Amino Acids (2009) 37:751–760
DOI 10.1007/s00726-008-0218-3

ORIGINAL ARTICLE

Colonic luminal ammonia and portal blood L-glutamine and L-arginine concentrations: a possible link between colon mucosa and liver ureagenesis

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Received: 14 October 2008 / Accepted: 18 November 2008 / Published online: 11 December 2008

Abstract The highest ammonia concentration in the body is found in the colon lumen and although there is evidence that this metabolite can be absorbed through the colonic epithelium, there is little information on the capacity of the colonic mucosa to transfer and metabolize this compound. In the present study, we used a model of conscious pig with a canula implanted into the proximal colon to inject endoluminally increasing amounts of ammonium chloride and to measure during 5 h the kinetics of ammonia and amino acid concentration changes in the portal and arterial blood. By injecting as a single dose from 1 to 5 g ammonia into the colonic lumen, a dose-related increase in ammonia concentration in the portal blood was recorded. Ammonia concentration remained unchanged in the arterial blood except for the highest dose tested, i.e. 5 g which thus apparently exceeds the hepatic ureagenesis capacity. By calculating the apparent net ammonia absorption, it was determined that the pig colonic epithelium has the capacity to absorb 4 g ammonia. Ammonia absorption through the colonic epithelium was concomitant with increase of L-glutamine and L-arginine concentrations in the portal blood. This coincided with the expression of both glutamate dehydrogenase and glutamine synthetase in isolated colonic epithelial cells. Since L-glutamine and L-arginine are known to represent activators for liver ureagenesis, we propose that increased portal concentrations of these amino acids following increased ammonia colonic luminal concentration represent a metabolic link between colon mucosa and liver urea biosynthesis.

Keywords Ammonia absorption · Pig colon mucosa

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Introduction

The highest ammonia (considered as the sum of NH_4^+ and NH_3) concentration in the body is found in the large intestine lumen. Indeed ammonia is found at millimolar concentrations in the rat large intestine (Lin and Visek 1991b; Mouille et al. 2004) and in human faecal dialysates (Wrong et al. 1965). Manipulation of dietary pattern such as an increase in the amount of dietary proteins leads to a marked rise of the large intestine ammonia luminal content in animals (Mouille et al. 2004) and in faecal ammonia concentrations in humans (Geypens et al. 1997). In contrast, high resistant starch consumption results in decreased ammonia concentrations in the colon lumen (Birkett et al. 1996; Heijnen et al. 1997; Govers et al. 1999) indicating that the dietary conditions have a marked influence on such parameter (De Preter et al. 2006). Ammonia is produced through bacterial fermentation of nitrogenous compounds, i.e. mainly proteins and peptides (Chacko and Cummings 1988) from both dietary and endogenous origins. From 6 to 18 g nitrogenous material (depending on the alimentary

supply) enter into the large intestine through the ileocaecal junction every day (Smiddy et al. 1960; Kramer 1966; Gibson et al. 1976). Indeed, substantial amounts of even highly digestible proteins may escape digestion in the human small intestine (Evenepoel et al. 1999; Bos et al. 2007). When present in the large intestine lumen, this nitrogen-containing material undergoes intense proteolysis by the colonic flora resulting in free amino acid release followed by the production of numerous bacterial metabolites including ammonia (Blachier et al. 2007). This end product is produced by bacterial deamination of amino acids and by urea hydrolysis (Vince and Burridge 1980; Moran and Jackson 1990). Urea transporters are expressed in the colonic mucosa (Stewart et al. 2004; Smith et al. 2004; Inoue et al. 2004), where they are likely to participate in the transfer of urea from the blood to the intestinal lumen. The luminal concentration of ammonia in the large intestine lumen is the net result of bacterial ammonia production and utilization as well as absorption through the large intestine epithelium. The colonic lumen is the major site of ammonia production, and the large intestine mucosa is able to absorb significant amounts of luminal ammonia. Indeed, approximately 4 g of ammonia are absorbed daily by the human colon (Summerskill and Wolpert 1970; Wolpert et al. 1971).

In healthy individuals, ammonia is transported to the liver by the portal vein, where it is mostly removed through urea synthesis in the hepatic ornithine cycle in periportal hepatocytes (Meijer et al. 1990) and incorporation of ammonia into glutamate leading to L-glutamine synthesis in perivenous hepatocytes (Haussinger 1990). An increase in blood ammonia concentration is detrimental because this compound is toxic to the central nervous system (Raabe 1990).

In rat distal colon, NH_3 is 400 times more permeant than NH_4^+ (Cohen et al. 1988). Members of the ammonia transporter family, i.e. RhBG and RhCG have been found to be expressed in absorptive fully differentiated colonocytes but not in crypt cells and mucous cells indicating that they may participate in luminal ammonia transport (Handlogten et al. 2005). Furthermore, in apical membranes from rat distal colon, it has been shown that the H^+, K^+-ATPase can function as a Na^+-dependent NH_4^+-ATPase indicating that this ATPase may participate in the ammonia transport (Codina et al. 1999). Interestingly, Singh et al. (1995) found that the apical membranes of crypt colonocytes are characterized by a low permeability for NH_4^+/NH_3, a result that may be linked to an adaptation of these cells to the luminal environment of the colonic epithelium.

