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Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences

Review Article

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Summary. Depending on the amount of alimentary proteins, between 6 and 18 g nitrogenous material per day enter the large intestine lumen through the ileocaecal junction. This material is used as substrates by the flora resulting eventually in the presence of a complex mixture of metabolites including ammonia, hydrogen sulfide, short and branched-chain fatty acids, amines; phenolic, indolic and N-nitroso compounds. The beneficial versus deleterious effects of these compounds on the colonic epithelium depend on parameters such as their luminal concentrations, the duration of the colonic stasis, the detoxication capacity of epithelial cells in response to increase of metabolite concentrations, the cellular metabolic utilization of these metabolites as well as their effects on colonocyte intermediary and oxidative metabolism. Furthermore, the effects of metabolites on electrolyte movements through the colonic epithelium must as well be taken into consideration for such an evaluation. The situation is further complicated by the fact that other non-nitrogenous compounds are believed to interfere with these various phenomenons. Finally, the pathological consequences of the presence of excessive concentrations of these compounds are related to the short- and, most important, long-term effects of these compounds on the rapid colonic epithelium renewing and homeostasis.

Keywords: Amino acids – Bacterial metabolites – Colon epithelium – Adaptative metabolism

Introduction

Although alimentary protein digestion followed by amino acids and oligopeptides absorption in the small intestine is an efficient process, substancial amounts of nitrogenous compounds from both exogenous and endogenous origins can enter the large intestine through the ileocaecal junction. This nitrogenous material which consists mainly in proteins and peptides (Chacko and Cummings, 1988) is quantitatively related to the amount of ingested proteins (Silvester and Cummings, 1995) and represents between 6 and 18 g per 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).

When present in the large intestine lumen, this material undergoes intense proteolysis by the colonic flora resulting in peptide and amino acid release followed by the production of numerous bacterial metabolites (Table 1) including ammonia, hydrogen sulfide, amines, short-chain and branched-chain fatty acids, phenols and organic acids (Macfarlane and Cummings, 1991). Several among them and depending on their luminal concentrations are believed to exert various effects on the colonic epithelial layer.

The aim of the present review is to give an overview on nitrogenous metabolism in the large intestine lumen in relationship with dietary conditions and to present some evidences that strongly suggest that the production of several metabolites at this site have consequences in term of colon physiopathology.

Nitrogenous metabolism in the lumen of the large intestine

The level of dietary protein consumption in the different countries is vastly different according to food availability and cultural dietary habits ranging from approximately 40 to more than 100 g per day (Claudian, 1992). In Western countries, alimentary proteins represent 80–100 g per day.

Table 1. Luminal metabolites which can be formed from amino acid precursors in the large intestine

Amino acid precursors	Metabolites formed
Alanine	acetate, ethylamine, propionate
Arginine	agmatine, nitric oxide, putrescine
Aspartate	acetate, succinate
Cystéine	sulfides
Glutamate	acetate, butyrate
Glycine	acetate, methylamine
Histidine	histamine
Isoleucine	2-methylbutyrate
Leucine	isovalerate
Lysine	acetate, butyrate, cadaverine
Méthionine	sulfides
Phenylalanine	phenylacetate, phenylethylamine, phenyllactate, phenylpropionate, phenylpyruvate
Threonine	acetate, propionate
Tryptophane	indole, indoleacetate, indolepropionate, 3-methylindole, tryptamine
Tyrosine	4-ethylphenol, hydroxyphenylacetate, hydroxyphenyllactate, hydroxyphenylpropionate, hydroxyphenylpyruvate, p-cresol, phenol, tyramine
Valine	isobutyrate, 2-methylbutylamine
Deamination of amino acids	ammonium
Deamination and fermentation of amino acids	H_2, CO_2, CH_4, lactate, succinate, formate, oxaloacetate

The protein digestibility for most proteins is equal or even higher than 90% (Baglieri et al., 1994; Gausseres et al., 1996; Gaudichon et al., 1999; Mariotti et al., 2001; Bos et al., 2005).

The undigested proteins together with endogenous proteins (i.e. pancretic secretory products, desquamated enterocytes, mucous proteins (Hoskins and Boulding, 1981; Macfarlane et al., 1986) enter the caecum. Then the luminal content is transferred to the right (or ascending) colon, to the transverse colon and to the left (or descending) colon before reaching the sigmoid colon and rectum (Christensen, 1991). In constrast to the rapid passage of luminal material in the small intestine, the transit of content in the large intestine is slowed down allowing large populations of bacteria to develop (Cummings, 1978). Interestingly, a longer colon transit time appears to lead to more extensive proteinaceous substrate breakdown (Macfarlane et al., 1989). The first event in protein degradation is hydrolysis of the polypeptides by proteases and peptidases. Large intestine proteases are more active at neutral or slightly alkaline pH (Macfarlane et al., 1988). Since luminal pH is more acid in the right than in the left colon, this is likely to play a role in protein degradation according to the anatomical part of the large intestine.

