of ammonia and urea production when ammonia plus lactate was given, were 57 and 14 %.

Simultaneous supply with ammonia and lactate slightly increased the fixation of ammonia in the liver and its incorporation into urea, but the net

hepatic urea formation in this case was declined with comparison to ammonia alone treatment.

The delivery of ammonia from endogenous sources for hepatic urea formation appeared when ammonia passed through the liver to the peripheral blood. Lactate abolished the peripheral hyperammonaemia (table 1), decreasing the hepatic ureagenesis from endogenous ammonia.

Peripheral hyperammonaemia delivers the additional ammonia and its stimulates ureagenesis, probably, through adrenaline mediation. Such a suggestion was made by Emmanuel *et al* (1982) and Barej *et al* (1982). Adrenalectomy in sheep causes a decrease in plasma urea concentration,

Table 3. — The utilization of lactate and production of glucose in the liver

	lactate utilization ($\mu\text{mol}/\text{min}/\text{kg}$ bw)	glucose production ($\mu\text{mol}/\text{min}/\text{kg}$ bw)
(mean \pm SD)		
<i>Control</i> (2 samplings \times 3 different conditions \times 6 sheep)		
	10.5 \pm 9.0 a	27.4 \pm 5.5 a
<i>Treatment</i> (2 samplings \times 6 sheep)		
NH ₄ Cl	- 33.9 \pm 11.7 b	32.9 \pm 9.2 a
Lactate	29.6 \pm 20.8 c	37.7 \pm 4.8 a
NH ₄ Cl plus lactate	97.6 \pm 40.6 d	46.3 \pm 6.9 b
<i>2 h after treatment</i> (6 sheep)		
NH ₄ Cl	- 8.3 \pm 2.4 b	39.0 \pm 7.0 b
Lactate	3.1 \pm 1.2 a	37.5 \pm 6.0 a
NH ₄ Cl plus lactate	13.2 \pm 5.8 a	52.4 \pm 11.5 b

Means in the same column with different letter are different ($P \leq 0.05$)

Table 4. — The concentration of insulin and glucagon in the portal blood of sheep

	insulin (mU/l)	glucagon (ng/l)
(mean \pm SD)		
<i>Control</i> (2 samplings \times 3 different conditions \times 6 sheep)		
	20.5 \pm 5.4	179 \pm 29
<i>Treatment</i> (2 samplings \times 6 sheep)		
NH ₄ Cl	15.5 \pm 3.9 a	161 \pm 28
Lactate	26.1 \pm 5.0 b	201 \pm 27
NH ₄ Cl plus lactate	26.2 \pm 8.3 b	218 \pm 30
<i>2 h after treatment</i> (6 sheep)		
NH ₄ Cl	16.2 \pm 5.5	186 \pm 38
Lactate	18.4 \pm 5.9	184 \pm 28
NH ₄ Cl plus lactate	17.3 \pm 9.2	195 \pm 41

Means in the same column with different letter are different ($P \leq 0.05$)

which can be related to low urea formation (Slawski *et al* 1984).

The rate of hepatic urea formation increased a few hours after the end of ammonia treatment, when the blood ammonia concentration was very low. Similar results were obtained in many of our previous experiments on sheep, in which high concentration of blood ammonia disappeared in 20 minutes after ammonia infusion while the increase of urea concentration was observed 2-3 hours later (Barej *et al* 1982).

Glucogenic precursors like lactate stimulates the ureagenesis as well as glutamate and glutamine synthesis in hepatocytes (Krebs *et al* 1979). One can expect that many compounds, other than urea are produced by the ovine liver during its perfusion with ammonia and lactate. For example both agents stimulate the synthesis of some amino acids and pyrimidine (Motyl unpublished).

The liver glucose formation originates from gluconeogenesis and glycogenolysis. Lactate as well as ammonia given together or separately to

the mesenteric vein stimulated glucose release from the liver (table 3). Lactate as a source of aspartate and oxoglutarate (Krebs *et al* 1979) increases the glucose synthesis *de novo* (gluconeogenesis). Ammonia acts in most cases as a hyperglycaemic agent. The infusion of ammonium chloride to the mesenteric vein increased the release of glucose in the liver without simultaneous uptake of lactate (table 3). The hyperglycaemic effect of ammonia in sheep was observed several times in our previous experiments (Barej *et al* 1982, Slawski *et al* 1984). Those experiments showed that insulin and adrenaline are mediators in the glycaemic action of ammonia ion. Peripheral hyperammonaemia stimulated the release of adrenaline in the medullary gland and decrease in the plasma insulin concentration. Data in table 4 in the present experiment also suggest a fall in the concentration of plasma insulin after ammonia administration. Since ammonia itself does not influence glycogenolysis and inhibits gluconeogenesis (Krebs *et al* 1979) the hyperglycaemic effect of ammonia can be explained by the mediation of adrenaline and insulin in the glycogen breakdown.

Abstract

Ammonium chloride (30 $\mu\text{mol}/\text{min}/\text{kg}$ bw), lactate (50 $\mu\text{mol}/\text{min}/\text{kg}$ bw) or ammonium chloride plus lactate were infused for two hours into the mesenteric vein of sheep. Blood samples were taken before, during, and two hours after infusion from portal, hepatic and jugular veins for estimation of ammonia, urea, lactate and glucose. Average portal and hepatic blood flow was 59 ± 35 and 89 ± 48 ml/min/kg bw respectively without any regular changes when ammonium chloride or lactate alone were administered. The treatment with ammonium chloride increased the hepatic ammonia fixation from 9.1 ± 2.1 to 25.2 ± 5.7 $\mu\text{mol}/\text{min}/\text{kg}$ bw and urea formation from 17.6 ± 14.2 to 56.4 ± 43.2 $\mu\text{mol}/\text{min}/\text{kg}$ bw. The infusion of ammonium chloride alone provoked a peripheral hyperammonaemia and it increased the endogenous sources of ammonia for urea production. Both lactate and ammonia caused an increase of hepatic glucose release. It was suggested that lactate stimulated mainly gluconeogenesis, and ammonium-glycogenolysis. The effect of ammonia in the last reaction as well as an increased net urea formation in the liver, was probably mediated by the hypoinsulinaemia and/or an increase of adrenaline secretion.

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