Despite the fact that, as described above, there are published biochemical data on the presence of different ammonia transport systems in colonic epithelial cells, there is little information on the in vivo capacity of the colonic epithelium to absorb and metabolize ammonia in the process of transfer from the lumen to the bloodstream. Our working hypothesis was that the metabolic end-product ammonia is partly recycled by the colon mucosa in order to provide nitrogen moieties of L-glutamine and L-arginine, the latter being considered as signaling molecules for liver ureagenesis (Meijer et al. 1990). To document such putative colonic epithelium capacity, we used the pig model that is considered relevant for gastrointestinal physiological studies (Jonbloed 1997) to measure the kinetics of ammonia and amino acid concentrations in blood originating from venous and arterial sources after endoluminal injection of increasing amounts of ammonia in the pig proximal colon. In fact, ammonia is involved in amino acid metabolism, and ammonia has been shown by us to be metabolized in isolated colonocytes (Mouille et al. 1999). We found significant difference in L-glutamine and L-arginine portal concentrations. Lastly, we found that glutamate dehydrogenase and glutamine synthetase are expressed in colonic epithelial cells raising the view that these metabolic pathways may play a role in the observed ammonia provoked increase in portal L-glutamine concentration.

Materials and methods

Animals

The experiments were performed using Large White pigs (mean mass 51 ± 9 kg, $n = 13$). For in vivo ammonia intestinal absorption measurement, a 8–10 day wash-out period was used between experiments. The pigs were fed twice a day with 800 g of semi-synthetic diet (Table 1) containing 178 g/kg dry matter casein (Darcy-Vrillon et al. 1993).

Surgical procedure for in vivo study

All procedures related to care and use of animals were approved by the French Ministry of Agriculture (order No.

Table 1 Composition of the experimental diet

Dietary component	Content (g/kg)
Casein	178
Purified corn starch	516
Sucrose	65
Soybean oil	150
Purified cellulose	50
Mineral and vitamin supplement[a]	41

[a] As described in Darcy-Vrillon et al. (1993)

87-848). After a fasting period of 24 h, animals received an intravenous injection of 1 g sodium thiopental (Nesdonal, Merial, Lyon, France). The induction and maintenance of anesthesia was performed using isoflurane. Under aseptic conditions, a catheter was surgically implanted in the portal vein and in the right carotid artery and in some experiments with a portal blood flow probe, i.e. Transonic probe (Transonic Systems, Ithaca, NY). A canula was implanted into the lumen of the proximal colon. During 3 days after surgery, pigs received an analgesic treatment (Metacam, 0.2 mg/kg per day). The recovery of a normal food intake was considered as a parameter for the recovery from the surgical stress before the start of the experiments. This happened approximately 1 week after surgery. The animals were starved the evening before the ammonia injection experiments that was performed the morning after at 9.00 AM. Blood samples (2 ml) were collected from the portal vein and artery 10 and 1 min before the endoluminal injection into the proximal colon (time zero) of 60 ml of a freshly prepared aqueous solution (pH 6.0) maintained at 37°C and containing NH_4Cl or NaCl (control experiments). Subsequently, 2.0 ml of portal and arterial blood samples were collected after different period of times following colonic injection and mixed with 2.5 ml perchloric acid (6%). After centrifugation, the supernatant was neutralized with KOH solutions and after an additional centrifugation, the supernatants were used for ammonia concentration measurement (see below) that was always performed within 48 h after sampling. Aliquots were frozen at −80°C up to the amino acid and urea analysis (see below).

Ammonia luminal colonic content analysis

For the measurement of the ammonia colonic content, the animals were starved the evening before the experiment and the animals were anaesthetized at 9.00 AM. Forty centimeters of proximal and distal colon were surgically removed and the luminal content was centrifuged at 12,000*g* for 15 min. Supernatants were measured for their pH and then aliquots were treated with perchloric acid and neutralized with KOH as described above before ammonia measurement (see below) that was also performed within 48 h following the experiments.

Ammonia assay

Ammonia was assayed in duplicate by an enzymatic assay using glutamate dehydrogenase. Samples of blood and colonic luminal content extracts were mixed with distilled water up to 1 ml volume and then with 1 ml of the assay buffer, i.e. Tris buffer (200 mM, pH 8) containing 20 mM alpha-ketoglutarate and 0.3 mM NADH. Then the NADH absorbance decrease was measured at 340 nm before and 1 h after addition of 5 units glutamate dehydrogenase (Sigma chemicals, St Louis, MO).

Amino acid and urea measurement in venous and arterial blood

Amino acids and urea in deproteinized neutralized blood were determined with an amino acid autoanalyzer (Jeol, Tokyo, Japan) by ion-exchange chromatography with ninhydrin detection after dilution of the samples with a lithium citrate buffer containing D-glucosaminic acid and amino-ethylcysteine as internal standards. Participation to the European quality control scheme (ERNDIM, Brussels, Belgium) ensures the accuracy of our amino acid determinations.