It is generally considered that colonic luminal amino acids are not available for significant absorption by the colonic epithelium (Darragh et al., 1994; Hume et al., 1993) and, if this proposition is correct, this would mean that amino acid nitrogen entering the large intestine is lost for protein synthesis in the body (Schaafsma, 2000). However, there is evidence which suggests that some absorption from the large intestine cannot be totally excluded (Metges, 2000). Firstly, experiments performed on pigs after infusion of proteins or amino acids into the large intestine lumen indicate whole body N balance improvement which may indicate amino acid colonic absorption (Fuller and Reeds, 1998). Furthermore, some absorption of microbial amino acids from the pig colon was suggested based on the appearance of ^{15}N-labeled amino acids in the venous blood after infusion of ^{15}N-labeled bacteria into the caecum (Niiyama et al., 1979). More recently, it has been documented that amino acids synthetized by the intestinal flora may be absorbed (Torrallardona et al., 1996; Metges et al., 1999; Millward et al., 2000) even if it remains unclear whether this absorption occurs exclusively in the small or also in the large intestine in humans. There is however some biochemical data in support of absorption of amino acids in the large intestine. The ATBo+ neutral and cationic amino acid transporter is found to be expressed in the colon (Nakanishi et al., 2001) being localized at the luminal membranes of colonocytes (Hatanaka, 2002). An other neutral and cationic amino acid transporter B^{o+} system is expressed on the apical surface of colonic absorptive cells (Ugawa et al., 2001). In any case, the amplitude of putative amino acid colonic absorption remains to be determined. In addition, the possibility that luminal amino acid would be used locally for protein synthesis in the rapidly renewed colonic epithelium is an important possibility that has been little evaluated (Backes et al., 2002). In contrast, there is a large body of litterature which indicates that amino acids are intensely metabolized by luminal bacteria and this represents most likely the main fate of luminal amino acids at this site. Bacterial amino acid metabolism is performed through a series of oxidative and reductive reactions including deamination, decarboxylation, fermentation and alpha and beta-elimination. From in vitro protein fermentation studies, there is some indication that reductive deamination represents the major route of amino acids degradation (Macfarlane and Allison, 1986).

In the next paragraph we present various metabolites derived from amino acids which are recovered in the large

intestine lumen with indication on the way they are produced and utilized, their physiopathological effects on the epithelium, the metabolic pathways involved in their intestinal detoxification and the capacity of epithelial cells to adapt to changes in their luminal concentrations.

Ammonia

The highest concentration of ammonia in the body is found in the large intestine lumen. Since NH_3/NH_4^+ has a pKa equal to 9.02 (Visek, 1968), the vast majority of total ammonia (considered as the sum of NH_4^+ and NH_3) is in the form of ammonium at pH found in the colonic lumen. NH_4^+/NH_3 concentrations are found to be between 10 and 70 mM in rats (Lin and Visek, 1991b; Mouillé et al., 2004) and between 2 and 34 mM in human faecal dialysates (Wrong et al., 1965). An increase in the amount of dietary proteins leads to a marked rise in large intestine NH_4^+/NH_3 content in animals (Mouillé et al., 2004) and in faecal NH_4^+/NH_3 concentrations in humans (Cummings et al., 1979; Geypens et al., 1997). In humans, ammonia luminal concentration progressively increase from the right to the left colon (Macfarlane et al., 1992). The luminal NH_4^+/NH_3 concentration in the large intestine is mainly the net result of the bacterial production and utilization as well as absorption through the large intestine epithelium. It is also possible, but remains to be demonstrated, that ammonia generated inside colonocytes from arterial L-glutamine may partly reach the lumen. In fact colonocytes strongly metabolize this amino acid resulting in ammonia production (Darcy-Vrillon et al., 1993). Bacterial flora produces NH_4^+/NH_3 through deamination of amino acids and to a lesser extent through urea hydrolysis catalysed by urease activity which is high in colon (Vince and Burridge, 1980; Wolpert et al., 1971; Cummings, 1975; Moran and Jackson, 1990; Wrong et al., 1985; Smith et al., 2004). Urea transporters are expressed in 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 circulation to the intestinal lumen. Several dietary compounds may affect ammonia concentration in the large intestine. High resistant starch consumption or a mixture of resistant starch together whith wheat bran resulted in decreased ammonia concentration in the colon lumen (Birkett et al., 1996; Heijnen et al., 1997; Govers et al., 1999). The fiber type is in addition an important parameter for the modulation of ammonia concentration since pectin increased colonic concentration and cellulose in contrast decreased it (Lupton and Marchant, 1989). Protein-restricted diet together with lactulose consumption has been reported to be a nutritionnal way to treat hyperammonemia (Weber, 1997) based on the fact that colon is the major site which generates ammonia. Lactulose increases the bacterial incorporation of ammonia (De Preter et al., 2006). Acidification which follows the metabolism of lactulose may have an additional effect on ammonia production and absorption (Vince and Burridge, 1980). However, as recently pointed out (Shawcross and Jalan, 2005), other factors including ammonia metabolism in the small intestine, kidneys and muscles are likely to be important in modulating the effects of hyperammonemia.

In any case, it is clear that large amounts of NH_4^+/NH_3 are absorbed through the large intestine mucosa. Approximately 4 g of ammonia are absorbed daily by the human colon (Summerskill and Wolpert, 1970; Wolpert et al., 1971). In rat distal colon, NH_3 is 400 times more permeant than NH_4^+ (Cohen et al., 1988). Colonic HCO_3^- secretion can modify NH_4^+/NH_3 absorption. It has been proposed that colonic HCO_3^- secretion titrates luminal NH_4^+ to NH_3 allowing the diffusion of this lipophilic weak base through membranes of surface epithelial cells (Wrong and Vince, 1984; Cohen et al., 1988). Recently, 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 can substitute NH_4^+ for K^+ (Codina et al., 1999). Interestingly, Singh et al. (1995) found that the apical membranes of crypt colonocytes had 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.

