Amino Acids (2008) 34:683–685
DOI 10.1007/s00726-007-0002-9

SHORT COMMUNICATION

Glutamine enhances glucose-induced mesangial cell proliferation

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Received: 1 October 2007 / Accepted: 9 November 2007 / Published online: 4 December 2007

Abstract The proliferation of mesangial cells (MC) in the presence of glutamine (0–20 mM) was determined in both low (5 mM) and high (25 mM) glucose-containing medium. Glutamine in a high glucose (HG) environment increased cell proliferation in a dose-dependent manner. Inhibition of glutamine:fructose 6-phosphate amidotransferase (GFAT) and of phosphodiesterase significantly reduced glutamine-induced proliferation. Supraphysiologic levels of glutamine increase MC proliferation in a HG milieu via GFAT and cAMP-dependent pathways, suggesting that glutamine could pose a risk for diabetic nephropathy.

Keywords Glutamine · Supplementation · Kidney disease · Mesangial cell · Cell proliferation

Introduction

Glutamine, derived both from endogenous production (primarily skeletal muscle) and from dietary sources, is the most abundant free amino acid in the body. Of the glutamine entering via the diet, approximately one-third reaches the circulation and down regulates endogenous glutamine synthesis (Boza et al. 2001) to maintain serum levels within the narrow range (0.5–0.9 mM) necessary for the maintenance of cell division and cell metabolism. However, a dietary glutamine overload may increase its intestinal absorption and lead to supraphysiologic serum concentrations (Souba et al. 1990).

As a result of studies such as those showing improvement in immune function following administration of glutamine to patients under severe catabolic conditions and in marathon runners (Castell and Newsholme 1997; Souba et al. 1990) glutamine has been placed on a list of "immunonutrients" (Huang et al. 2003) and has found increased use as a dietary supplement.

In the normal kidney, the mesangial cell (MC) renewal rate is usually less than 1%, but in diabetes, it is increased significantly by high glucose (HG) via the hexosamine biosynthetic pathway in which glutamine takes part as an amino donor in a reaction catalyzed by glutamine:fructose-6-phosphate amido-transferase (GFAT) (Nerlich et al. 1998).

To assess whether excess glutamine could potentially play a role in exacerbating the pathologic role of HG in MC function, we studied the effect of glutamine on mouse MC division in vitro under low glucose (LG) and HG conditions, and in the presence of inhibitors of GFAT.

Materials and methods

Cell culture

A primary culture of mouse MC was a gift of the late Dr. Liliane Striker and has been previously characterized in detail (MacKay et al. 1988). Cells (passage 15–16) were

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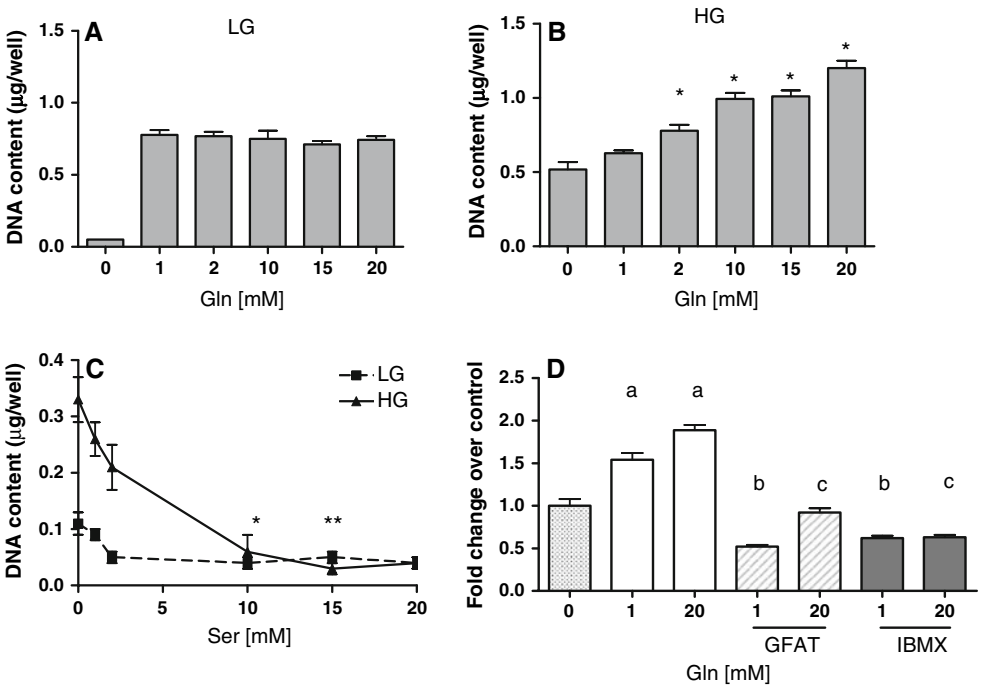