Epithelial colonic cell isolation

Colonocytes were isolated from proximal and distal colon as described previously (Darcy-Vrillon et al. 1993). Briefly, after extensive luminal washing with NaCl solution (9 g/l), the mucosa was rinsed with a Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 10 mM HEPES, 5 mM dithiothreitol, 2.5 mg/ml bovine serum albumin and equilibrated against a mixture of O_2/CO_2 (19/1, v/v). The colonic segments were then filled with the same buffer containing 10 mM EDTA and incubated for 20 min at 37°C. The luminal fluids containing epithelial cells were centrifuged and washed twice in the same bicarbonate buffer medium (pH 7.4) without EDTA and dithiothreitol but containing 1.3 mM $CaCl_2$ and 2 mM $MgCl_2$ and 10 mg/ml bovine serum albumin. The cell pellets obtained were then stored at −80°C until Western blot and enzymatic activity analysis (see below). Samples of pig liver used for positive control experiments in Western blot analysis of glutamine synthetase were kept at −80°C.

Western blot analysis of glutamine synthetase

Isolated colonocytes were sonicated three times 10 s at 4°C in a Tris–HCl 10 mM buffer containing 20 mM NaCl, 5 mM $MgCl_2$, 0.5% NonidetP40 (Sigma Chemicals, St Louis, MO), and a protease inhibitor cocktail (Roche, Penzberg, Germany). The same buffer was used to homogenize pig liver samples using an ultraturax. The crude extracts were centrifuged at 12,000*g* for 30 min and the supernatants were stored at −20°C up to Western blot analysis.

For such a purpose, an aliquot of colonocyte extract corresponding to 150 μg proteins was mixed with an equal volume of sample buffer (2×) i.e. Tris–HCl 125 mM buffer (pH 6.8) containing 4% SDS, 20% glycerol, 200 mM dithiothreitol and 0.002% bromophenol blue. Liver extracts

corresponding to 30 μg proteins were treated exactly by the same way. Then, these samples were heated in boiling water for 5 min, cooled on ice and separated together with molecular weight markers on a 12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then electrotransferred onto a Hybond-*P*-polyvinyldiene difluoride membranes purchased from Amersham (Little Chalfont, UK). Immunoblots were pre-incubated with blocking solution [5% skimmed milk in TBST (0.10% Tween 20 in Tris–buffered saline solution]. The membrane was hybridized with a rabbit polyclonal IgG anti-glutamine synthetase (1/200, Santa Cruz Biotech, CA) and after washing, was incubated with a secondary anti-rabbit peroxidase antibody (1/30,000, Jackson Immunoresearch Laboratory, West Grove, PA). Glutamine synthetase was detected using ECL+ (Enhanced ChemiLuminescence) kit (Amersham, Little Chalfont, UK).

Measurement of glutamine synthetase and glutamate dehydrogenase activities in colonocytes

Glutamine synthetase activity was assayed in colonocytes isolated from both proximal and distal part of the colon by a modification of the method of Meynial-Denis et al. (1996). Briefly, colonocytes were sonicated at 4°C and the homogenates were incubated at 37°C for 60 min in an imidazole/HCl buffer (50 mM, pH 7.5) containing 15 mM L-glutamate, L-(U-14C) glutamate (Amersham, Little Chalfont, UK; 0.25 μCi per assay), 150 mM KCl, 2.5 mM dithiothreitol, 10 mM EDTA, 10 mM ATP, 20 mM $MgCl_2$ and 40 mM NH_4Cl. At the end of incubation, an aliquot of incubation medium was loaded on anion exchange column (AG1-X8, 200–400 mesh) and the radioactive L-glutamine was eluted with Tris buffer (10 mM, pH 7). Radioactivity in the samples was determined by liquid scintillation and the glutamine synthesis was calculated after correction for the recovery of standard amounts of L-(U-14C) glutamine (Perkin-Elmer, Boston, MA) by reference to the specific radioactivity of the L-glutamate precursor in the incubation medium. Glutamate dehydrogenase activity was measured spectrophotometrically by incubating colonocyte homogenates in a phosphate buffer (100 mM, pH 7.6) containing 10 mM alpha-ketoglutarate, 2.6 mM EDTA, 1.0 mM ADP, 100 mM NH_4Cl and 0.2 mM NADH. The decrease in NADH absorbance measured at 340 nm was followed during 20 min. The enzymatic specific activity was determined after protein measurement in the assay using the Lowry's procedure and bovine serum albumin as standard.

Calculations

Apparent net ammonia absorption was calculated according to the Fick principle as the difference between portal and arterial concentrations multiplied by the flow rate in the portal vein (Vaugelade et al. 1994). The mean portal flow-rate averaged 1.99 ± 0.20 l/min. The results are presented as the mean ± SE together with the number of independent experiments (n) performed in different animals. Differences between doses of injected ammonia with respect to metabolite concentrations in portal and arterial blood were analyzed using mixed models for repeated-measure analysis (SAS Institute, Cary, NC, USA) with time and ammonia doses as independent factors. Differences with P values <0.05 were considered as statistically significant.

