Amino Acids (2012) 42:375–383
DOI 10.1007/s00726-010-0814-x

ORIGINAL ARTICLE

Effects of essential amino acids or glutamine deprivation on intestinal permeability and protein synthesis in HCT-8 cells: involvement of GCN2 and mTOR pathways

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Received: 1 September 2010 / Accepted: 16 November 2010 / Published online: 28 November 2010

Abstract GCN2 and mTOR pathways are involved in the regulation of protein metabolism in response to amino acid availability in different tissues. However, regulation at intestinal level is poorly documented. The aim of the study was to evaluate the effects of a deprivation of essential amino acids (EAA) or glutamine (Gln) on these pathways in intestinal epithelial cells. Intestinal epithelial cell, HCT-8, were incubated during 6 h with 1/DMEM culture medium containing EAA, non EAA and Gln, 2/with saline as positive control of nutritional deprivation, 3/DMEM without EAA, 4/DMEM without Gln or 5/DMEM without Gln and supplemented with a glutamine synthase inhibitor (MSO, 4 mM). Intestinal permeability was evaluated by the measure of transepithelial electric resistance (TEER). Using [L-$^{2}H_{3}$]-leucine incorporation, fractional synthesis rate (FSR) was calculated from the assessed enrichment in proteins and free amino acid pool by GCMS. Expression of eiF2α (phosphorylated or not), used as marker of GCN2 pathway, and of 4E-BP1 (phosphorylated or not), used as a marker of mTOR pathway, was evaluated by immunoblot. Results were compared by ANOVA. Six-hours EAA deprivation did not significantly affect TEER and FSR but decreased p-4E-BP1 and increased p-eiF2α. In contrast, Gln deprivation decreased FSR and p-4E-BP1. MSO induced a marked decrease of TEER and FSR and an increase of p-eiF2α, whereas mTOR pathway remained activated. These results suggest that both mTOR and GCN2 pathways can mediate the limiting effects of Gln deprivation on protein synthesis according to its severity.

Keywords Amino acids · Cellular signaling · Glutamine · Intestine · Nutrition

Abbreviations

EAA	Essential amino acid
eaaF	Essential amino acid-free medium
GlnF	Glutamine-free medium
GlnF(+)	Glutamine-free medium supplemented with glutamine synthase inhibitor
MSO	Methionine sulfoximine

Introduction

The regulation of intestinal permeability plays a major role in the organism defense. Stress events, chronic inflammatory diseases or infections are mainly associated with an increase of intestinal permeability and then to a deregulation of gut homeostasis (Turner 2009). Intestinal barrier is regulated by a balance on one hand between cell proliferation and apoptosis and, on the other hand, between protein synthesis and degradation. Protein fractional synthesis rate (FSR) approached 50%/day in the human duodenal mucosa (Nakshabendi et al. 1996; Coeffier et al. 2003), a value much higher than that of other major tissues such as liver or muscle. Previous studies reported that nutritional states or

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interventions can affect gut protein synthesis (Bouteloup-Demange et al. 1998; Adegoke et al. 2003; Winter et al. 2007; Coeffier et al. 2003; Coeffier et al. 2008; Le Bacquer et al. 2003). However, the effects of amino acids deprivation on intestinal protein synthesis are poorly documented. Gastric and duodenal FSR are not affected by a severe malnutrition in anorectic patients (Winter et al. 2007). In contrast, Le Bacquer et al. (2001) reported that glutamine (Gln) deprivation decreases FSR in Caco-2 cells. Threonine limitation limits protein synthesis in jejunal mucosa of growing pigs (Wang et al. 2007) but not in rats (Faure et al. 2005). In addition, involved signaling pathways remain unknown.

Two major pathways are involved in the regulation of initiation and elongation of protein synthesis: mTOR (mammalian target of rapamycin) and GCN2 (general controller non-derepressible 2 kinase) pathways. In response to growth factors or hormones, mTOR pathway stimulates protein synthesis. Briefly, activated mTORC1 complex causes phosphorylation of S6 kinase and translation factor 4E-BP1. Phosphorylated 4E-BP1 releases eIF4E, which initiates with other factors translation. Nutrients and in particularly amino acids are able to activate mTOR pathway (Kim et al. 2008; Sancak et al. 2008). In intestinal cells, leucine and arginine have been shown to activate mTOR pathway (Nakajo et al. 2005; Rhoads et al. 2006) but not glutamine (Nakajo et al. 2005; Rhoads and Wu 2009). In contrast, GCN2 pathway is activated in response to a limitation of amino acids availability. The kinase GCN2 is activated by free tRNA accumulation during amino acid deprivation. Then, activated GCN2 phosphorylates the initiation factor eiF2α that impairs protein synthesis. GCN2 activation by amino acids deprivation have been previously reported for instance in the brain (Maurin et al. 2005) or in myoblasts (Deval et al. 2008). In astrocytes, GCN2 activation by limitation of arginine availability has been also reported (Lee et al. 2003). In intestinal cells, to our knowledge, there are no data showing GCN2 activation in response to amino acids limitation.

