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ORIGINAL CONTRIBUTION

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Role of glutamine on the *de novo* purine nucleotide synthesis in Caco-2 cells

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Summary *Background:* The body's nucleotide pool derives from three potential sources: *de novo* synthesis, salvage of preformednucleosides/ bases or the diet. The relative contributions of these pathways of assimilation are poorly understood *in vivo.* Dietary nucleotides have been suggested to have beneficial effects an the development and repair of the gastrointestinal tract. Tissues with a rapid turnover, such as the gut and the immune system cells, may utilise preformed nucleotides (coming from the diet), in situations in which there is a high demand of nucleotides for nucleic acid synthesis. Therefore, nucleotides could be onsidered as conditionally essential nutrients.

Aim of the work and methods:- Development of a method to measure synthesis de novo of RNA-purine nucleotides in Caco-2 cells, relying an the incorporation of ^{14}C-glycine into the purine ring of the nucleotide.

To establish the fractional synthesis rate of RNA purine nucleotides in Caco-2 cells, grown in culture medium containing different concentrations of glutamine, in the presence or abscence of added nucleotides.

To investigate the degree to which tissue ribonucleosides are derived from the culture medium or from de novo synthesis in the presence of different concentrations of glutamine, using undifferentiated Caco-2 cells, stressed or not by the addition of IL-1β to the medium.

Results and conclusions: The presence of high levels of glutamine in the culture medium is essential for cell proliferation (estimated by measurement of the fractional synthesis rate of purine nucleotides) and the presence of nucleotides cannot replace the glutaminedependence of Caco-2 cell proliferation. The incorporation of exogenous purine nucleotides into RNA of Caco-2 cells is rather limited, and it becomes important when cells are stressed by glutamine deprivation.

Stress by addition of interleukin-1β resulted in the maintenance or the increase in *de novo* synthesised RNA-purine nucleotides, even in the presence of exogenous nucleotides. However, the addition of interleukin-1β to the culture medium led to an enhanced salvage of preformed pyrimidine nucleotides for nucleic acid synthesis when glutamine was present in the medium at a concentration of 0.5 mmol/L.

Key words Caco-2 cells – nucleotides – *de novo* synthesis – glutamine – ^{14}C-glycine

Introduction

Under physiological conditions, the small intestine exhibits a high net glutamine utilisation in many species, including man, exceeding that of all other organs measured [1]. Apart from being an important respiratory fuel for tissues with a rapid turnover (such as the small intestine and the immune system), glutamine acts as the nitrogen donor for *de novo* synthesis of purine and pyrimidine nucleotides [2].

Glutamine can be made available in the lumen of the intestine from the digestion of food protein. Human studies, using stable isotope approaches, have demonstrated that approximately 50 % of dietary glutamine is retained in the splanchnic tissues (small intestine and liver), suggesting that the absorptive cells of the small intestine would, therefore, utilise a great portion of the glutamine coming from the diet [3]. Hence, to satisfy the high demand for glutamine by the immune system and the intestine in the postabsorptive state, it must be provided from the body (skeletal muscle and liver).

In humans, during the stresses associated with injury, sepsis and inflammation, there is a marked increase in glutamine consumption by the gastrointestinal tract, immunologic cells, inflammatory tissue, and kidney. Requirements for glutamine by these tissues may outstrip the synthetic capacity of the skeletal muscle. Thus, in these situations, the intracellular pools of glutamine in muscle are markedly reduced [4]. In other words, glutamine transport would no longer be flux-generating for glutamine release and utilisation by cells of the immune function and by enterocytes. Several studies have demonstrated that glutamine may be a conditionally essential amino acid during critical illness, particularly as it relates to supporting the metabolic requirements of the intestinal mucosa [5].

Cellular nucleic acids can derive from three potential sources: diet, salvage of preformed nucleosides (coming from nucleic acid degradation) and endogenous synthesis (especially from amino acid precursors: glutamine, glycine and aspartate) (Fig. 1). The vast majority of the studies related to dietary nucleotides have been done to show their biological effects. Up to date, very few studies have focused an the absorption, metabolism and incorporation into tissues of dietary nucleotides, probably due to methodological difficulties. Moreover, there are not enough data about the contribution in the body's pools of nucleic acids of each one of the three potential sources above mentioned, and how this contribution changes depending either on the tissue studied or under special metabolic conditions.