Results

Ammonia concentrations in colonic luminal content

Ammonia luminal concentrations in the proximal and distal segments of the fasted pig colon were not significantly different. Therefore, the data were pooled together. Ammonia luminal concentration was averaging 27.2 ± 17.5 mM ($n = 4$). The mean colonic luminal pH in the liquid supernatant of the centrifuged colonic lumen was equal to 6.4 ± 0.4 ($n = 3$).

Kinetics of ammonia concentrations in portal and arterial blood following endoluminal ammonia injection

As indicated in Fig. 1, when NH_4Cl was injected in increasing amounts in the lumen of the proximal colon of conscious fasted pigs, a dose-related increase of ammonia concentration in portal blood was recorded when compared with the control experiment in which 16.25 g NaCl was injected via the endoluminal way (Fig. 1a). This dose of NaCl was chosen in order to represent the same amount of chloride than the amount present in the highest NH_4Cl dose, i.e. 14.86 g NH_4Cl (corresponding to 5 g NH_4^+). As reported in Fig. 1a, without ammonia colonic luminal injection, the ammonia concentrations were higher at all time points in the portal blood than in the arterial blood. However, the differences were relatively modest suggesting minor ammonia colonic absorption under these experimental conditions. After acute endoluminal injection of 1 g NH_4^+, and as compared to control experiments, the ammonia portal blood concentrations were rapidly increased as soon as 10 min after ammonia colonic injection and remained higher for 2 h before progressively returning back to basal values (Fig. 1b). In that case, a global increase of portal ammonia concentration was observed ($P < 0.001$). A much higher increase in ammonia portal blood

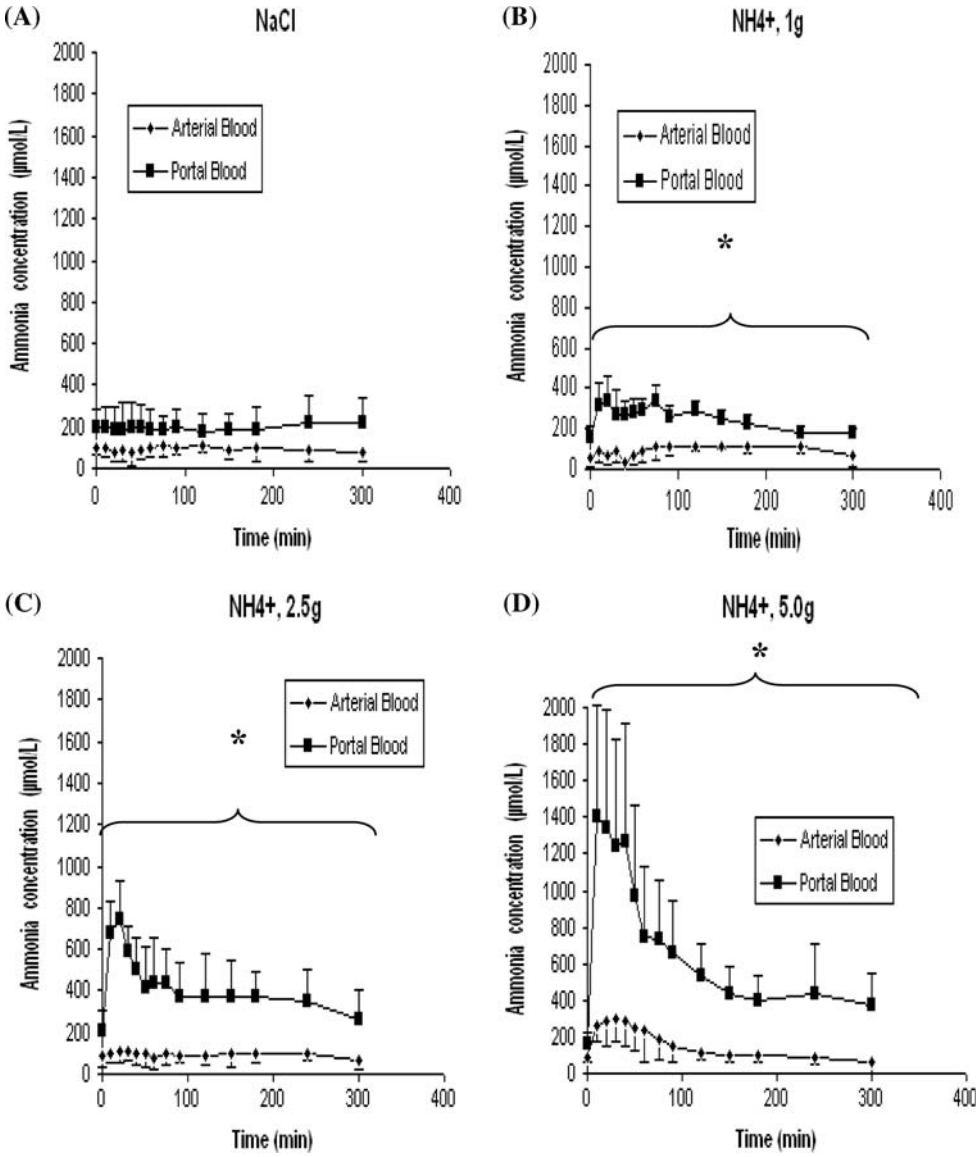

Fig. 1 Ammonia concentrations in portal and arterial blood following colonic intraluminal injection of increasing amounts of NH_4Cl (**b**, **c**, **d**) or NaCl (control experiments, **a**) into the proximal colon. At zero time, conscious pigs received an intraluminal injection of NH_4Cl or NaCl and portal and arterial blood samples were recovered at different time for ammonia assay using an enzymatic test. The results are expressed as mean ± SE and represent 3–5 independent experiments. *Indicates significant differences ($P < 0.05$) between arterial and portal blood (mixed models for repeated-measure analysis)

