Amino acid signaling in the intestine: The roles of glutamine, leucine and arginine

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ABSTRACT Amino acids have an influence on the function of organs, glands, tendons and arteries. Some of them play crucial roles in the control of gene expression by controlling the initiation phase of mRNA translation. Furthermore, recent studies have revealed that some kinds of amino acids directly participate in important signal transduction in the immune system. Glutamine, leucine and arginine play crucial roles in intestinal growth, integrity, and function through cellular signaling mechanisms. In this paper, we review amino acid signal transduction in the intestinal function.

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INTRODUCTION

Twenty percent of the human body is composed of protein. Protein consists of one or more chains of amino acids[1]. Amino acids contribute to homeostasis of the human body through their important role in the network between glucose and lipid metabolism. Furthermore, recent studies have revealed that some amino acids such as glutamine, arginine and leucine directly participate in important signal transduction in the intestinal immune system[2]. For example, a number of studies have reported that arginine plus nucleic acid and ω-3 fatty acids is effective in protection against infection after surgery. Glutamine has become a major energy source for the intestinal mucosal cells and lymphocytes, which is essential for maintaining the structure of the intestinal mucosa and hyperactivated lymphocytes. In this paper, we describe amino acid signal transduction in the intestinal immune system.

Glutamine signaling in the intestine

Glutamine was originally categorized as a non-essential amino acid[2]. However, glutamine is the primary metabolic fuel of the small intestine[3]

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and during stress the body's requirements for glutamine appear to exceed the individual's ability to produce sufficient amounts of this amino acid[4]. So it is now called a conditionally essential amino acid. We previously showed that newborn mice fed with otherwise complete milk lacking only glutamine died more frequently from intestinal hemorrhage than mice fed with complete milk with adequate glutamine[5]. Glutamine is an important energy source for neutrophils, lymphocytes and macrophages and is essential for the differentiation and maturation of various kinds of cells. Glutamine increases the percentage of helper T cells and regulatory T cells, activates intestinal flora, suppresses the production of inflammatory and suppressor cytokines, improves the intestinal barrier and the potentiation of the function of immunocompetent cells. Glutamine signaling in the intestinal immune system is easily broken down by a lack of enteral nutrition[2,3]. For example, sepsis caused by bacterial translocation frequently occurs in patients treated with only parenteral nutrition. On the contrary, early enteral nutrition contributes to relatively immediate recovery from major surgery. Enhanced recovery after surgery is based on this concept.

It has been previously demonstrated that L-glutamine inhibits the activation of p70S6 kinase and the phosphorylation of 4E-BP1 induced by arginine or leucine in rat intestinal epithelial cells[6]. Based on those results, another study confirmed the effect of each amino acid, including glutamine, in an *in vivo* model using newborn mice[5]. Until recently, it was quite difficult to study the physiological and cyto-biological effects of amino acids *in vivo*, especially for neonatal animals, because of the lack of methods of alimentation or of preparing a diet for neonatal mice.

A previous study used a totally new milk feeding system and amino acid milk to successfully observe the physiological effects of glutamine depletion in newborn mice[5]. Interestingly, a significant high incidence of colonic hemorrhage occurred in mice fed with glutamine deleted milk compared to the complete amino acid milk or glutamine-rich amino acid milk groups. There was only one complete amino acid milk-fed mouse that had a similar colonic hemorrhage on day six. This mouse might have had a genetic/physiological susceptibility to hemorrhage. It could also have been caused by the fact that the amount of amino acid contained in the complete amino acid milk was 50% of the ordinary amount because emulsified fat was added to the artificial amino acid milk at a ratio of 1:1 to yield complete amino acid milk. The proteins in the milk used in the study were in the form of amino acids, resulting in a very high osmotic pressure with a low amount of fat. If the artificial amino acid milk is given directly to newborn mice without adding fat, which is a major nutrient for them, a fat deficiency might occur. Therefore, the melena that occurred in the complete amino acid milk-fed mouse may actually be explained by an insufficient intake of glutamine.