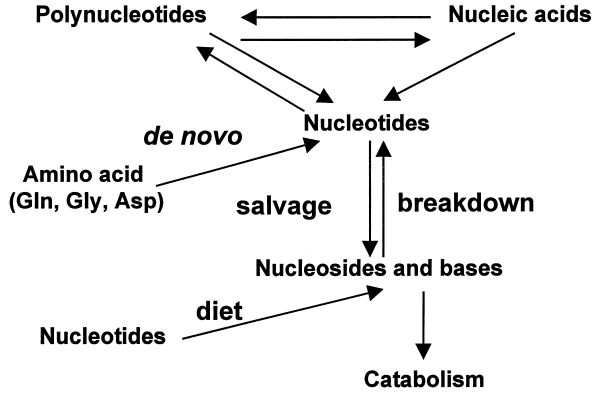

Fig. 1 Pathways of nucleotides metabolism.

In well-nourished animals, *de novo* synthesis of both purines and pyrimidines from amino acid precursors is capable of supporting the cellular needs for nucleic acid synthesis [6]. According to this observation, when glutamine status is depleted or in situations in which there are a high demand of nucleotide synthesis (after gut injury, after surgical trauma, sepsis, rapid growth: pregnancy or newborn infants, etc.), the small intestine may increase salvage of exogenous nucleotides by reducing catabolism [7] and, hence, may spare the cell's needs for glutamine and other amino acid precursors for the *de novo* pathway of nucleotide synthesis [8].

In rat studies, it has been shown that 90 % of the ingested nucleotides are absorbed; yet, according to studies using either radio- or stable-isotope approaches, less than 5 % of the purine nucleotides absorbed are incorporated into intestinal nucleic acids and a relatively small amount appears in hepatic cells [9–11]. Therefore, dietary nucleotides are extensively metabolised by gastrointestinal and liver tissues prior to their entry into the systemic circulation. Whether these observations are applicable to earlier developmental stages or under more physiological dietary conditions remains undetermined [12, 13].

In vitro studies, using Caco-2 cells, have shown that nucleotides are taken up by the cells and extensively metabolised during absorption by epithelial monolayers. Moreover, the addition of nucleotides to the culture medium was associated with enhanced specific brush border enzymatic activities, but only when cells were stressed by glutamine deprivation [14, 15]. These data suggest that exogenous nucleotides may increase the growth and maturation of enterocytes as well as reduce their dependence upon exogenous glutamine [16]. Szondy and Newsholme [17] have studied the effect of various concentrations of nucleobases, nucleosides or glutamine an the proliferation of lymphocytes stimulated by phytohaemagglutinin. They observed that the addition of nucleosides to culture medium that already contained glutamine increased the rate of incorporation of labelled thymidine into DNA, but these rate values were very low, compared with the values obtained when medium contained high glutamine concentrations.

The main aims of the present study were the following:

- To establish the fractional synthesis rate of RNA purine nucleotides in Caco-2 cells, grown in culture media

containing different concentrations of glutamine, in the presence or abscence of added nucleotides. This approach was possible due to the fact that a single molecule of glycine contributes two adjacent carbon atoms to the purine ring, adding U-^{14}C-glycine to the culture medium.

- To investigate the degree to which tissue ribonucleosides are derived from the culture medium or from endogenous synthesis. This was performed in the presence of different concentrations of glutamine, using undifferentiated Caco-2 cells, stressed or not by the addition of IL-1β to the culture medium.

Material and methods

Cell culture

Intestinal Caco-2 cells were obtained from the ATCC (passages 40 to 52). Cells grown routinely at 3×10^6 cells in T75 cm^2 culture flasks (Costar, Cambridge, MA) in Dulbecco's modified minimum essential medium containing 25 mmol/L glucose, 30.8 mmol/L $NaHCO_3$ and supplemented with 20 % heat-inactivated foetal bovine serum, 2 mmol/L glutamine, 1 mg/L fungizone, 10^5 U/L penicillin-streptomycin, 150 mg/L gentamycin and 1 % non-essential amino acid solution (containining in mg/L, L-alanine, 890; L-asparagine, 1500; L-aspartic acid, 1330; L-glutamic acid, 1470; glycine, 750; L-proline, 150 mg; L-serine, 1050). All culture media and suppiements were purchased from GIBCO (Life Technologies, Basel, Switzerland). Cells were maintained in a 10 % CO_2 air atmosphere at 37 °C, and media were changed every other day. At confluency, cells were passaged by detaching them with 0.25 % trypsin. For experiments, cells were plated at 2×10^6 cells in 10 cm diameter Petri dishes (Costar) and media changed every other day.