Thus, the major aim of the present study was to assess the effects of a deprivation of essential amino acids (EAA) or of Gln on protein synthesis and on mTOR and GCN2 pathways in intestinal epithelial cells.

Materials and methods

Cell culture

Cultures of the human intestinal epithelial adenocarcinoma cell line HCT-8 (European Collection of Animal Cell Cultures, Salisbury, UK) were used between passages 30 and 40. Cell culture reagents, Dulbecco's modified Eagle medium (DMEM), and amino acids (AA)-free DMEM, Gln, and fetal calf serum were supplied by Eurobio (Les Ulis, France). Non-essential amino acids (NEAA solution 100X), essential amino acids, and methionine sulfoximine (MSO) were supplied by Sigma-Aldrich (St Quentin Fallavier, France). EAA solution 100X (L-isoleucine 10.5 g/L; L-leucine 10.5 g/L; L-lysine 14.6 g/L; L-methionine 3 g/L); L-phenylalanine 6.6 g/L; L-threonine 9.5 g/L; L-tryptophan 1.6 g/L; L-valine 9.4 g/L) was prepared, filtered and stored at −20°C until use. Cells were routinely grown in supplemented DMEM as previously described (Hubert-Buron et al. 2006; Marion et al. 2005). Then, cells were seeded in 6-well plates on microporous filters to obtain cell monolayer. Briefly, 1×10^6 cells were suspended in 1 ml of culture medium and added to the upper chamber of each porous filter (0.4 µm pore size; Millipore Merck). Four milliliters of cell-free culture medium was added to the lower chamber. The Transwell plates were then incubated at 37°C in an atmosphere of 5% CO2 in air and 95% humidity. The culture media were changed every day. Confluent cell monolayers were obtained within 12–14 days and checked by the measure of the transepithelial electrical resistance (TEER).

Treatments

Culture medium was replaced at the apical side during 6 h with: 1/AA-free DMEM supplemented with 1% of EAA, 1% of NEAA and 2 mM of Gln (called control), 2/with saline as positive control of nutritional deprivation (called saline), 3/AA-free DMEM supplemented with 1% of NEAA and 2 mM of Gln but without EAA (called eaaF), 4/AA-free DMEM supplemented with 1% of EAA, 1% of NEAA but no Gln (called GlnF) or 5/AA-free DMEM supplemented with 1% of EAA, 1% of NEAA but no Gln and supplemented with a glutamine synthase inhibitor, MSO at 4 mM (called GlnF(+)). The cells were washed with PBS then centrifuged at 1,000*g* for 10 min and the cell pellets stored at −80°C for protein measurement.

Assessment of TEER

Before and after treatment, TEER was measured using an epithelial volt-ohm meter (Millipore Merck).

Protein FSR

To calculate fractional synthesis rate, [L-$^2H_3$]-leucine (99% MPE; Mass Trace) was added in the culture medium as previously described (Le Bacquer et al. 2001). Briefly, 1 µmol/mL [L-$^2H_3$]-leucine was simultaneously added during the nutritional treatments during 6 h. After treatment, cells were washed three times with ice-cold PBS and

then scrapped in 500 µL of per-chloro acetic acid. The protein pellet containing isotopically enriched proteins was dissolved in 1 M NaOH and then hydrolysed in 6 M HCl at 110°C for 18 h to allow analysis of the enrichment of amino acid released from protein hydrolysis.

The enrichments of [L-$^2H_3$]-leucine were determined in the intracellular free amino acid pools and in the proteins by gas-chromatography-mass spectrometry (GC–MS) (MSD 5972, Hewlett Packard, Palo Alto, CA, USA), using *tert*-butyldimethylsilyl (*t*-BDMS) derivatives as previously (Bouteloup-Demange et al. 2000; Coeffier et al. 2008). Appropriate standard curves were run simultaneously for determination of the enrichments. The fractional synthesis rate of protein was calculated as follows: FSR (%/day) = $[(E_t - E_o)/E_p] \times 1/t \times 24 \times 100$, where E_t is the enrichment in proteins at time t, in %. E_o is the natural abundance of the labeled amino acid in cells, in %. E_p is the enrichment of the intracellular free amino acid precursor pool at time t, in %. "t" is the duration of the tracer incubation, in hour.