Ammonium chloride, when perfused at 35 mM concentration in the rat colon can exert deleterious effects on the colonic epithelium. After 3 h-treatment with NH_4Cl, the distal colon mucosa displayed histological damages. The epithelium was found to be disorganized and sloughing of epithelia cells was evidenced (Lin and Visek, 1991a). However, from experiments with isolated pig colonic-crypts, 50 mM NH_4Cl does not induce epithelial cell necrosis after 4 h-treatment (Leschelle et al., 2002). Intraperitoneal injection of increasing doses of urease for 24 h in mice resulted in a marked but reversible depression of tritiated thymidine incorporation into DNA of colon (Zimber and Visek, 1972), a result possibly due to

increased ammonia concentration in the colon lumen although this parameter was not measured in this study. Using the human colon adenocarcinoma cell line HT-29 $Glc^{-/+}$ as a model of colonocytes, it was shown that ammonium chloride at concentrations close to that found in the large intestine lumen i.e. 20 mM was able to increase the volume of vacuolar lysosomes and to repress markedly cell proliferation without affecting cell viability (Mouillé et al., 2003). Ingestion of high amounts of dietary proteins for 5 months which is associated with increased luminal concentration of ammonia, increases thymidine incorporation in colon mucosal cells (Lin and Visek, 1991b). However, in these experiments, it was not possible to attribute the observed effect solely to ammonia. Injection of 75 mM NH_4Cl in the isolated colon for 7 days resulted in a greater number of mitoses per crypt, a result which was interpreted as a compensatory phenomenom for the deleterious effect of ammonia (Ichikawa and Sakata, 1998).

Ammonia interferes with the oxidative metabolism of colonocytes since this metabolite is able to inhibit butyrate activation and/or butyrate β-oxidation in mitochondria (Darcy-Vrillon et al., 1996) and also acetate and propionate oxidation (Cremin et al., 2003). These latter substrates, which are produced within the large intestine lumen, are known as major fuels for colonic epithelial cells (Roediger, 1982; Ardawi and Newsholme, 1985). NH_4Cl, when provided at the luminal side of colonocytes induces slight modifications of the intracellular pH (Hasselblatt et al., 2000; Busche et al., 2002) in colonic epithelial cells and diminishes both chloride secretion (Prasad et al., 1995) and Na^+ absorption (Cermak, 2000). In a model of rodent with chemically-induced colon carcinogenesis, the intrarectal ammonium acetate infusion during 52 weeks resulted in an increase of colonic adenocarninoma (Clinton et al., 1988). It should be noted that in these experiments, it was not possible to distinguish between the effects of ammonium and acetate. Interestingly, in humans, ammonia concentration is highest in the distal part of the large intestine (Macfarlane and Cummings, 1991) where the highest incidence of neoplasms is found (Scheiden et al., 2000). However, this type of correlation obviously does not allow to propose any causal relationship between these two parameters but should stimulate novel investigations on that matter.

The capacity of isolated rat colonocytes to metabolize ammonia into L-citrulline has been demonstrated and is related to the presence of arginase, carbamoyphosphate synthetase I (CPS I) and ornithine carbamyol transferase (OCT) activities in these cells (Mouillé et al., 1999, 2004). However, the CPSI activity in rat colonocytes is very low since it represents only 0.3% of the activity measured in liver. Using isolated human colonocytes and colon biopsies collected from the healthy mucosa of patients undergoing colorectal tumour surgery, it was determined by real time quantitative PCR that CPS I and OCT mRNA were present in all biological preparations ($n = 10$). However, mRNA contents in these colonic samples were low when compared with the abundant mRNA contents in human liver ($n = 4$). Indeed, it represented 0.005 and 0.1% of hepatic CPSI and OTC mRNA, respectively (Blottière H, Jarry A and Blachier F; unpublished data).

By immunohistochemistry, carbonic anhydrase was found to be largely expressed in colonocytes from the surface epithelium with a predominant fraction of the enzyme being localized in the mitochondria (Saarnio et al., 1999). The authors proposed that the carbonic anhydrase function is to provide bicarbonate ions to CPS I allowing ammonia detoxication in colonocytes. The physiological relevance of such metabolic pathways for the control of mitochondrial NH_4^+/NH_3 concentration inside colonocytes requires further studies.

Hydrogen sulfide

Hydrogen sulfide (H_2S) is a bacterial metabolite present in the lumen of the large intestine which is produced through fermentation of sulfur-containing amino acids, through the reduction of inorganic sulfate and sulfited additives and through intestinal sulfomucin metabolism (Florin et al., 1991; Gibson et al., 1988; Roediger et al., 1997). The H_2S production appears to depend on dietary conditions since fasting was associated in rats with a marked reduction of caecal H_2S concentration (Suarez et al., 1998) and since in humans, faecal sulfide excretion was roughtly proportional to dietary protein intake (Magee et al., 2000). The H_2S concentration in the lumen of the human large intestine varies from 1.0 to 2.4 mM (Macfarlane et al., 1992) and sulfide faecal concentration in humans consumming a high meat diet represents as much as 3.4 mM (Magee et al., 2000). However, unbound H_2S in the lumen of the colon may represent a considerably lower concentration since there is a large capacity of faecal components to bind hydrogen sulfide (Jorgensen and Mortensen, 2001a) and since H_2S can bind to divalent cations (Suarez et al., 1998).