concentration was observed in the first 20 min following endoluminal injection of 2.5 g ammonia (Fig. 1c); and then decreased up to the end of blood sampling, i.e. 5 h after ammonia injection. Using this dose of ammonia, the portal ammonia concentration was significantly higher than the values recorded using 1 g ammonia dose whatever the kinetics time ($P < 0.025$). When the highest dose of ammonia, i.e. 5 g was injected into the proximal colon lumen, a tremendous increase was recorded as soon as 10 min after ammonia injection (Fig. 1d). This very high ammonia concentration was relatively stable for up to 40 min following ammonia injection and then progressively decreases up to the end of blood sampling. In that latter case, there was a measurable increase of ammonia concentration in the arterial blood between 10 and 60 min when compared to values obtained when no ammonia or lower doses of ammonia were used ($P < 0.05$). Ammonia portal concentrations after 5 g ammonia colonic injection were significantly higher than the one measured with 2.5 g dose ($P < 0.05$) and a significant interaction between time and dose of injected ammonia was recorded ($P < 0.0001$).

When the net apparent ammonia absorption was calculated as described in "Material and methods" using porto-arterial differences in ammonia concentrations together with the measured portal blood flow, there was a relatively good consistency between the quantity of ammonia injected into the proximal colon and the quantity of absorbed ammonia (Fig. 2). Furthermore, and interestingly, this calculation raises the view that 5 h after ammonia colonic

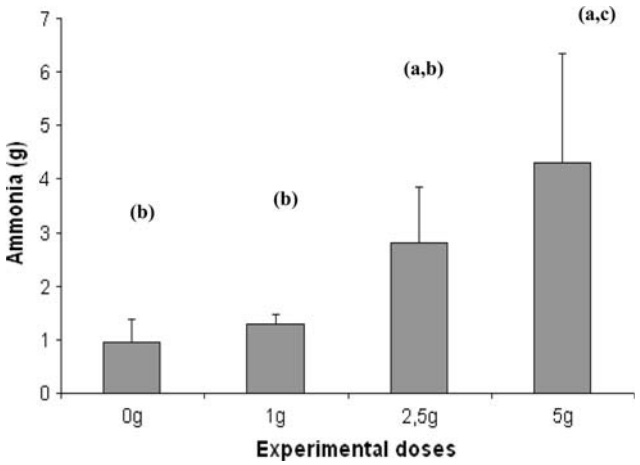

Fig. 2 Net amounts of ammonia absorbed within 5 h. The apparent net absorption of ammonia was calculated from the differences between portal and arterial ammonia concentrations multiplied by the venous blood flow after intraluminal injection of increasing amount of NH_4Cl or NaCl (control experiments). The results are expressed as mean ± SE and represent 3–5 independent experiments. Columns not sharing the same superscript are statistically different ($P < 0.05$)

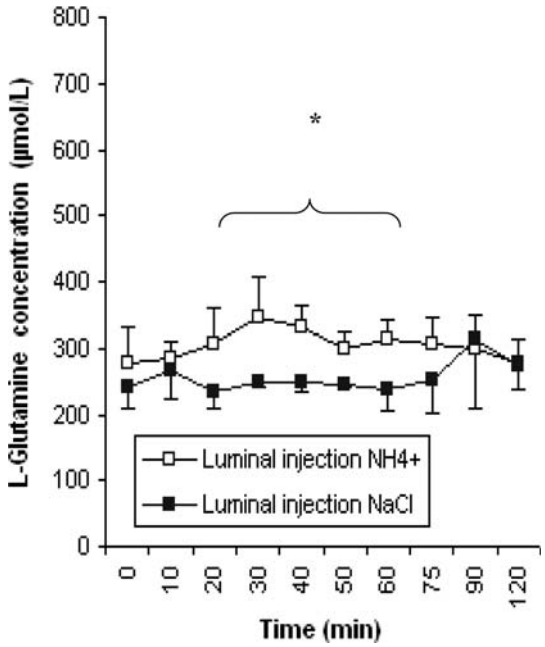

Fig. 3 L-glutamine concentrations in portal vein following colonic intraluminal injection of NH_4Cl or NaCl. At zero time, conscious pigs received an intraluminal injection of 2.5 g ammonia (or NaCl in control experiments) and portal blood samples were recovered at different times for the determination of L-glutamine concentrations. The results are expressed as mean ± SE and represent 3–5 independent experiments. *Indicates significant differences ($P < 0.05$) between portal blood L-glutamine concentration after ammonia or NaCl intraluminal injection

injection, a large part of injected ammonia was absorbed whatever the dose used.