Fig. 1 Mesangial cell proliferation with increasing concentration of glutamine: **a** 0–20 mM in LG, and **b** in HG medium; or **c** with serine 0–20 mM in LG and HG. **d** Inhibition of glutamine-induced proliferation with 40 µM DON and 0.5 mM IBMX. Level of significance $P < 0.05$. *Asterisk* compared to control (0 glutamine); *a* compared to 0 glutamine, *b* compared to 1 mM glutamine, *c* compared to 20 mM glutamine

grown as previously published (Doi et al. 2000) with a 3:1 mixture of DMEM/Ham's F12 media containing 6 mM glucose, 1 mM glutamine, 0.075% $NaHCO_3$, penicillin (100 U/ml)/streptomycin (100 µg/ml), and 20% fetal bovine serum (FBS, Life Technologies, Rockville, MD, USA) in a humidified incubator at 37°C, 5% CO_2. The cells were plated at a density of 2×10^4 cells per well in 24-well culture dishes pre-coated with human fibronectin in 1 ml of DMEM media containing 0.075% $NaHCO_3$, penicillin (100 U/ml)/streptomycin (100 µg/ml), and 10% fetal bovine serum (FBS, Life Technologies, Rockville, MD, USA) in a humidified incubator at 37°C, 5% CO_2. Glucose and glutamine were added as needed according to each study.

Cell proliferation assay

Cell proliferation was measured by DNA content using a colorimetric assay as previously described (Sellitti et al. 2001). Cells were grown in LG (5 mM) or HG (25 mM) medium in the presence of glutamine or serine for 72 h. After washing the cells with ice cold Hank's balanced salt solution (HBSS), DNA was precipitated with 10% trichloroacetic acid for 10 min at 4°C, and 0.5 ml of a solution containing 20 volumes of a stock (1 g of diphenylamine, 90 ml of glacial acetic acid and 2 ml of sulfuric acid), 8 volumes of H_20 and 0.1 volume of 1% acetaldehyde was added to each well for 48 h. DNA content was determined in triplicate wells by reading OD 580 nm and comparing to a standard curve established with calf thymus DNA.

Inhibition of glutamine-induced cell proliferation

A glutamine analog, 5-diazo-3-oxonorleucine (DON) that competitively inhibits the GFAT pathway was added to MC cultures at a concentration of 40 µM. In a separate study, the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 0.5 mM) was added to MC to maintain high levels of cAMP. MC proliferation was determined as described above in cultures with or without inhibitors.

Statistical analysis

All values represent mean ± SEM of replicates. Comparisons between groups were performed using one-way ANOVA followed by Welch's *t* test.

Results

Mesangial cell proliferation was studied in both LG and HG milieux at glutamine concentrations ranging from zero to supraphysiologic values (up to 20 mM). Whereas cell proliferation reached a plateau at a low glutamine concentration (1 mM) in LG medium, it increased in a dose-dependent manner with glutamine in HG medium (Fig. 1a,

b). In contrast, serine, an amino acid with structural and chemical characteristics comparable to glutamine, significantly decreased HG-dependent MC proliferation in a dose-dependent manner (Fig. 1c). The glutamine-induced elevation of MC proliferation was significantly inhibited by blocking either GFAT activity with DON or phosphodiesterase activity with IBMX (Fig. 1d).

Discussion

Chronically elevated dietary intake of amino acids and protein has been associated with hyperfiltration and reduced glomerular filtration rate that in turn results in glomerular ECM expansion and development of glomerulosclerosis. It has also been demonstrated that a high concentration of an amino acid mixture increases expression of glomerulosclerosis markers and MC proliferation (^{3}H-thymidine incorporation) in vitro (Meek et al. 2003). We have recently confirmed that glutamine alone can up regulate markers of glomerulosclerosis in vitro (Pithon-Curi et al. 2006).

Since conventional experimental protein diets used in animal models of glomerulosclerosis are generally formulated with casein, a glutamine-rich protein (Dumas et al. 1970), it is possible that the well-known association of high protein diet with glomerulosclerosis may owe much to the presence of excess glutamine.

Compared with LG, HG actually had a suppressive effect on MC proliferation at normal glutamine concentrations (1–2 mM) (Fig. 1a, b). This is in agreement with earlier findings of time-dependent effect of glucose on cell growth (Romen and Takahashi 1982), and also shows that supraphysiological glutamine concentrations are able to reverse the HG inhibition of growth at 72 h.

Previous studies have shown that an increase in cAMP both inhibits cell proliferation through cAMP-dependent protein kinase (PKA) activation (Rhoads et al. 2000) and down regulates GFAT activity (Chang et al. 2000). Our study showing significant inhibition of MC growth with either DON or IBMX is consistent with the existence of such a cAMP/GFAT pathway in the regulation of mouse MC proliferation. These data would suggest that in vivo, high dietary intake of glutamine, especially in diabetics (i.e., high glucose state) would increase MC proliferation via the hexosamine biosynthetic pathway.

The results of this study point to a risk of inducing chronic kidney disease with high glutamine intake. This is likely to be greatest in professional athletes frequently taking large amounts of glutamine supplements. The benefit of glutamine supplementation when this amino acid becomes insufficient is irrefutable. However, there is a need for awareness regarding risks of indiscriminate intake of glutamine in the sports community, especially among those who are diabetics or have transiently higher blood glucose levels. The clinical relevance of the in vitro findings reported herein is supported by preliminary results with a diabetic rat model of glutamine supplementation (Lagranha et al, unpublished observation).

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