H_2S which is a toxicant known to disturb lung and brain functions (Nicholson et al., 1998; Grieshaber and Vökel, 1998) has been implicated in the etiology of ulcerative

colitis from several lines of evidence. Firstly, it has been documented that hydrogen sulfide has a number of adverse effects that could play a part in the pathogenesis of ulcerative colitis (reviewed in Pitcher and Pitcher, 1996). Secondly, faecal sulfide concentration and production were found to be elevated in patients with ulcerative colitis (Pitcher et al., 2000; Levine et al., 1998) even if in one study, no difference was found in faecal sulfide concentration between ulcerative colitis patients and control subjects (Moore et al., 1998). Thirdly, in experimental colitis animal models, it is possible to induce a pathological state similar to the one observed in ulcerative colitis using two forms of undigestible sulfates i.e. dextran sulfate sodium (Gaudio et al., 1999; Tamaru et al., 1993; Leung et al., 2000) and sulfate-containing carraghenan (Watt and Marcus, 1973).

Because of its lipid solubility, H_2S penetrates biological membranes (Reiffenstein et al., 1992) and has the capacity to severely inhibit cytochrome c oxidase activity; an effect characterized by an inhibitory constant similar to the one measured with cyanide (Petersen, 1977; Nicholls, 1975). In colonic epithelial cell homogenates, micromolar concentrations of the sodium salt of H_2S inhibit cytochrome c oxidase activity (Leschelle et al., 2005). Therefore, it appears that, as determined in the rat model (Levitt et al., 2002), the vast majority of luminal H_2S is most likely in a bound form which is presumed to exert little or no toxic effect on the colonic epithelium. An other known feature of sulfide is its capacity to inhibit n-butyrate oxidation in human epithelial colonic cells (Roediger et al., 1993; Hulin et al., 2002). Since butyrate is a major fuel in colonocytes (Ardawi and Newsholme, 1985; Roediger, 1982), this led to the hypothesis that sulfide may be an initiating event in ulcerative colitis (Roediger et al., 1993). Although it is clear that sulfide is able to inhibit short chain acyl-CoA dehydrogenase activity (Moore et al., 1997; Babidge et al., 1998; Shaw and Engel, 1987), this agent can act independently of the β-oxidation since it inhibits also L-glutamine and acetate oxidation in colonic epithelial cells (Leschelle et al., 2005). Several reports have shown that butyrate oxidation and ketone body production are decreased in colonocytes and biopsy specimens recovered from ulcerative colitis patients (Roediger, 1980; Chapman et al., 1994) and in colonocytes isolated from rodents after experimental colitis induction (Ahmad et al., 2000). However, it is worth noting that other authors found no difference in butyrate metabolism in colonocytes obtained from ulcerative colitis patients when compared with control subjects (Finnie et al., 1993; Clausen and Mortensen, 1995; Jorgensen et al., 2001b). Interestingly, using an in vivo test i.e. $^{14}CO_2$-breath test after rectal instillation of labelled butyrate, Den Hond et al. (1998) found that patients with active extensive ulcerative colitis have a decreased colonic butyrate oxidation. However, the fact that remission was associated with normal oxidation strongly suggests that ulcerative colitis mucosa is not intrinsically altered for butyrate oxidation. In other words, decrease in butyrate oxidation is probably not a primary defect in ulcerative colitis.

An important feature of the large intestine is its high capacity for sulfide detoxification. According to several studies, the primary large intestine metabolism of sulfide is done through methylation catalyzed by thiol S-methyltransferase in the presence of S-adenosyl-L-methionine (Roediger and Babidge, 2000; Weisiger et al., 1980). However, conversion of sulfide to thiosulfate appears to be the main process for sulfide detoxication. In fact, analysis of caecal venous blood obtained after intracaecal instillation of radioactive H_2S in rats reveals that H_2S is largely oxidized to thiosulfate (Levitt et al., 1999). The nature of such a conversion (i.e. spontaneous and/or enzymatic process) is not known but since heated caecal mucosal homogenate was unable to metabolize H_2S (Levitt et al., 1999), it is likely that thiosulfate synthesis is done through enzymatic catalysis. Lastly, rhodanese is an enzyme that has been detected in the submucosa and crypts of the colon and which is able to metabolize H_2S in vitro (Picton et al., 2002). However, since this enzyme requires cyanide for its activity, the relative physiological importance of such an enzyme for sulfide detoxification remains to be established. A recent in vitro study reported several adaptative capacities of human epithelial colonic cells (HT-29 cells) after 7 h-pretreatment in the presence of sulfide. Under such conditions, the cells shifted oxidative metabolism towards glycolysis and increased the proton leakage through the inner mitochondrial membrane (Leschelle et al., 2005). This latter effect was associated with an increased expression of the UCP2 protein. In the light of recent findings, these results may be interpreted as an adaptation which allows reduction of superoxide anion mitochondrial production which is favored in case of cytochrome c oxidase inhibition (Dawson et al., 1993; Papa et al., 1997; Ricquier and Bouillaud, 2000).