Effects of endoluminal colonic ammonia injection on blood amino acid concentration

Since ammonia is a substrate for several amino acid metabolic pathways and has been shown to be metabolized by isolated colonocytes, the effects of the colonic injection of the intermediate dose of ammonia, i.e. 2.5 g ammonia on amino acid concentration were measured in both portal and arterial blood. As indicated in Fig. 3, ammonia intraluminal injection led to a significant transient increase (global ammonia effect: $P < 0.05$) in L-glutamine concentration in portal blood between 20 and 60 min when compared with control experiment (NaCl injection). In contrast, L-glutamine concentrations in arterial blood were not significantly different after ammonia or NaCl colonic injection (not shown).

The injection of 2.5 g ammonia into the colonic lumen provoked also a significant measurable increase in L-arginine concentration in portal blood when compared to control experiments (global ammonia effect: $P < 0.05$, significant differences between 20 and 60 min, Fig. 4). However, there was no significant difference of L-arginine concentrations in the arterial blood after ammonia and NaCl endoluminal injection.

The other amino acids related to L-glutamine and L-arginine metabolism analyzed in the venous and arterial blood (e.g. aspartate, alanine, proline, citrulline and ornithine) were not markedly modified following ammonia colonic injection when compared to the zero time of the kinetics and with the control experiment using NaCl (data not shown).

Lastly, when urea concentrations were measured in arterial blood 30, 60 and 120 min after injection of 2.5 g ammonia into the colonic lumen, the recorded values were found markedly increased ($P < 0.01$ at 30 and 60 min and $P < 0.025$ at 120 min) when compared to the values recorded following NaCl injection (Fig. 5).

Glutamate dehydrogenase and glutamine synthetase expression in colonic epithelial cells

Since, we measured an increased L-glutamine concentration in the portal blood following ammonia injection in the proximal colon, we measured in the colonocytes the activities of glutamate dehydrogenase and of glutamine synthetase which can use ammonia, respectively, for L-glutamate synthesis from alpha-ketoglutarate and for L-glutamine synthesis from L-glutamate. Glutamate dehydrogenase activity was detected in colonocytes from both proximal colon (18.10 ± 9.73 nmoles/min per milligram proteins, $n = 5$) and from distal colon (15.93 ± 5.36 nmoles/min per milligram proteins, $n = 4$).

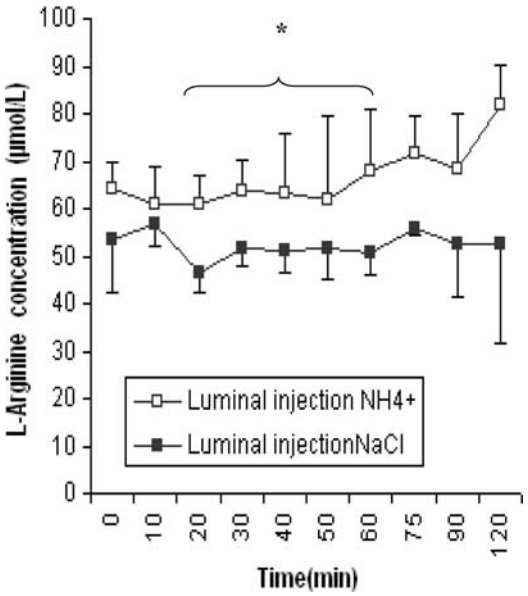

Fig. 4 L-arginine concentrations in portal vein following colonic intraluminal injection of NH_4Cl or NaCl. At zero time, conscious pigs received an intraluminal injection of 2.5 g ammonia (or NaCl in control experiments) and portal blood samples were recovered at different times for the determination of L-arginine concentrations. The results are expressed as mean ± SE and represent 3–5 independent experiments. *Indicates significant differences ($P < 0.05$) between portal blood L-arginine concentration after ammonia or NaCl intraluminal injection

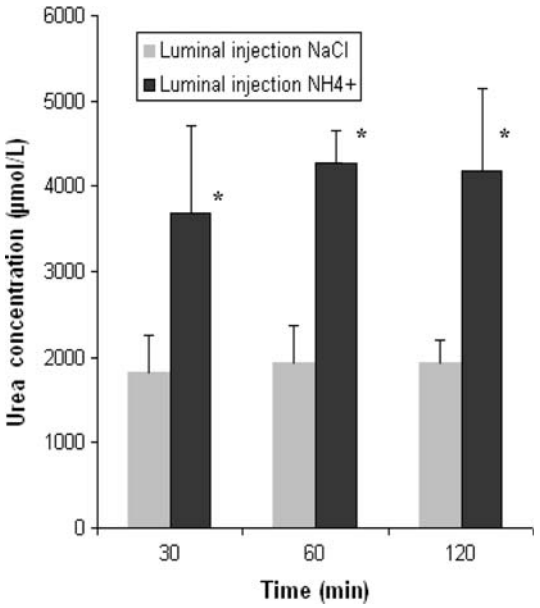

Fig. 5 Urea concentration in arterial blood following colonic intraluminal injection of NH_4Cl or NaCl. At zero time, conscious pigs received an intraluminal injection of 2.5 g ammonia (or NaCl in control experiments) and arterial blood samples were recovered at different times for the determination of urea concentrations. The results are expressed as mean ± SE and represent three independent experiments *Indicates significant differences ($P < 0.05$) between arterial blood urea concentration after ammonia or NaCl intraluminal injection