Western blot analysis

Proteins (25 µg) were separated on 4–12% Tris–Glycine resolving gels (Invitrogen, Cergy-Pontoise, France) and transferred to a nitrocellulose membrane (GE Healthcare, Orsay, France), which was blocked for 1 h at room temperature with 5% (w/v) non-fat dry milk in TBS (10 mmol/L Tris, pH 8; 150 mmol/L NaCl) plus 0.05% (w/v) Tween 20. Then, an overnight incubation at 4°C was done with goat polyclonal antibody anti-4E-BP1 (sc-6025, SantaCruz Biotechnology, Tebu-bio, Le Perray en Yvelines, France), rabbit polyclonal antibodies anti-eiF2α (sc-11386, SantaCruz Biotechnology), anti-phosphorylated-4E-BP1 (Ser65, sc-18091-R, SantaCruz Biotechnology) and anti-phosphorylated-eiF2α (Ser51, #9722, Cell Signaling Technology), and mouse polyclonal antibody anti-β-actin (Sigma-Aldrich). After three washes in a blocking solution of 5% (w/v) non-fat dry milk in TBS/0.05% Tween 20, 1 h incubation with peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (1:5,000, SantaCruz Biotechnology) was performed. After three additional washes, immuno-complexes were revealed by using the ECL detection system (GE Healthcare). Protein bands were quantified by densitometry using ImageScanner III and ImageQuant TL software (GE Healthcare).

qRT-PCR

After reverse transcription of 1.5 µg total RNA into cDNA by using 200 units of SuperScript™ II Reverse Transcriptase (Invitrogen) as previously described (Leblond et al. 2006), qPCR was performed by SYBR™ Green technology on Bio-Rad CFX96 real time PCR system (Bio-Rad Laboratories, Marnes la Coquette, France) in duplicate for each sample as previously described (Coeffier et al. 2010). GAPDH was used as the endogenous reference gene. Specific primers were for ATF4, 5′-TGAAGGAGTTCGACTTGGATGCC-3′ and 5′-CAGAAGGTCATCTGGCATGGTTTC-3′ and for GAPDH, 5′-TGCCATCAATGACCCCTTCA-3′ and 5′-TGACCTTGCCCACAGCCTTG-3′.

Statistical analysis

Results are expressed as means ± SEM for the indicated number of independent experiments. Statistical analysis was performed using GraphPad Prism 5.01 (GraphPad software Inc, San Diego, CA, USA) and consisted in one-way ANOVA with Tukey post-tests. The level of statistical significance was fixed at $p < 0.05$.

Results

Transepithelial electric resistance and protein fractional synthesis rate

As positive control of deprivation, 6-h incubation of cells with saline induced a marked decreased of TEER (−196 ± 21 Ω, Fig. 1a) and of FSR (−2.2 fold change, Fig. 1b). Surprisingly, EAA deprivation did not significantly affect TEER and FSR (Fig. 1). In contrast, Gln deprivation (GlnF) did not significantly affect TEER but decreased FSR (−1.3 fold change, Fig. 1). A complete Gln depletion by the addition of MSO (GlnF(+)) decreased TEER (−136 ± 12Ω) and more markedly FSR (−3.7 fold change, Fig. 1). TEER was similar between complete Gln depletion and saline conditions, whereas FSR was more markedly decreased after complete Gln depletion compared with saline.

eiF2α expression

In control conditions, eiF2α was constitutively expressed and mainly in unphosphorylated form (Fig. 2a), suggesting that GCN2 pathway was not activated. The expression of p-eiF2α was similarly increased by saline, eaaF and GlnF(+) conditions (Fig. 2a, b). In contrast, Gln deprivation (GlnF) did not modify p-eiF2α expression. The expression of unphosphorylated eiF2α was not affected by any treatments (Fig. 2c). We also assessed ATF4 transcription factor mRNA level which presented the same pattern than p-eiF2α (data not shown). These results suggest that EAA depletion or severe Gln deprivation may activate the GCN2 pathway. However, to our knowledge, eiF2α expression was only reported in cancerous intestinal cell lines (Hu et al. 2010) or colonic epithelial cells (Yang et al. 2010). We thus determined the expression of p-eiF2α in several tissues and in particularly in the intestinal tract of