Meanwhile, no animal developed any colonic hemorrhage in the glutamine-rich amino acid milk group, so it was assumed that the glutamine level in that milk was high enough to maintain the intestinal epithelium. Other macroscopic findings in the hemorrhagic intestines of the glutamine deleted amino acid milk-fed mice were inflammatory changes of the entire intestine, with intestinal wall thickening by edema. Microscopic observations supported these findings, with infiltration of inflammatory cells in and around the destroyed colonic mucosa at the site of the hemorrhage. On the other hand, the height of the colonic mucosa of the glutamine rich amino acid milk-fed mice was well conserved and was higher than that of the complete amino acid-fed mice[5].

Experimental data from cultured intestinal epithelial cells of normal rat, IEC6 cells supported

the findings of the animal experiments, with the reduced cell proliferation, an accumulation of cell population in the sub-G0 phase, and the cleavage of caspase-3 under glutamine-depleted culture conditions. According to the results, glutamine depletion induces cell death of colonic mucosa, presumably due to an acute induction of apoptosis[7], followed by the destruction of mucosa maintenance, which eventually leads to colonic hemorrhage. It was remarkable that colonic hemorrhage could be induced simply by removing one amino acid, glutamine[5].

Glutamine has attracted close attention as an amino acid nutrient and as an immunopotentiating factor for intestinal cells[1,2]. However, no case of intestinal hemorrhage induced by glutamine deficiency has been reported to date. Although glutamine is a nonessential amino acid and can be produced in the living body, neonatal mice are actively growing and are very dependent on external alimentation due to a heavier consumption of nutrients, including glutamine, compared to adults. It seems likely that the consumption of glutamine by these neonatal mice might exceed the amount of glutamine pooled and formed in the living body, leading to the emergent status of glutamine deficiency[5].

In terms of molecular/biological effects of glutamine, there are several outstanding issues that remain to be clarified. One is to explore the possible involvement of certain signaling pathways. It has been reported that glutamine stimulates the mitogen-activated protein kinase (MAPK) pathway[8] and the synthesis of heat shock proteins in intestinal cell lines[9]. It has recently been unveiled that amino acids are involved in the control of the mammalian target of rapamycin (designated mTOR and also called FRAP, RAFT-1, and RAPT-1) signaling pathway. It was also found that glutamine regulates the activation of the mTOR pathway in a small bowel epithelial cell line derived from rats[6].

Future studies should focus on the mechanism for the induction of colonic hemorrhage and how glutamine is involved in the intracellular signaling pathway, such as in mTOR. To sum up, feeding neonatal mice with glutamine deleted amino acid milk induced a high incidence of colonic hemorrhage within a week, and this was due to an induction of epithelial cell death[5].

Cells regulate amino acid pools in part by the degradation of endogenous proteins. A major mechanism for degradation of intracellular proteins when exogenous amino acids are unavailable is autophagy. The protein kinase mTOR is a key regulator of autophagic mechanism.

Mammalian cells express two mTOR-containing complexes, mTORC1 and mTORC2. mTORC1 is active and exerts a direct suppressive effect on autophagy. A recent study indicates that extracellular glutamine suppresses autophagy by stimulating mTORC1[10].

Arginine signaling in the intestine

Arginine is a major trigger for the release of specific hormones[11], and is the major amino acid precursor of polyamines, which are essential for healing in the gastrointestinal tract[12]. It is the substrate for the synthesis of nitric oxide (NO)[13], which is necessary for host defenses against viruses, bacteria and malignant cells. It also stimulates the proliferation and maturation of lymphocytes, cytokines and antibodies[14]. Arginine reduces the incidence of infection from pathogenic microorganisms and the death rate from infection in pregnant women and infants[15].

Arginine induce activation of p70 S6 kinase and phosphorylation of 4E-BP1 in a rapamycin-sensitive manner in rat intestinal epithelial cells, which suggested arginine enhances cell migration and activates the immediate downstream mTOR, p70S6k, but not ERKs in enterocytes[15]. Immunohistochemically, abundant p70S6k locates

in the cytoplasm, whereas the activated form, phospho-p70S6k, is localized entirely in the nucleus of resting cells[15]. Arginine ingestion stimulates thymic lymphocytes, the proliferation of T cells, and the production of IL-2, IL-2 receptor expression on T cells and macrophage and NK cell activity[16]. This indicates that arginine ingestion is important in the development of the intestinal immune system in infants and small children.