Altogether, it appears that the deleterious effect of sulfide on the large intestine epithelium depends on several parameters including the luminal concentration of unbound sulfide, the enzymatic detoxification potential of the mucosa and the adaptative capacities of the epithelial cells towards this metabolite. In support of the influence of dietary factors on the clinical course of

ulcerative colitis, it has been shown that consumption of proteins in the highest tertile of intake as well as high sulfur and sulfate consumption were associated with an increased risk of relapse in ulcerative colitis patients (Jowett et al., 2005).

Branched-chain fatty acids

Short-chain fatty acids (including branched chain fatty acids) are end products of bacterial fermentation in the large intestine in man and animal species (Elsden et al., 1946; Rechkemmer et al., 1988). Dietary substrates for short shain fatty acid production are mainly fibres, resistant starch and proteins (Mortensen and Clausen, 1996). The major short-chain fatty acids found in the human colon are acetate, propionate and butyrate (Macfarlane and Cumming, 1991). They can all be produced from amino-acids. Acetate can be produced by bacteria which are found in the human large intestine from glycine, alanine, threonine, glutamate, lysine, and aspartate (Barker, 1981; Elsden and Hilton, 1978). Butyrate can be produced from glutamate and lysine (Macfarlane and Cummings, 1991) and propionate can be synthetized from alanine and threonine (Macfarlane and Gibson, 1995). We will not discuss in this paper of acetate, propionate and butyrate which can originate from several alimentary compounds and which have been the subjects of several reviews (Sheppach, 1994; Kim, 2000; Lupton, 2004; Cuff and Shirazi-Beechey, 2004) but will concentrate on branched chain fatty acids. These latter luminal compounds originate exclusively from the breakdown of proteins and are not produced from carbohydrates (Rasmussen et al., 1998; Noorgaard et al., 1995) representing therefore good markers of protein breakdown. The branched-chain fatty acids i.e. isobutyrate, 2-methybutyrate and isovalerate are formed from valine, isoleucine and leucine, respectively. Branched-chain fatty acid concentrations increase progressively from the lumen of the proximal colon to the lumen of the distal part of the colon (Macfarlane and Cummings, 1991). They represent between 5 and 10% of total short-shain fatty acids (Mortensen and Clausen, 1996) which themselves average in the colon and faeces between 80 and 130 mmol/Kg contents (Macfarlane and Cummings, 1991). Faecal branched-chain fatty acid concentrations can be modified according to the presence of different dietary compounds. In humans, the consumption of 3 g fructooligosaccharides twice daily for 4 weeks resulted in a decreased faecal isovalerate concentration (Swanson et al., 2002). In an in vitro faecal incubation system, lactulose was able to completely inhibit isobutyrate and isovalerate production from albumin (Mortensen et al., 1990).

Little is known about the metabolism of branched chain fatty acids in colonic epithelial cells. Jackiewiecz et al. (1996) have shown that isolated colonocytes oxidize isobutyrate and produce 3-hydroxyisobutyrate as a metabolic end product. In this study, butyrate inhibited isobutyrate catabolism by colonocytes probably through competitive inhibition for the activation of isobutyrate to its CoA ester. This latter result led the authors to propose that isobutyrate may serve as a fuel in colonocytes when butyrate availability is defective.

Several reports indicate effects of branched-chain fatty acids on ionic movements through the colonic epithelial layer. Diener et al. (1993) have shown that incubation of rat isolated colonic crypts with isobutyrate activated the Na^+/H^+ exchanger resulting in an increase of the crypt diameter due to swelling of crypt cells. In mouse distal colon, apical incubation with isobutyrate produced an alkalinization of the crypt lumen and an acidification of the subepithelial tissue; a process which was related to the role of the colonic crypt lumen acting as a pH microdomain (Chu and Montrose, 1995). In the rat distal colon, isobutyrate is able to prevent the c-AMP-dependent induction of serosal to mucosal chloride flux (Dagher et al., 1996), to reverse the cGMP-induced chloride secretion in both proximal and distal colon of the rat (Charney et al., 1999) and to stimulate Na^+ absorption in the proximal colon (Zaharia et al., 2001). In a model of epithelial colonic cells, Mush et al. (2001) have demonstrated that isobutyrate can increase the activity and protein content of the Na^+/H^+ exchanger NHE_3 indicating that in vitro, this branched-chain fatty acid may act as a regulator of colonic Na^+ absorption.

When 5 mM isobutyrate or isovalerate were added to the culture medium, and in contrast with the effects of n-butyrate used at the same concentration, these branched-chain fatty acids exerted no measurable effect on proliferation, differentiation or apoptosis using in vitro models of colonic adenocarcinoma cells (Heerdt et al., 1994; Mac Bain et al., 1997; Siavoshian et al., 1997).

Polyamines

Numerous amines (including polyamines) have been detected in the gut luminal contents. Polyamines are polycationic compounds which are present in all tested mammalian cells. They have been the subject of numerous studies due to their strong requirement for cell growth (Pegg and Mc Cann, 1982), their involvement in cellu-

lar malignant transformation (Auvinen et al., 1992) and their ability to interfere with many cellular processes including stimulation of DNA, RNA and protein synthesis (Ginty et al., 1989; Balasundaram and Tyagi, 1991), stabilization of cellular membranes (Schuber, 1989) and DNA conformation (Howell et al., 1996; Thomas and Thomas, 2001). Intracellular polyamine concentrations are the net result of biosynthesis, degradation, recycling, uptake and release occuring through a complex serie of highly regulated molecular events (Heby and Persson, 1990; Pegg et al., 1989; Seidel and Scemama, 1997; Wallace and Caslake, 2001).