Glutamine synthetase activity was also detected in colonocytes isolated from the proximal colon (64 ± 13 nmoles/h per milligram proteins, $n = 3$) and isolated from the distal colon (78 ± 24 nmoles/h/mg proteins, $n = 3$). When liver homogenates was used as a matter of comparison, glutamine synthetase activity averaged 2.1 ± 0.6 µmoles/h/mg of proteins. The presence of glutamine synthetase in colonic epithelial cells was confirmed by Western blot analysis (Fig. 6) using a polyclonal antibody. Glutamine synthetase was detected in both colonocytes isolated from proximal and distal colon but the apparent molecular weight (49 kDa, $n = 3$) was found to be slightly lower than the isoform detected in pig liver (50 kDa, $n = 3$).

Discussion

The results of the present study clearly demonstrate that the acute ammonia injection into the lumen of the proximal colon resulted in increased portal ammonia concentration that is concomitant with increase L-glutamine and L-arginine concentrations. For ammonia, this increase was however transient since with all ammonia concentration tested, after an initial increase of portal blood ammonia concentrations, there was a progressive return back to lower values. This is an indication that 5 h after ammonia endoluminal injection, a large proportion of this compound was absorbed through the colonic mucosa. In addition, the net amount of absorbed ammonia was increasing with the amount of ammonia present in the colonic lumen indicating a dose-related transfer of ammonia through the colonic epithelium. Interestingly, the control experiments (i.e. performed with endoluminal injection of NaCl) indicate that the endogenous ammonia present in the lumen of the colon (i.e. present at an average value of 27 mM concentration) is absorbed at a relatively modest rate. Indeed, in that later case, it can be calculated that approximately 1 g of ammonia is absorbed within 5 h. The injection of 1 g exogenous ammonia into the colon lumen led to an additional 0.3 g (30% of the dose) absorption above the basal endogenous ammonia absorption. At the intermediary ammonia dose (i.e. 2.5 g), it can be calculated that 1.8 g (thus 72% of the injected dose) was absorbed. At the highest dose (5 g ammonia), 84% of the exogenous ammonia was absorbed 5 h after colonic injection. This indicates that ammonia is proportionally more efficiently absorbed when the ammonia luminal content is highly increased than when this increase is more limited and thus suggest that in vivo the transport systems in colonic epithelial absorptive cells is of low affinity and high capacity.

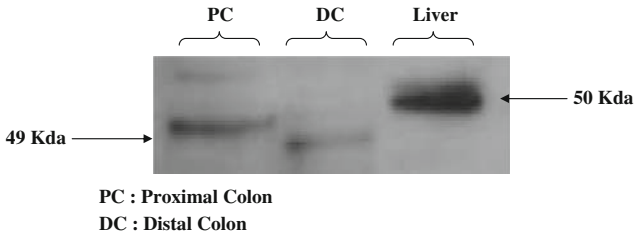

Fig. 6 Western blot analysis of glutamine synthetase in isolated colonocytes recovered from the proximal and distal colon. Colon epithelial cells were isolated from the pig proximal and distal colon and colonocyte extracts corresponding to 150 µg proteins were separated on a 12.5% polyacrylamide gel. Pig liver proteins (30 µg) used as a matter of comparison were treated exactly the same way. After transfer on membrane, glutamine synthetase was revealed using a rabbit polyclonal IgG antibody. *Arrows* indicate the molecular weights of glutamine synthetase in colon and liver as determined using the MW markers

Interestingly, when 5 g ammonia was injected into the proximal colon of the pig, there was a significant but transient increase of ammonia concentration in the arterial blood. This result means that in the present experimental model of starved pig, the absorption of 4.2 g luminal ammonia through the colonic epithelium led to a saturation of the main metabolic pathways responsible for ammonia detoxication, i.e. periportal liver ornithine cycle and perivenous glutamine synthetase pathway.