To establish the fractional synthesis rate of RNA purine nucleotides, Caco-2 cells were grown in culture media containing different concentrations of glutamine, in the presence or absence of added nucleotides. Cells were grown for 6 days in basal medium containing 0, 0.5, 1 or 2 mmol/L glutamine and with or without added nucleotides (10 mg/L each: AMP, CMP, GMP and UMP). At day 6, 0.37 MBq of U-^{14}C-glycine (3.66 GBq/mmol, Amersham, England) was added per dish and cells were further incubated for 24 h. In a second experiment, 20 ng of recombinant human interleukin 1β (IL-1β) (Genzyme, Cambridge, MA) were also added at day 6 to determine the fractional synthesis rate of RNA purine nucleotides in Caco-2 cells, after stimulation with IL-1β. In a third experiment, to investigate the degree to which tissue ribonucleosides derived from the culture medium instead of from endogenous synthesis, we added 74 kBq of 2-^{14}C-cytidine (2.03 GBq/mmol, Sigma, St. Louis, MO) and 74 kBq of 2-^{14}C-uridine (2.07 GBq/mmol, Sigma) instead of U-^{14}C-glycine. Therefore, we measured the specific radioactive of RNA-uridine or RNA-cytidine in cells grown in medium containing 0, 0.5, 1 or 2 mmol/L glutamine, added nucleotides (10 mg/L each: AMP, CMP, GMP and UMP) in the presence or not of IL-1β, at the same levels as mentioned above.

Twenty-four hours after the addition of the radioactive precursors (glycine or pyrimidine nucleosides) the culture medium was aspirated (0.5 mL were quickly frozen at – 80 °C for measurements of glycine and glutamine concentration). Cells were washed 3 times with Hanks' balanced salt solution (HBSS: 137 mmol/L NaCl, 5.36 mmol/L KCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 0.44 mmol/L KH_2PO_4, 0.34 mmol/L NaH_2PO_4, 5.6 mmol/L glucose and 10 mmol/L HEPES, pH 7.4) (GIBCO) and gently scraped in HBSS with the aid of a cell scraper. Finally, cells were pelleted by centrifugation 2000×g, 5 min. The pellet was stored at –80 °C until analysis.

Cell pellets were resuspended in 1 mL of deionised water and homogenised at 15000 rpm for 30 seconds with the aid of a Polytron. Of this homogenate, 500 µ was ultrafiltered (10000 Da. cut-off membrane) and the ultrafiltrate was stored at – 80 °C for determination of intracellular free glycine and glutamine concentration and ^{14}C-glycine counting. The remainder of the supernatant was dried under vacuum for RNA isolation.

HPLC determination of glycine

Free glycine (intracellular and in the medium culture) concentration was determined by reversed-phase HPLC, after derivatisation with phenylisothiocyanate (Pico-Tag method, Waters, Milford, MA). These derivatives were separated on an octadecylsilyl reversed-phase column. The elution solvent consisted of solvent A, containing 70 mmol/L sodium acetate in water (pH : 6.45), and solvent B, containing 450 mL/L acetonitrile and 150 mL/L methanol in water. The gradient mixing was as follows: 0–13.5 min, 3 % B, 13.5–14 min, 100 % B, 14–34 min 100 % B, 34–35 min 100 % A, 45 min 100 % A. The column effluent was spectrophotometrically monitored at 254 nm. The outlet of the UV detector was connected to a radioactivity detector (Radiomatic 500TR, Packard, Meriden, CT) to measure the ^{14}C-glycine.

HPLC analysis of nucleosides

Caco-2 cell RNA was isolated using the RNeasy mini kit (QIAGEN, Basel, Switzerland). The dried RNA pellets were dissolved in autoclaved diethyl pyrocarbonate-treated water and digested to nucleosides as described by Crain [18], using Nuclease P1, phosphodiesterase I and alkaline phosphatase (Sigma). RNA hydrolysates were analysed by reversed-phased HPLC as described by Pomerantz & Mc-

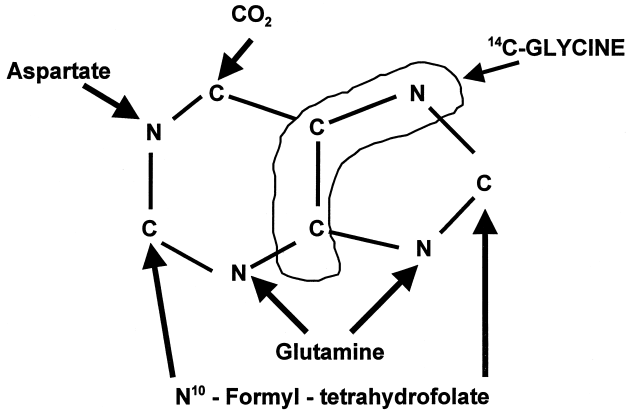

Fig. 2 Synthesis *de novo* of purine bases in the presence of ^{14}C-glycine.