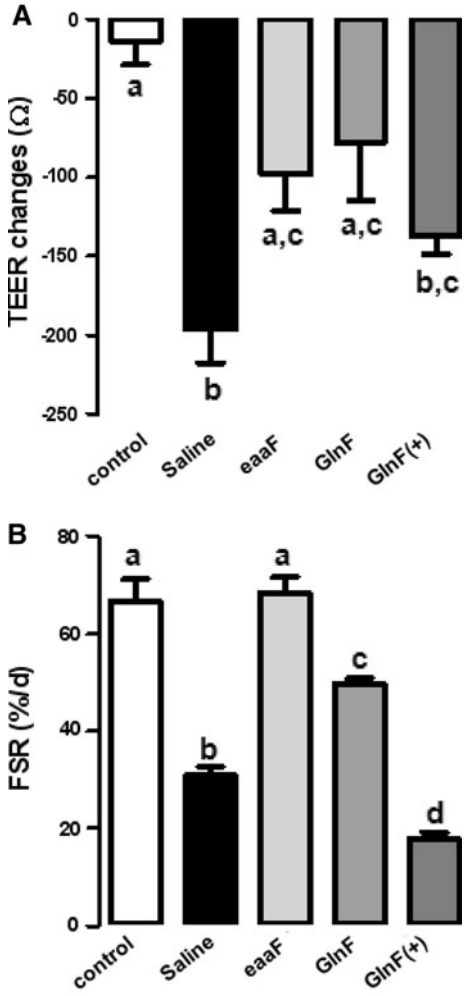

Fig. 1 Effects of essential amino acid or glutamine deprivation on transepithelial electric resistance variation and protein synthesis in HCT-8 cells. **a** Transepithelial electric resistance (TEER, Ω) was measured before and after 6-h treatments. **b** Protein fractional synthesis rate (FSR, %/day) was calculated after 6 h treatments using [L-$^{2}H_{3}$]-leucine incorporation. Values (means ± SEM, $n = 6$) without a common letter differ ($p < 0.05$). eaaF, essential amino acid-free medium; GlnF, glutamine-free medium; GlnF(+), glutamine-free medium supplemented with glutamine synthase inhibitor