L-glutamine concentration in the portal blood was found increased from 20 to 60 min after the endoluminal injection of the intermediary ammonia dose (2.5 g). However, L-glutamine concentration was not modified in the arterial blood. Two hypotheses can be made to explain this result: (1) a net synthesis of L-glutamine has been performed by the bacterial flora followed by further absorption through the colonic epithelium. Bacteria are known to be able to incorporate ammonia into both L-glutamate and L-glutamine in processes involving, respectively, glutamate dehydrogenase and glutamine synthetase (Leigh and Dodsworth 2007). However, it is generally believed that colonic luminal amino acids are not absorbed to any major extent by the colonic epithelium (Darragh et al. 1994; Hume et al. 1993) except within the first hours following piglet birth (Smith and James 1976). (2) L-glutamine has been synthesized from ammonia in the colonic epithelial cells and then released in the portal vein. The fact that we found that glutamine synthetase was detected in colonocytes is an evidence that makes this latter hypothesis likely. In support of such an hypothesis, glutamine synthetase was detected in colonic epithelial cells both as enzymatic activity and as immunoreactive protein with a molecular weight compatible with that of the monomer (Eisenberg et al. 2000). Colonic epithelial cells are primarily considered as L-glutamine consuming cells (Watford et al. 2000; Darcy-Vrillon et al. 1993) through the stepwise synthesis of L-glutamate and alpha-ketoglutarate and entry of this latter metabolite into the Krebs's cycle. Indeed, this amino acid represents a major oxidative fuel in colonocytes (Ardawi and Newsholme 1985). Yet, glutamine synthetase activity has also been measured in human colonic biopsy samples (James et al. 1998) and in the human adenocarcinoma epithelial Caco-2 colonic cells (Le Bacquer et al. 2001). The presence of glutamine synthetase in colonocytes suggests the presence of a futile cycle with both ATP-dependent glutamine synthesizing capacity (glutamine synthetase) and glutamine degrading capacity (glutaminase). We propose that a marked increase of ammonia content in the colon lumen would partly shift through a mere mass action phenomenon colonocytes from L-glutamine consumption to L-glutamine production. L-glutamine can then be degraded in ammonia and L-glutamate in periportal hepatocytes (Meijer et al. 1990). These metabolites are respectively precursors for ureagenesis and *N*-acetylglutamate. This latter compound is an allosteric activator of the first enzyme in the urea cycle, i.e. carbamoylphosphate synthetase 1 (Meijer et al. 1990). Another consequence of such a shift would be a reduction of the colonocyte intracellular accumulation of ammonia through an ATP-consuming pathway. Since glutamate dehydrogenase, a reversible enzyme able to catalyse the synthesis of glutamate from alpha-ketoglutarate and ammonia was also detected in isolated colonocytes (this study), this would represent another metabolic pathway for ammonia detoxication. Indeed, there is evidence that ammonia in excess in the colon lumen can act as a metabolic troublemaker. In in vitro experiments, it has been shown that a 40 mM ammonia concentration is able to markedly decrease the oxidative metabolism of the bacterial luminal compounds butyrate and acetate in colonic epithelial cells (Cremin et al. 2003). Since these short chain fatty acids which are produced in the colonic lumen from alimentary fibers, resistant starch and proteins (Mortensen and Clausen 1996) are considered as luminal fuels for colonocytes (Ardawi and Newsholme 1985; Roediger 1982), the intracellular removal of ammonia through the stepwise synthesis of L-glutamine, although representing an energy-consuming process, would reduce the ammonia-provoked inhibition of the oxidative metabolism of short-chain fatty acids. Further work is obviously required to decipher more precisely the complex metabolic interactions between substrates from both luminal and plasma origins. Other differences in the portal blood amino acid concentration changes after injection of ammonia in the colon lumen were related to L-arginine concentrations. We documented in the present study increases of this amino acid after ammonia injection without detectable changes in arterial L-arginine concentrations. L-arginine is both the direct urea precursor after transport into the hepatocytes by a low affinity system

(Closs et al. 1993) and an activator of *N*-acetylglutamate synthase; and thus of the synthesis of carbamoylphosphate through the catalytic activity of carbamoylphosphate synthetase (Meijer et al. 1990). It thus appears that the colonic mucosa is able, following an ammonia luminal load, to produce the two main amino acids known to be involved in ureagenesis stimulation. We have previously shown that rat isolated colonocytes are able to metabolize ammonia to L-citrulline through the sequential action of carbamoylphosphate synthetase 1 and ornithine carbamoyltransferase (Mouille et al. 2004); and to a minor extent to L-arginine (Mouille et al. 1999) through the action of argininosuccinate synthetase and argininosuccinate lyase (although these latter two enzymes are little expressed in such cells).

Altogether, the present data indicate that in vivo the colonic mucosa is able to transfer ammonia from the lumen to the bloodstream using a system with low affinity and high capacity. Furthermore, our data are compatible with the view that colonic epithelial cells are able to partially metabolize in vivo ammonia to L-glutamine and L-arginine. We propose that these metabolic capacities are physiologically related to supply of activators for liver ureagenesis in a context of increased colonic luminal ammonia concentration. This corresponds to an emerging new interorgan metabolism between the colonic mucosa and liver cells. In addition, these metabolic capacities would play a role locally in the colonic epithelial cells by allowing a control of ammonia concentration in these cells during its transcellular journey. Indeed, since ammonia at excessive concentrations is able to interfere (at least in vitro) with colonocyte short-chain fatty acid oxidative metabolism (Cremin et al. 2003; Darcy-Vrillon et al. 1996) and ex vivo to disorganize the colonic epithelium morphology (Lin and Visek 1991a), this detoxication metabolism would represent a way to control ammonia intracellular concentration in colonocytes therefore limiting its deleterious effect at the colonic epithelial level.

Acknowledgments The authors wish to thank Jean-Claude Bernardin, Pierre Vaugelade and Christian Poirier for their expert contribution in animal experimentation. Mamy Eklou-Lawson was a recipient of a grant from the French Ministry of Foreign Affairs (Service de Coopération et d'Action Culturelle SCAC-French Embassy in Togo).

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