Closkey [19]. The elution solvent consisted of solvent A, containing 250 mmol/L ammonium acetate in water (pH : 6.0), and solvent B, containing 600 mL/L acetonitrile in water. The gradient mixing was as follows: 0–8.6 min, 100 % A; 8.6–10 min, 5 % B; 10–25 min, 25 % B; 25–26 min, 100 % B; 26–30 min, 100 % B; 30–31 min, 100 % A; 45 min, 100 % A. The column effluent was spectrophotometrically monitored at 254 nm. The outlet of the UV detector was connected to a radioactivity detector (Radiomatic 500TR, Packard, Meriden, CT) to measure the ^{14}C-adenosine and ^{14}C-guanosine (synthesised *de novo* from ^{14}C-glycine) and ^{14}C-uridine and ^{14}C-cytidine (in the experiments in which radiolabelled nucleosides were added to the culture medium).

Determination of the synthesis *de novo* of RNA purine nucleotides

Biosynthesis of the purine base involves the incorporation of two carbons derived from a single glycine molecule, so that in the presence of U-^{14}C-glycine, purine base synthesis will result in the synthesis of a base containing 2-^{14}C atoms (Fig. 2). In the present work, we assumed that the precursor pools of glycine for protein and RNA-nucleotide synthesis were the same; thus, the contribution of *de novo* synthesis (in other words, the fractional synthesis *de novo* rate of RNA-purine nucleotides in 24 hours) was estimated as

$$\text{FSR (\%/d)} = \frac{\text{Specific radioactivity of RNA-purine nucleoside (adenosine or guanosine)} \times 100}{\text{Specific radioactivity of intracellular free glycine}}$$

Data presentation and statistical analysis

Data are presented as mean values ± SEM. Data were analysed by one-way ANOVA followed by a post hoc Tukey test to determine mean differences between the groups for all the parameters studied. A difference was considered significant at $p < 0.05$.

Results

De novo synthesis of purine nucleotides from ^{14}C-glycine

This experiment was conducted to quantify the contribution of *de novo* nucleotide synthesis to the Caco-2 cell RNA pool and to study, to what extent, the addition of exogenous nucleotides to the medium could decrease this contribution. Likewise, we studied the effect of the addition of different concentrations of glutamine to the medium. The approach used relies an the fact that a single molecule of glycine contributes two adjacent carbon atoms to the purine ring. Therefore, in principle, the radioactivity found in Caco-2 cells RNA-purine nucleosides should come from newly synthesised purine nucleosides, incorporated into RNA.

This study was designed to achieve isotopic equilibrium between RNA and its precursors. Unfortunately, we were unable to have sufficient quantities of free purine nucleosides to measure their isotopic enrichments. Therefore, we assumed that there was a state of isotopic equilibrium between intracellular free glycine (in equilibrium with the extracellular one) and free purine nucleosides. However, if isotopic equilibrium had not been achieved, it would have led to an under- rather than an overestimate of the contribution of nucleosides synthesised *de novo* to the RNA precursor pool [11].

The specific radioactivity of glycine (cpm/nmol) in the extracellular medium and in the intracellular pool were very similar (data not shown), showing that after 24 h of incubation, both pools were approaching isotopic equilibrium, regardless of the level of glutamine supplementation or the presence or not of added nucleotides. Figure 3 shows the ^{14}C incorporation into RNA-purine nucleotides from ^{14}C-labelled glycine in Caco-2 cells, grown in media containing different concentrations of glutamine, in the presence or not of added nucleotides. In other words, the specific radioactivity of RNA-bound purine nucleosides (guanosine and adenosine) after 24 h of incubation of Caco-2 cells with ^{14}C-glycine was measured.