Surprisingly, polyamine metabolism in colonic epithelial cells has been the subject of a little number of studies despite their potential relevance for the physio-pathology of the large intestine. In human colonocytes, intracellular polyamine concentrations are in the range of 100–300 nmoles per mg protein with the following hierarchy spermine > spermidine > putrescine (Elitsur et al., 1992). Although colonocytes are able to convert arginine to ornithine and urea through arginase activity (Mouillé et al., 1999), little ornithine is used for putrescine spermidine and spermine synthesis due to a very low ornithine decarboxylase (ODC) activity (Elitsur et al., 1992; Mouillé et al., 2004). Similar ODC activities were measured in human colonocytes recovered from the upper and lower crypt regions (Elitsur et al., 1993). Therefore, it appears that intracellular polyamine content in colonocytes would originate mainly from exogenous sources. Blood polyamines are probably a minor source for colonocytes since in serum, polyamines represent not more than 0.5 μM concentration (Bartos et al., 1977). In contrast, luminal putrescine, spermidine and spermine which average approximately 700, 100 and 10 μM, respectively in the rat chyme (Osborne and Seidel, 1990) represent an extracellular source of these polycations for colonocytes. Luminal polyamines can originate from 4 sources: 1. Dietary origin i.e. polyamines which have not been absorbed and which pass the ileo-caecal junction 2. Polyamines in endogenous secretion (Loret et al., 2000). 3. Net synthesis of polyamines from the colonia flora 4. Polyamines released from desquamated colonocytes in the course of epithelium renewal (Mc Cormack and Johnson, 1991). Although the relative contribution of these 4 sources is not known, the experiments performed with rats receiving a polyamine-deficient diet for long term periods and which indicate that it resulted in hypoplasia of colonic mucosa with an associated decreased DNA and protein contents (Löser et al., 1999) indicate that luminal polyamines from dietary origin are important factors for colonic mucosa renewal. In humans, it has been determined that between 350 and 500 μmol polyamines are ingested daily for individuals on a Western diet (Bardocz et al., 1995).

Colonic bacteria may also represent a source of luminal amines since the microbial flora present in the distal part of intestine is able to produce various compounds including putrescine, agmatine, cadaverine, tyramine and histamine (Gale, 1940; Macfarlane and Cummings, 1991). However, when large doses of antibiotics were administered to rats together with a polyamine-deficient diet, the effect on the colonic mucosa i.e. hypoplasia was not much different than the one obtained using solely polyamine-deficient diet (Löser et al., 1999) indicating that bacterially-derived polyamines are less important than those derived from the diet. Luminal polyamines are known to be involved in large intestine physiology. They are implicated in fluid secretion by colonic crypts (Cheng et al., 2004) and in postprandial colonic motility (Fioramonti et al., 1994).

From a pathological point of view, several studies have reported that dietary polyamines are involved in the growth of chemically-induced preneoplastic colonic lesions in experimental animals (Paulsen et al., 1997; Duranton et al., 1997) indicating that luminal polyamines are likely to favour such epithelial modifications. These results are consistent with the fact that colonocytes isolated from cancerous areas had higher ODC activity and polyamine content than colonocytes isolated from the adjacent normal colonic mucosa (Elitsur et al., 1992); a situation which itself is in accordance with the strict requirement of polyamines for human colon adenocarcinoma cell growth (Gamet et al., 1991; Blachier et al., 1995).

In addition to putrescine, spermidine and spermine which may be considered as ''classical'' polyamines, agmatine has been the subject of recent studies regarding its metabolism in the body and its physiological functions (reviewed in Grillo and Colombatto, 2004; Morris, 2006). Agmatine is present in food from vegetal and animal origins especially in fermented food products (Novella-Rodriguez et al., 2000; Okamoto et al., 1997; Kirsbaum et al., 2000) and can be produced and degraded by several bacterial strains (Roon and Barker, 1972; Simon and Stalon, 1982; Shaibe et al., 1985; Satishchandran and Boyle, 1986; Panagiotis et al., 1987; Karlsson et al., 1988; Arena and Manca de Nadra, 2001). Agmatine is detected in human faecal material (Molderings et al., 2003) and we were able to measure luminal agmatine in the different anatomical parts of the large intestine of rats fed standard rat dry food containing 23% proteins (V Carbon and F Blachier, unpublished data) using per-

chloric extraction and HPLC with o-phthaldialdehyde derivatization procedure. Thus agmatine concentrations in the caecum, proximal, median and distal colon lumen averaged 180 ± 80; 130 ± 40; 360 ± 70 and 160 ± 80 μM, respectively ($n = 4$ animals).

To the best of our knowledge, there is no data on the metabolism of agmatine in normal colonocytes but experiments with colonic epithelial cells originating from human colonic adenocarninomas have revealed that agmatine is able to accumulate in such cells and to slow down cell growth at concentrations between 100 and 1000 μM (Heinen et al., 2003; Mayeur et al., 2005). This effect on cell proliferation coincided with modification of cell cycle effector expression, accumulation in the S and G2/M phases and reduction of the rate of DNA synthesis (Mayeur et al., 2005). Interestingly, agmatine content in human colon carcinoma tissue was reduced when compared with adjacent macroscopically normal colonic tissue (Molderings et al., 2004). Using HT-29 colonic tumoral cells, it was determined that these cells did not synthetize agmatine from arginine and little degraded agmatine through agmatinase activity (Mayeur et al., 2005).