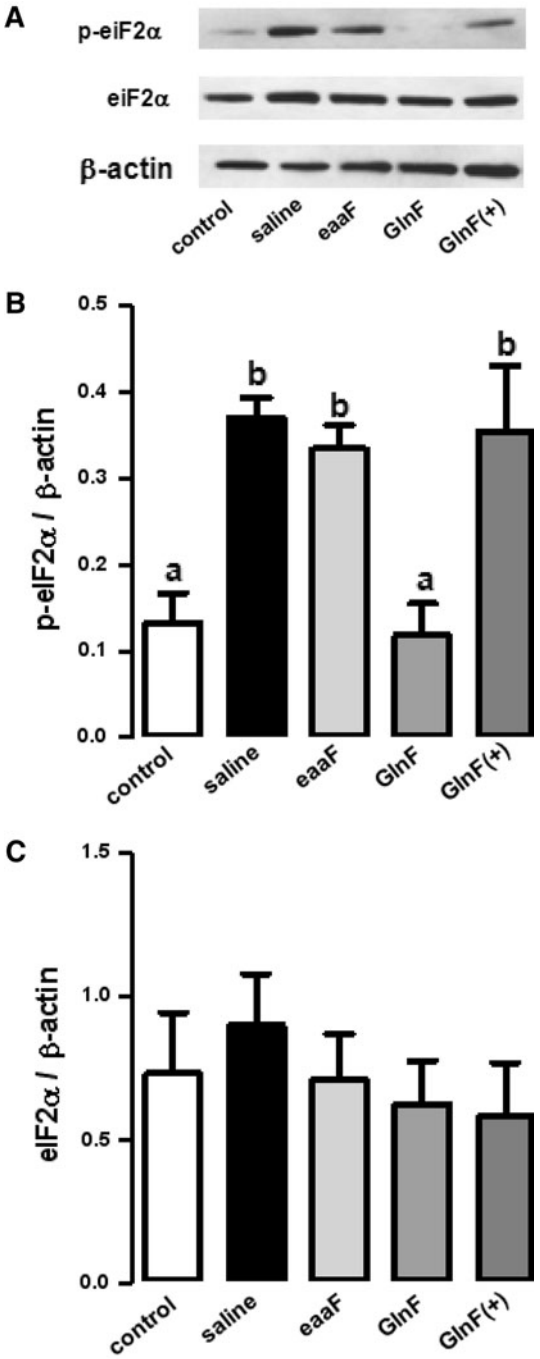

Fig. 2 Effects of essential amino acid or glutamine deprivation on eiF2α expression. **a** Representative pictures of p-eiF2α, eiF2α and β-actin immunoblots. **b** Densitometric analysis of p-eiF2α expression and **c** eiF2α expression. Values (means ± SEM, $n = 6$) without a common letter differ ($p < 0.05$). eaaF, essential amino acid-free medium; GlnF, glutamine-free medium; GlnF(+), glutamine-free medium supplemented with glutamine synthase inhibitor

12-h fasted rats and in human duodenal and colonic mucosa. As shown in the Fig. 3, p-eiF2α was expressed in brain, heart, lung and liver as previously reported (Maurin et al. 2005; Chotechuang et al. 2009; Drogat et al. 2007; Crozier et al. 2005) and in stomach, jejunum, ileum and colon from fasted rats but not in the duodenum. We also observed p-eiF2α in the colon but not in the duodenum of fasted humans.

4E-BP1 expression

In control conditions, 4E-BP1 was mainly expressed in its phosphorylated form (Fig. 4), suggesting that the mTOR pathway was constitutively activated. The expression of p-4E-BP1 was markedly decreased in saline, eaaF and GlnF conditions compared with control and unphosphorylated form of 4E-BP1 was significantly increased after

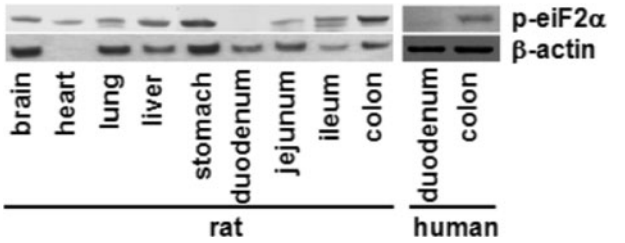

Fig. 3 p-eiF2α expression in the intestinal tract. Representative immunoblots of p-eiF2α and β-actin expression in different tissues from fasted rats ($n = 3$) and in human duodenum and colon

saline but not in other conditions. In contrast, p-4E-BP1 expression was not affected by complete Gln deprivation (GlnF(+)). Thus, during a complete nutritional deprivation (saline), 4E-BP1 was mainly present in its unphosphorylated form in accordance with the marked decrease of FSR and TEER. We also observed that 4E-BP1 was mainly present in its phosphorylated form after incubation of cells with 2 mM Gln (control) and after severe Gln depletion (GlnF(+)) but not after incubation of cells with 0 mM Gln (GlnF). In order to better understand the effects of Gln on mTOR pathway, we incubated HCT-8 cells with different doses of Gln and measured FSR and p-4E-BP1 expression.

Effects of Gln on protein fractional synthesis rate and p-4E-BP1 expression

The lowest FSR was observed after complete Gln depletion (0 mM + MSO) and then increased with increasing doses of Gln (Fig. 5, dark line). In contrast, the lowest expression of p-4E-BP1 was measured after incubation of cells with 0 mM Gln (Fig. 5, bars). Expression of p-4E-BP1 increased after complete Gln depletion (0 mM + MSO) compared with 0 mM Gln. Increasing doses of Gln from 0 to 10 mM were also associated with an increase of p-4E-BP1 expression. Thus, there is no correlation between mTOR activation by Gln availability and protein synthesis in HCT-8 cells including all tested conditions (Spearman $r = -0.03$, $p = 0.8$). However, if we excluded GlnF(+) conditions from the analysis, there was a strong correlation between p-4E-BP1 and FSR ($r = 0.58$, $p = 0.0218$).

Discussion

Intestinal protein synthesis rate that can influence gut barrier is highly dependent of nutritional status or interventions (Adegoke et al. 2003; Bouteloup-Demange et al. 1998; Coeffier et al. 2003; Coeffier et al. 2008; Winter et al. 2007; Boukhettala et al. 2010) or of the pathophysiological conditions (Nakshabendi et al. 1996; El Yousfi