Based on the assumption that the specific radioactivity of intracellular free glycine represents that of the precursor glycine for purine biosynthesis, we found an increase in the purine synthesis when glutamine was present in the culture medium independent of the concentration used. The nucleotide supplementation of the culture medium led to a

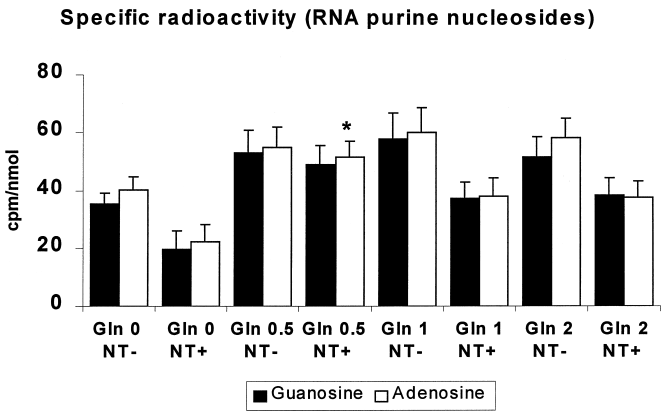

Fig. 3 Incorporation of ^{14}C-glycine added to the medium in RNA-bound purine nucleosides (guanosine and adenosine) from Caco-2 cells grown in cultures containing different concentrations of glutamine supplemented or not with nucleotides. Data are mean values ± SEM for 6 experiments. *Significantly different from Gln 0 NT+; $p < 0.05$.

general trend to decrease the specific radioactivity of RNA-purine nucleosides at all glutamine levels of supplementation, especially when glutamine was not present in the medium, albeit differences were not significant.

Figure 4 shows the fractional synthesis rate (FSR) (%/d) of de novo synthesised purine nucleosides incorporated into RNA of Caco-2 cells after 24 h of incubation with ^{14}C-glycine. Glutamine supplementation resulted in higher FSR, irrespective of whether the medium contained nucleotides for both RNA-guanosine and -adenosine or not. Differences were significant at all levels of glutamine supplementation ($p < 0.05$), compared to the FSR values observed in the cells deprived of glutamine. It was also observed that the addition of nucleotides to the culture led to a general trend to decrease the FSR of purine nucleosides, which was significant ($p < 0.05$) in the case of RNA-adenosine from cells grown in media containing 2 mmol/L glutamine and for both purine nucleosides, when medium was deprived of glutamine.

Synthesis *de novo* of purine nucleotides in Caco-2 cells stimulated by the addition of interleukin 1β

Figure 5 shows the specific radioactivity of RNA-bound purine nucleosides (guanosine and adenosine) after 24 h of incubation of Caco-2 cell with ^{14}C-glycine and interleukin 1 β. As we already observed in experiment 1 (Fig. 2) that specific radioactivity values of both purine nucleosides increased when glutamine was present in the medium, reaching significance at 1 and 2 mmol/L glutamine supplementation. As we indicated above, when nucleotides were present in the medium, there was a slight decrease in the specific radioactivity of RNA-bound purine nucleosides, but it was not significant.

Figure 6 shows the fractional synthesis rate (FSR) (%/d) of de novo synthesised purine nucleosides incorporated into RNA of Caco-2 cells after 24 h of incubation with ^{14}C-glycine and interleukin 1β. Once again, the nucleotide supplementation of the medium culture led to a small decrease in the FSR of both RNA purine nucleosides, but neither was significant.

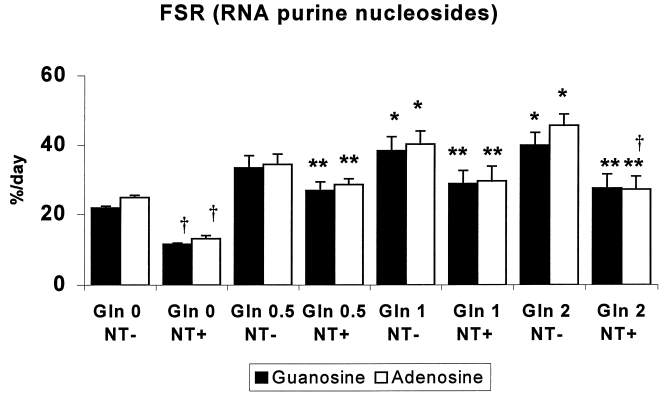

Fig. 4 Fractional synthesis rate (%/d) of RNA-bound purine nucleosides in Caco-2 cells grown in cultures containing different concentrations of glutamine supplemented or not with nucleotides. Data are mean values ± SEM for 6 experiments. *Significantly different from Gln 0 NT–; **Significantly different from Gln 0 NT+; ‡Significantly different from Gln 2 NT–; $p < 0.05$.