Phenolic and indolic compounds

Degradation of aromatic amino acids in the large intestine lumen produces phenolic and indolic compounds. Phenylalanine catabolism leads to phenyl-containing compounds i.e. phenylpyruvate, phenyllactate, phenylacetate, and phenylpropionate (Macfarlane and Cummings, 1991). Tyrosine luminal degradation leads to hydroxyphenyl-containing molecules including hydroxyphenylpyruvate, hydroxyphenyllactate, hydroxyphenylpropionate and hydroxyphenylacetate. Other metabolites are also formed from tyrosine like phenol, p-cresol and 4-ethylphenol (Bone et al., 1976). Phenols are the major products of aromatic amino acid metabolism in the distal bowel and phenol concentrations appears to be higher in the left (distal) colon than in the proximal colon (Hughes et al., 2000). In vitro, phenol can react with nitrite to produce ρ-diazoquinone with mutagenic activity (Kigugawa and Kato, 1988). Tryptophan bacterial metabolism generates low concentrations of 3-methyl indole (skatole), indole acetate and indole propionate together with relatively high concentrations of indole (Claus and Raab, 1999; Yokoyama and Carlson, 1979; Smith and Macfarlane, 1996; Karlin et al., 1985). Indole concentration is higher in the mouse distal large intestine than in the caecum and proximal colon (Whitt and Demoss, 1975). Skatole concentration increases in the large intestine lumen after a high meat diet consumtion or when luminal fermentation increases as a result of longer intestinal stasis (Yokoyama and Carlson, 1979). The faecal phenol and p-cresol excretion and concentration can be diminished by a high resistant starch diet (Birkett et al., 1996) and p-cresol faecal concentration is found to be increased when a 25% casein diet is ingested versus a 15% casein diet (Toden et al., 2005). It has been proposed that conjugation of phenol with sulphate in the colonic mucosa represent a detoxification process against the putative deleterious effect of this compound against colonic epithelial cells (Ramakrishna et al., 1991). Overall, there is a paucity of data on the effects of phenolic and indolic compounds on the colon epithelium.

Nitric oxide, nitrite and N-nitroso compounds

Nitric oxide (NO) can be detected in the lumen of the large intestine (Lundberg et al., 1994). This metabolite is produced mainly in the colonic mucosa through the conversion of L-arginine to NO by NO synthase (NOS) activity. Indeed, a ''constitutive'' activity of the inducible isoform of NOS (i NOS) has been detected in the normal colonic mucosa (Boughton-Smith et al., 1993; Guihot et al., 2000). There is also a possibility that some luminal NO may be from bacterial origin since bacteria are known to produce NO from nitrate and nitrite (Brittain et al., 1992; Goretski et al., 1990; Sobko et al., 2004). Furthermore, using colon epithelial (Caco-2) cells, Witthöf et al. (1998) have shown that some strains of bacteria are able to upregulate i NOS expression in epithelial cells, a phenomenom which was associated with increased release of the stable end product nitrite through the apical membranes.

There is clear evidence that the expression of the inducible form of NOS is increased in the colonic mucosa of ulcerative colitis patients and experimental primates (Rachmilewitz et al., 1995b; Ribbons et al., 1995; Boughton-Smith et al., 1993; Dijkstra et al., 1998; Leonard et al., 1998; Kolios et al., 1998; Guihot et al., 2000). This coincides with increased colonic luminal NO concentration in ulcerative colitis patients compared with control subjects (Lundberg et al., 1994) and increased L-citrulline concentration (i.e. the co-product of NOS activity) in rectal biopsy specimens recovered from patients with active ulcerative colitis when compared in those from patients with quiescient disease or with normal mucosa histology (Middleton et al., 1993). Induction of i NOS in the inflamed human colonic epithelium is associated with the formation of peroxynitrite and nitration of

cellular proteins (Singer et al., 1996). Peroxynitrite is an oxidant formed through reaction of NO with superoxide which reacts with tyrosine creating nitrotyrosine (Beckman and Koppenol, 1996). Studies with animal models of chemically-induced colitis are in accordance with human data since experimental colitis is associated with increased i NOS expression in the colonic mucosa (Boughton-Smith et al., 1994; Seo et al., 1995; Ferretti et al., 1997). In such models, pharmacological inhibition of NO synthesis is associated with an amelioration of the tissue injury (Rachmilewitz et al., 1995a; Naito et al., 2001; Hogaboam et al., 1995) raising the view that an excess mucosal NO production may be implicated in ulcerative colitis etiology. This view has however been challenged since i NOS-deficient mice were found to present increased colonic macroscopic damage when treated chemically in order to induce colonic inflammation when compared with wild-type mice (Mc Cafferty et al., 1997). A plausible explanation for such discrepancy would be that a drastic reduction of mucosal NO production would favour gut inflammation. This hypothesis would be in accordance with the known effect of NO on the maintenance of intestinal epithelium integrity (Mac Kendrick et al., 1993; Miller et al., 1993) and permeability (Kubes, 1992, 1993).