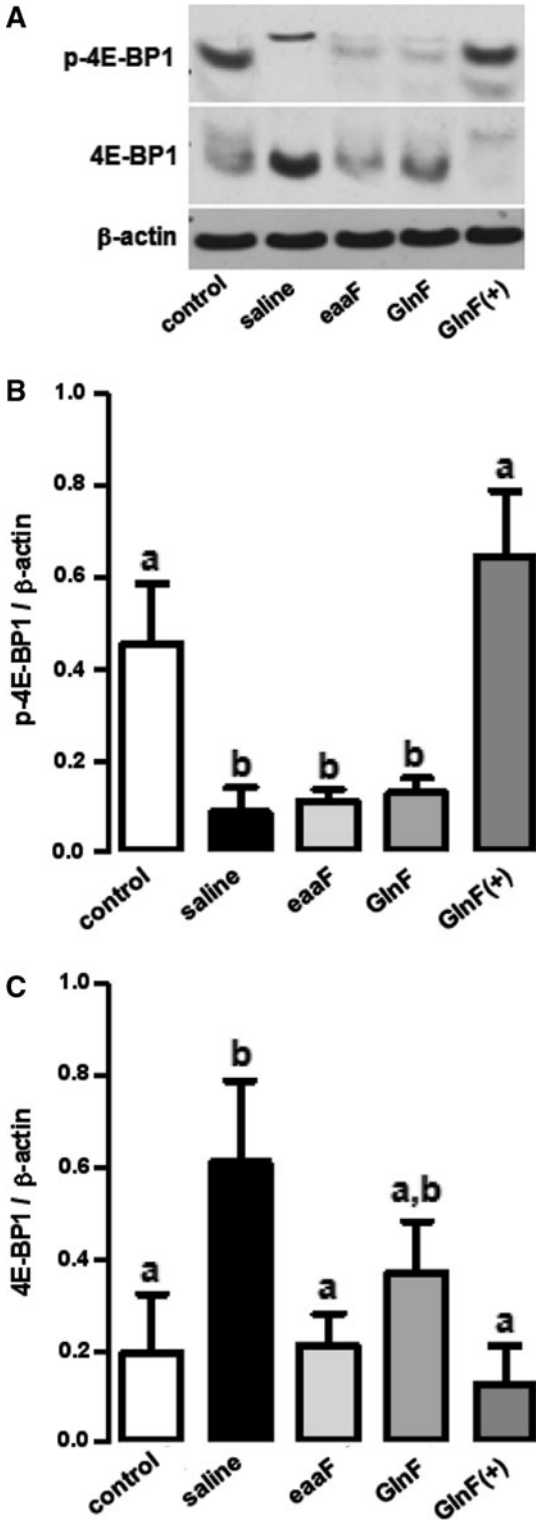

Fig. 4 Effects of essential amino acid or glutamine deprivation on 4E-BP1 expression. **a** Representative experiments of p-4E-BP1, 4E-BP1 and β-actin immunoblots. **b** Densitometric analysis of p-4EBP1 expression and **c** 4E-BP1 expression. Values (means ± SEM, $n = 4$) without a common letter differ ($p < 0.05$). eaaF, essential amino acid-free medium; GlnF, glutamine-free medium; GlnF(+), glutamine-free medium supplemented with glutamine synthase inhibitor

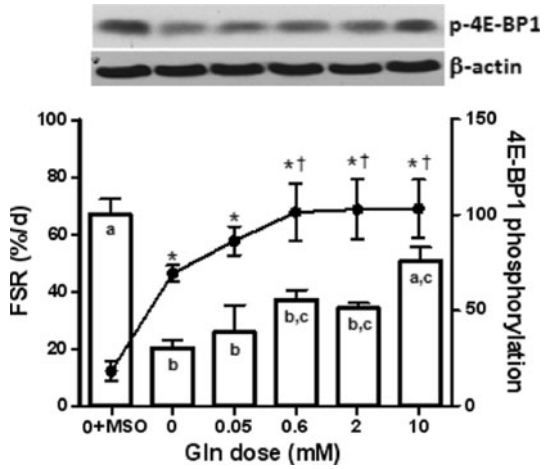

Fig. 5 Effects of glutamine availability on p-4E-BP1 expression and protein synthesis. p-4E-BP1 immunoblot and densitometric analysis (*bars*) and protein fractional synthesis rate (FSR, %/day, *dark line*) in HCT-8 cells treated with increasing concentrations of glutamine (from 0 to 10 mM) and glutamine inhibitor, MSO. Values are means ± SEM from 3 independent experiments. Values of p-4E-BP1 without a common letter differ ($p < 0.05$). $^{*}p < 0.05$ versus 0 + MSO and $^{\dagger}p < 0.05$ versus 0

et al. 2003; Boukhettala et al. 2009). However, little is known about signaling pathways regulating initiation and elongation of protein synthesis in response to amino acid availability at the intestinal level. It has been described in vitro that arginine and leucine supplementation can activate mTOR pathway (Nakajo et al. 2005; Rhoads et al. 2006). In vivo data in enterocolitic piglets also showed that arginine stimulates protein synthesis through mTOR pathway (Corl et al. 2008). The effects of amino acid limitation on intestinal protein synthesis are also poorly documented. Threonine limitation seems to have different effects according to the physiological conditions (Faure et al. 2005; Wang et al. 2007). Le Bacquer et al. (2001) clearly demonstrated in Caco-2 cells that glutamine deprivation limits protein synthesis, but did not assess involved signaling pathways.