Leucine signaling in the intestine

For the first time, Shigemitsu *et al.*[17,18] showed that the chirality, the structure of the four branched hydrocarbons of leucine, and the primary amine of leucine are required to stimulate p70S6k activity using various amino acids and derivatives of leucine. Additionally, l-Leucine have been revealed to regulate activation of p70 S6 kinase and phosphorylation of 4E-BP1 through mTOR signaling pathway in rat intestinal epithelial cells[15].

The mechanisms of leucine-induced p70S6k activation remain to be elucidated. Leucine added to amino acid-depleted H4IIE cells activated p70S6k. The activation of p70S6k by leucine was transient; however, the complete amino acid stimulated p70S6k more steadily. Leucine's effect on p70S6k was sensitive to rapamycin, but not very sensitive to wortmannin. Using various amino acids and derivatives of leucine, it was found that the chirality, the structure of the four branched hydrocarbons, and the primary amine are all required for leucine to stimulate p70S6k, indicating that the structural requirements for leucine to activate p70S6k are very precise[17,18]. If an acceptor molecule specific to leucine exists, then the recognition of this acceptor by leucine should be very narrowly defined. Amino acid transport system L has been known to incorporate histidine, isoleucine, methionine, phenylalanine and valine from the medium to the intracellular space as well as leucine. However, because the structural requirements of these amino acids by transport system L is not strict, it might not be the acceptor protein for leucine to induce the activation of p70S6k. Further study of the leucine-induced activation of translational effectors might show the mechanism of cellular events mediated by the mTOR signaling pathway, such as autophagy, the proliferation of T cells, or mRNA translation[18].

DISCUSSION

Amino acids are a large group of similar nutrients which compose proteins and perform a multitude of functions, such as comprising the structural components of tissues and cells, are a source of energy for muscles, and act as protein hormones, antibodies, and enzymes. Another crucial role of amino acids is the regulation of translational effectors, including p70S6k, and eIF-4E binding protein 1/PHAS1 (4E-BP1)[17,18].

p70S6k activated by multisite phosphorylation in response to insulin/mitogen, phosphorylates 40S ribosomal protein S6 *in vivo*. This protein kinase has been reported to play a critical role in regulating the selective translation of mRNAs containing a polypyrimidine tract at their 5' end. 4E-BP1 binds to the 7-methylguanosine cap-binding protein eIF-4E, and also stops eIF-4E from binding to p220/eIF-4G. Mitogens stimulate the phosphorylation of 4E-BP1, resulting in its dissociation from eIF-4E, which makes eIF-4E available for incorporation into the translational initiation complex and restoring translation[17].

4E-BP1 and p70S6k are both dephosphorylated *in vivo* in response to rapamycin, a macrolide immunosuppressant. This compound binds to FKBP12 and FKBP12-rapamycin forms a complex with mTOR. The protein kinase activity of mTOR is inhibited by the binding of FKBP12-rapamycin. Studies have reported that mTOR is an upstream regulator of both 4E-BP1 and p70S6k[17].

The phosphorylation and activation of 4E-BP1 and p70S6k *in vivo* has been shown to be

controlled by the availability of amino acids. A prior report showed that while amino acid withdrawal diminishes p70S6k activity and the phosphorylation of 4E-BP1 in Chinese hamster ovary cells overexpressing the insulin receptor (CHO-IR cells) and a cell line derived from human embryonic kidney cells grown in tissue culture (HEK293 cells), amino acid repletion restores those responses. It was also reported that these effects of amino acid repletion are blocked by rapamycin and that a p70S6k mutant called p70 Δ 2-46/ Δ CT104, which is rapamycin resistant, is also insensitive to inhibition by amino acid withdrawal. These results suggest that through the mTOR signaling pathway amino acids control both p70S6k and 4E-BP1[17,18]. Another study examined the ability of individual amino acids to stimulate p70S6k activation in rat H4IIE hepatoma cells, and it was previously shown that the addition of amino acids activates 4E-BP1 and p70S6k and that amino acid depletion from the culture medium can cause the generation of amino acids endogenously through autophagy, which is a major mechanism of facultative protein degradation in the liver.

In the intestine, autophagic proteolysis and its regulation by amino acids have not yet been unveiled. Several reports have claimed that autophagy regulates some inflammasome protein family in the intestinal immune system[19]. Recently, NLRP6 inflammasome, a regulator of colonic microbiota composition, play an important role for goblet cell mucus secretion[20]. These new evidence might open the horizon for the regulation of amino acid signal in the intestinal system in the future.

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