Luminal nitrite, in addition to represent and end-product of NO produced by the colonic mucosa can originate from other sources. If very little nitrate and nitrite seems to enter the colon from the small intestine (Florin et al., 1990), secretion of blood nitrate into the intestinal lumen may partly explain the faecal concentrations of nitrate and nitrite in humans (Witter et al., 1979). The situation is complicated by the fact that colonocytes can oxidize nitrite to nitrate (Roediger and Radcliffe, 1988). In addition, one has to consider the endogenous formation of nitrate (Green et al., 1981) and the L-arginine/NO pathway as a source of plasma nitrite (Rhodes et al., 1995). Nitrite and nitrate ions are involved in colon physiology since the former stimulates colonic bicarbonate secretion (Radcliffe et al., 1987) and the latter decreases colonic chloride absorption (Wurmli et al., 1987).

Nitrite can act as a substrate for nitrosamine production through its condensation with a secondary amine (e.g. L-proline) under acidic conditions which usually do not occur in the colon lumen or, more likely through the reaction catalysed at neutral pH by bacterial enzymes (Calmels et al., 1985). This nitrosation reaction depends on both luminal nitrite concentration and on bacterial reduction of nitrate to nitrite (Bingham, 1988). Nitrosamines have been detected in human faeces (Wang et al., 1978; Suzuki and Mitsuoka, 1981). In these early studies, the levels detected were low and artifactual interferences could not be excluded (Lee et al., 1981; Suzuki and Mitsuoka, 1985). In more recent studies, increased meat consumption was shown to increase faecal apparent total N-nitrosocompounds from precursors including N-glycosylamino acids and peptides (Mirvish et al., 2002; Hughes et al., 2001; Bingham et al., 1996). Tyramine, an amine produced by large intestine bacteria from tyrosine (Macfarlane and Cummings, 1991) can also react with nitrite to yield 3-diazotyramine with mutagenic properties (Sugimura, 2000). The toxicological consequences of increased faecal apparent individual N-nitroso-compounds on the colonic epithelium are not known since the methods used for detection give no information on the individual N-nitrosocompounds present (Hughes et al., 2000).

Overall effects of luminal protein degradation products on the colonic epithelium

If, as described above, there is no doubt that many individual luminal compounds generated from various amino acids can affect colonic epithelial cell metabolism and physiology, the long term in vivo effects of an increase of such compounds on the colonic epithelium as a consequence of a high intake of alimentary proteins remain an unresolved question. This latter question is clearly related to the protein consumption in areas such as Western Europe and United States which is relatively high (Metges and Barth, 2000) since it averages approximately two times the recommended intake (i.e. 0.83 g/kg/day; Rand et al., 2003) and may even represents four times this value in slimming high protein diet.

Concerning the possible relationship between the amount of alimentary proteins and the risk of developing colo-rectal neoplasms, epidemiological studies have failed so far to reach a consensus (American Institute for Cancer Research, 1997). Indeed, epidemiological studies report either a positive association between the level of protein intake and colorectal cancer (i.e. increased risk; Jain et al., 1980; Potter and Mc Michael, 1986; Lyon et al., 1987; Slattery et al., 1988; West et al., 1989; Gerhardsson de Verdier et al., 1990; Benito et al., 1991); no association (Tuyns et al., 1987; Whitemore et al., 1990; Peters et al., 1992; Meyer and White, 1993; Bostick et al., 1994; Giovanucci et al., 1994; Goldhom et al., 1994; Cross et al., 2003) or negative association (i.e. decreased risk; Lee et al., 1989). It is worth noting that the data presented are very mixed depending on the epidemiological nature

of the studies and on the source and amount of protein rich foods examined.

This is clearly not to deny that environmental factors (particularly alimentary factors) deeply interfere with the process of colorectal carcinogenesis as strongly suggested by several studies (Lichtenstein et al., 2000; Willett, 2002; Jänne and Mayer, 2000). However, given the complexity of a Western diet, it is not surprising that the epidemiological identification of alimentary compounds implicated in such a process remains a difficult task.

As reviewed by Mc Intosh and Le Leu (2001), experimental works with animal models of chemically-induced colonic preneoplasic or neoplasic lesions have evidenced that dietary proteins and individual amino acids can influence cancer risk, some having a promotional influence and others a preventive effect relative to an arbitrarily established standart diet.

Conclusion

Amino acids released from proteins originating from alimentary and endogenous sources serve as precursors for the production of numerous luminal metabolites by the colonic flora. Although only a relatively small part of these metabolites were studied, there are clear indications that some of them when present in excess can deeply interfere with colonic epithelial cell metabolism and physiology. In contrast, some of these metabolites have been shown to participate in the physiological functions of the colonic epithelium. The situation is however complicated by the fact that several alimentary compounds other than proteins are able to interfere with the process of production of these amino acid-derived metabolites. Furthermore, most of the studies have produced data related to the effects of individual metabolites, and very few studies have examined the overall effect on the colonic epithelium of several versus individual compounds in term of beneficial or deleterious effects.

Additional studies are then needed to decipher more exhaustively the complex luminal composition in the different colonic anatomical segments in amino acid-derived metabolites. Lastly, little is known on the metabolic capacities of colonic epithelial cells for detoxification of deleterious compounds and on the adaptability of such metabolic pathways for such a process. The effects of these compounds on cell metabolism and on the process of rapid colonic epithelium renewal (i.e. cell cycle, differentiation, migration along the colonic crypt and anoikis (detachment-induced apoptosis)) need also to be more deeply investigated.

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