In the present study, EAA deprivation decreased p-4E-BP1 and increased p-eiF2α suggesting a limitation of mTOR activation and an increase of GCN2 pathway. These results are in accordance with previously published data. Indeed, EAA limitation was associated with an activation of GCN2 pathway in the brain (Maurin et al. 2005) or in myoblasts (Deval et al. 2008). However, surprisingly, we did not observe a decrease of protein synthesis in the present study that could be mainly related to the short time of deprivation (6 h).

Gln deprivation induced a decrease of protein synthesis and more markedly after inhibition of glutamine synthase by MSO. These results confirmed the data obtained in Caco-2 cells (Le Bacquer et al. 2001) and indicated that HCT-8 cells expressed glutamine synthase. Our results also reported similar FSR (70%/day, approximately) between high dose of Gln and control media (2 mM Gln). Low Gln (0–0.05 mM) mildly decreased FSR (46–57%/day) whereas total Gln depletion markedly reduced FSR (12%/day). Le Bacquer et al. (2001) previously showed that increasing Gln from 0.1 to 5 mM did not affect protein synthesis that was markedly reduced by MSO. All these data suggest that, in physiological conditions, Gln supplementation was not able to enhance protein synthesis in intestinal epithelial cells. In vivo data showed controversial data and the effects of Gln were assessed not only on intestinal epithelial cells but on whole intestinal mucosa (Coeffier et al. 2003; Humbert et al. 2002; Tannus et al. 2009). In our study, while Gln depletion in the medium was only associated with a decrease of p-4E-BP1 expression, the complete Gln depletion was associated with an increase of p-eiF2α expression and no modification of p-4E-BP1 expression compared with control cells. All these results suggest that intracellular glutamine concentrations tightly regulate mTOR and GCN2 pathways to stimulate or not protein synthesis (Fig. 6). We could hypothesize that during Gln depletion as observed in trauma or burned patients (Coeffier and Dechelotte 2009), intestinal epithelial protein synthesis may be limited due to the decrease of mTOR activation. Increasing Gln concentrations increase protein synthesis as previously reported in Caco-2 cells (Le Bacquer et al. 2003) or in human duodenal mucosa (Coeffier et al. 2003) through activation of mTOR pathway. These conditions may reproduce luminal contents of Gln during nutritional interventions with Gln containing oral nutritional supplements. In contrast, complete Gln depletion inhibits protein synthesis through GCN2 pathway. The observed activation of mTOR pathway similar to control conditions (Fig. 6) could be mainly involved in the regulation of autophagy (Jung et al. 2010). Indeed, it was recently reported in Caco-2 cells that Gln deprivation blunted heat stress-induced autophagy by activation of mTOR pathway and limited cell survival (Sakiyama et al. 2009). Of course, this latter condition is extreme and does not occur in vivo. However, it has been showed in several pathologic conditions that glutamine synthase or intracellular glutamine content can be decreased in intestinal mucosa. For instance, in Crohn's disease, glutamine content was decreased about 31 and 26% in the ileum and colon, respectively (Sido et al. 2006) and in irritable bowel syndrome, glutamine synthase was less expressed in 42% of patients that was associated with increased intestinal permeability (Zhou et al. 2010). All these data underline the need of further studies evaluating these parameters according to exactly defined intracellular glutamine concentrations.