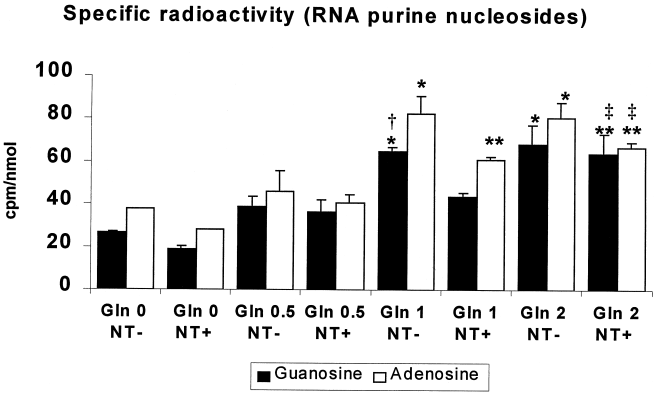

Fig. 5 Incorporation of ^{14}C-glycine added to the medium in RNA-bound purine nucleosides (guanosine and adenosine) from Caco-2 cells, grown in cultures containing different concentrations of glutamine supplemented or not with nucleotides, stimulated by the addition of interleukin-1β Data are mean values ± SEM for 3 experiments. *Significantly different from Gln 0 NT–; **Significantly different from Gin 0 NT+;.-347 †Significantly different from Gln 0.5 NT–; ± Significantly different from Gln 0.5 NT+; $p < 0.05$.

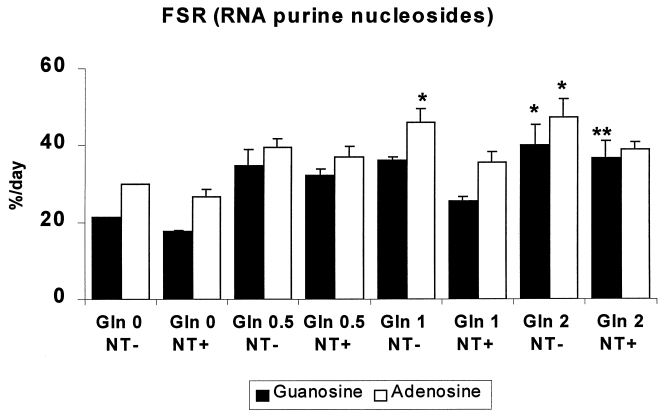

Fig. 6 Fractional synthesis rate (%/d) of RNA-bound purine nucleosides (guanosine and adenosine) in Caco-2 cells grown in cultures containing different concentrations of glutamine supplemented or not with nucleotides, stimulated by the addition of interleukin-1β. Data are mean values ± SEM for 3 experiments. *Significantly different from Gln 0 NT–; **Significantly different from Gin 0 NT+; $p < 0.05$.

Incorporation of ^{14}C-cytidine and ^{14}C-uridine in RNA-pyrimidine nucleosides

Figure 7 shows how ^{14}Crimidine nucleotides were incorporated into RNA of Caco-2 cells, regardless of whether the medium contained glutamine or not, but the incorporation was significantly higher when glutamine (1 mmol/L) was present for both cytidine and uridine. The addition of interleukin 1β produced a significant increase in the incorporation of ^{14}C-pyrimidine nucleotides to RNA, but only when glutamine was present in the medium (0.5 mmol/L).

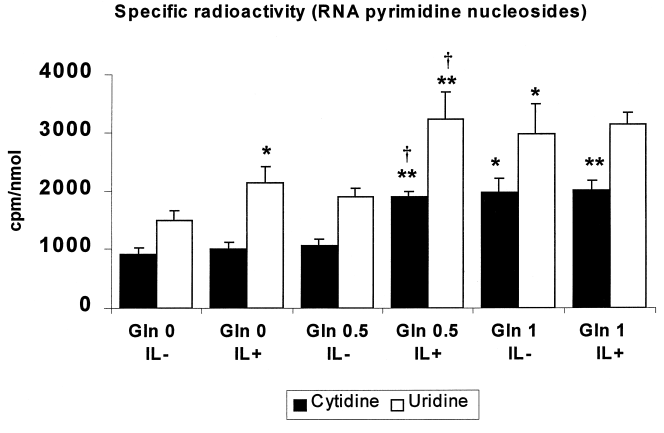

Fig. 7 Incorporation of ^{14}C-cytidine and ^{14}C-uridine added to the medium in RNA-bound pyrimidine nucleosides (cytidine and uridine) from Caco-2 cells, grown in cultures containing different concentrations of glutamine supplemented with nucleotides, stimulated or not by the addition of interleukin-1β. Data are mean values ± SEM for 4 experiments. *Significantly different from Gln 0 IL–; **Significantly different from Gln 0 IL+; †Significantly different from Gln 0.5 IL–; $p < 0.05$.