The mechanisms involved in the inhibitory effect of severe Gln depletion on protein synthesis deserve discussion. As Gln is a major source of energy for the small intestine (Windmueller and Spaeth 1974), it has been

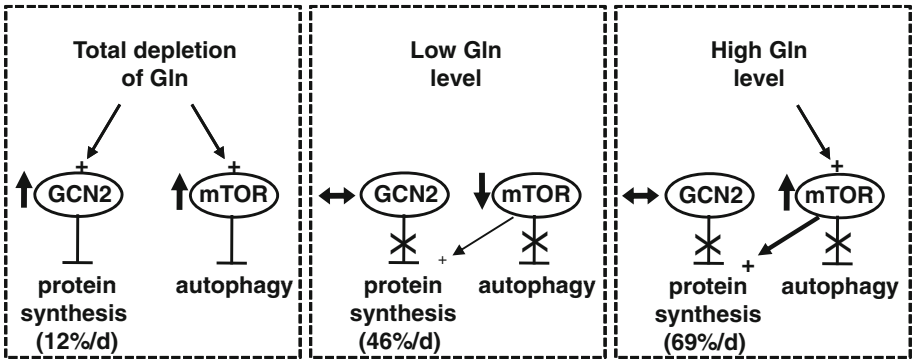

Fig. 6 Putative effects of glutamine availability on protein synthesis and autophagy trough GCN2 and mTOR pathways in intestinal epithelial cells adapted from our results and (Sakiyama et al. 2009). GCN2 and mTOR pathways are not activated in the presence of low concentrations of glutamine that was associated with FSR at 46%/day. When adding glutamine, protein synthesis is gradually increased through activation of mTOR pathway until 69%/day at 10 mM glutamine. In contrast, during complete glutamine depletion, protein synthesis is inhibited (12%/day) through GCN2 pathway. Activation of mTOR pathway during complete glutamine depletion may inhibit catabolic and survival process, autophagy

suggested that Gln stimulates protein synthesis by energy provision (Higashiguchi et al. 1993). However, glutamate fails to regulate protein synthesis in MSO-treated Caco-2 cells (Le Bacquer et al. 2001). Similarly, the drop in protein synthesis associated with MSO treatment could not be accounted to nitrogen deficiency, because DMEM culture medium supplied all amino acids in the present study. All these results suggest that severe Gln depletion per se was responsible of the decrease of protein FSR. In lung carcinoma cells, glutamine deprivation was reported to induce GCN2 pathway (Drogat et al. 2007). The use of L-asparaginase inducing Gln depletion activated GCN2 kinase in liver, pancreas, and spleen (Reinert et al. 2006). Our study is the first to report, in intestinal cells, that Gln depletion is able to induce phosphorylation of eiF2α, which might inhibit protein synthesis.

As previously described (Lee et al. 2003; Maurin et al. 2005), we observed that p-eiF2α was expressed in several tissues i.e. brain, heart, lung, liver. In addition, we observed that p-eiF2α is expressed in small intestine in fasted rats, in particularly in the jejunum and ileum and in the colonic mucosa of fasted rats and humans. However, p-eiF2α was not detectable both in the duodenal mucosa of rats and humans. As intestinal protein metabolism and gut barrier function were altered in various clinical conditions, the role of GCN2 pathway activation in the intestinal mucosa should be assessed in further studies, in particularly in response to luminal fasting.

The limitation of protein synthesis may affect intestinal barrier function. Le Bacquer et al. (2003) previously reported that decreased protein synthesis in Caco-2 cells was associated with a decrease of intestinal permeability. In this latter study, the decreases of protein synthesis and TEER were consecutive to a model of luminal fasting and Gln supplementation was able to restore these parameters. However, tight junctions were not studied (Le Bacquer et al. 2003). In addition, Li et al. (2004) showed that Gln depletion in the culture medium did not affect tight junction proteins, claudin-1, occludin or zonula occludens-1 compared with 0.1 or 0.6 mM Gln. However, complete Gln deprivation by using 0 mM Gln and MSO induced a reduction of tight junction protein expression, which was blunted by Gln supplementation (Li et al. 2004). Our results are in accordance with these data as we observed that Gln depletion (GlnF) did not affect TEER, whereas complete Gln deprivation (GlnF(+)) markedly decreased TEER and FSR, probably through GCN2 pathway activation. We can hypothesize that GlnF(+)-induced GCN2 activation may limit tight junction protein translation. However, the involvement of PI3K/Akt pathway (Li and Neu 2009) and the rate of protein degradation (Coeffier et al. 2010) could also be involved as recently described.

In conclusion, we showed in intestinal epithelial cells that (1) a short EAA deprivation modifies mTOR and GCN2 pathways without affecting protein synthesis and intestinal permeability, (2) glutamine availability, the major substrate of small intestine, appears to be a key factor to determine the protein synthesis rate through both mTOR and GCN2 pathways.

Acknowledgments NB was supported by a grant of the French Speaking Society of Clinical Nutrition and Metabolism (SFNEP) and Nutricia Clinical Nutrition (France).

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