Discussion

Synthesis *de novo* of purine nucleotides from ^{14}C-glycine, in the presence or abscence of exogenous nucleotides and different glutamine concentrations

Glutamine plays a major role in metabolism of enterocytes both as the main respiratory fuel for these rapidly dividing cells and as a source of nitrogen for the synthesis of nucleotides. The dependence of enterocytes and immunocompetent cells an glutamine supply has been shown in several studies [1, 17, 20]. In humans, ^{15}N-glutamine has been used to trace the fate of enteral glutamine. These studies found that 54 % of the glutamine administered was retained in the splanchnic bed [21]. *In vivo* studies, using stable isotopes, have shown that mucosa and liver RNA-purine nucleosides are derived almost exclusively from synthesis de novo from amino acid precursors [22]. Alternatively, previous studies have provided evidence that enterocytes do not carry out purine synthesis or only to a limited extent [23, 24]. It has been suggested [25, 26] that crypt cells are dependent an dietary nucleic acids to maintain nucleotide pools. Thus, it remains controversial whether enterocytes utilise dietary nucleotides. The results presented in this paper directly address this question. Caco-2 cells were able to synthesise purine nucleotides from amino acid precursors, and their fractional synthesis rate was higher when glutamine was included in the culture medium. FSR (%/d) of purine nucleosides varied from 20–25 % in the absence of glutamine and increased to 40–45 % when 2 mmol/L glutamine was added to the culture medium. The latter values are in good agreement with RNA synthesis rate data obtained *in vivo* in murine gut mucosa, 41 %/d [11]. Therefore, glutamine almost doubled RNA synthesis, the step prior to protein synthesis and cellular growth. Hartman et al. [1] studied protein synthesis rate in rat enterocytes, finding that synthesis was increased by 38 % when glutamine was included in the medium. These authors also indicated that when they replaced glutamine by equimolar amounts of its precursors, α-ketoglutarate plus ammonia, or glutamate, were equally ineffective. Higashiguchi et al. [27] also determined the effect of glutamine in isolated rat intestinal epithelial cells. They found that glutamine, but not other amino acids, stimulated protein synthesis in enterocytes from all levels of the villi. These same authors also observed that a maximal effect was found at a glutamine concentration of 0.67 mmol/L, which is the normal plasma concentration. Rhoads et al. [28], working in vitro with rat intestinal epithelial cells (IEC-6), have shown that glutamine promoted cell proliferation. They suggested that glutamine may be a unique nutrient for enterocytes, capable of dual signaling and augmenting the effects of the growth factors that govern cellular proliferation and repair.

As a logical progression, if glutamate and glutamine differ in their effects an enterocyte cell proliferation, the

glutamine *per se* or the amide-nitrogen of the glutamine molecule should be the responsible for this action. If the amide-nitrogen is the main factor, cell proliferation should follow nucleoside synthesis and glutamine is the donor of this amide-nitrogen for *de novo* synthesis of purine and pyrimidine bases.

The addition of nucleotides to the enterocytes in culture led to a decrease the FSR of purine nucleosides. It has been described previously using Caco-2 cells grown in media at different glutamine concentrations that nucleotides had an effect on growth proliferation and degree of proliferation, but only in the absence of glutamine [29]. Szondy & Newsholme [17] have shown that exogenous nucleotides can be partially replaced by nucleosides and, to some extent, by nucleobases for lymphocyte proliferation. However, these authors have also shown that the addition of nucleosides did not increase the incorporation of ^{3}H-thymidine to that observed at an optimal concentration of glutamine (0.3 mM). The authors concluded that the requirement of glutamine for lymphocyte proliferation is exerted through effects an the biosynthesis and hence the maintenance of the intracellular levels of nucleotides rather than through the glutamine molecule itself. According to our results, synthesis *de novo* is the main supplier of purine-nucleosides to nucleic acid synthesis. The present study supports the idea that an adequate supply of glutamine to the intestinal cell is more important than the supply of exogenous nucleotides.

Effects of the addition of interleukin 1b in the synthesis *de novo* of RNA-purine nucleotides

This experiment was performed to compare the addition of interleukin 1β to the synthesis rate of RNA-purine nucleosides and to study the effects of different concentrations of glutamine on that parameter. This study was also conducted to investigate if exogenous nucleotides can replace the requirement of glutamine in RNA synthesis.

It has been shown elsewhere [30] that many of the acute phase plasma proteins are expressed in human intestinal epithelial cell lines and their expression is induced or regulated by interleukin 1, interleukin 6, interferon and tumor necrosis factor in a manner characteristic of the acute phase response. Caco-2 cells express binding sites for interleukin 1 in both, the apical and basolateral poles. The authors conclude that enterocytes might be involved in a local response to injury/inflammation at the epithelial surface.

In situations of stress, intestinal cells have a higher than normal glutamine requirement. However, in this study differences in FSR-purine nucleotides due to the addition of interleukin-1β to the medium were at a minimum. These results then do not support the hypothesis that in a situation of a high demand for nucleotide synthesis, salvage of exogenous nucleotides could be increased, at least in Caco-2 cells grown in such conditions. In fact, the results have shown that stress by the addition of interleukin 1β resulted in the maintenance or increase in *de novo* synthesised RNA-purine nucleotides, even in the presence of exogenous nucleotides. In other words, *de novo* synthesis of purine nucleotides for nucleic acid synthesis predominates over salvage or use of preformed nucleotides.

Incorporation of ^{14}C-cytidine and ^{14}C-uridine in RNA-pyrimidine nucleosides

In this experiment the incorporation of exogenous ^{14}C-pyrimidine nucleotides was used to quantify the utilisation of exogenous nucleotides for nucleic acid synthesis in Caco-2 cells. These cells were stressed and compared to unstressed controls by the addition of interleukin-1 β to the medium.

^{14}C-pyrimidine nucleotides were incorporated into RNA of Caco-2 cells, regardless of whether the medium contained glutamine. The incorporation was significantly higher when glutamine (1 mmol/L) was present for both cytidine and uridine. Thus, glutamine stimulation of cell proliferation resulted in a greater utilisation of preformed pyrimidine nucleotides in Caco-2 cells. These results agreed with those reported by He et al. [14] who indicated that labelled pyrimidine nucleoside (10–15 %) was detected after a 2-h incubation with radiolabelled cytidine or uridine in intestinal cell lines (Caco-2 and IEC-6). Stable isotope approaches in mice, using ^{13}C-nucleotides have also shown evidence of a modest incorporation of pyrimidine nucleotides into tissue RNA [11, 31]. These results suggest that a modest, but perhaps critically important fraction of dietary pyrimidine nucleotides are incorporated into nucleic acids and it is tempting to speculate that this might represent a specific, and functionally important fraction or subfraction of RNA. Moreover, pyrimidine nucleotides are disproportionately high in human milk and are preserved during storage in the breast and digestion in the small intestine, as compared to purine nucleotides [32].

The addition of interleukin 1β produced a significant increase in the incorporation of ^{14}C-pyrimidine nucleotides into RNA, but only when glutamine was present in the medium (0.5 mmol/L). In contrast to purine nucleotides, salvage of preformed pyrimidine nucleotides was enhanced as response to interleukin 1β to synthesise nucleic acid. At higher glutamine concentrations (1 mmol/L) a stimulation was not evident. The only explanation for this fact is that at high doses of glutamine, *de novo* synthesis of pyrimidine nucleotides (from glutamine) are enough to exert the stimulating effect of interleukin 1β over Caco-2 cells.

Conclusions

The presence of optimal levels of glutamine in the culture medium is essential for cell proliferation (estimated by

measurement of the fractional synthesis rate of purine nucleotides). The presence of nucleotides cannot replace the glutamine-dependence of Caco-2-cell proliferation. The incorporation of exogenous purine nucleotides into RNA of Caco-2 cells is rather limited, and only becomes important when cells are stressed by glutamine deprivation.

The results in these studies have been obtained in a human colon carcinoma cell line, Caco-2 cells and may not be extrapolated to other nontumoral cell lines or *in vivo* situations. Weber [33] indicated that cancer cells are characterised by a much greater capacity of both salvage and *de novo* pathways than normal cells. Thus, from our results, it is premature to conclude that exogenous nucleotides are necessary to normal enterocyte cells during development or during periods of repair. He et al. [15] observed that the *de novo* biosynthesis is sufficient to support proliferation of Caco-2 cells but not of the normal rat small intestinal crypt cell line (IEC-6). These authors concluded that nucleotide supplements may enhance normal enterocyte growth and maturation as well as to spare the need of exogenous glutamine in cell maintenance and development. Therefore, these principles should now be tested *in vivo*.

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