Regulatory Effects of Polyamines on Animal Intestinal Homeostasis and Potential Mechanisms: Postprint

Authors: Fang Tingting, Liu Guangmang, Jia Gang, Zhao Hua, Chen Xiaoling, Wu Caimei, Cai Jingyi

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Abstract

Polyamines (putrescine, spermidine, spermine, etc.) are a class of biologically active low-molecular-weight aliphatic compounds in animals. Polyamines can regulate intestinal homeostasis and participate in physiological processes such as intestinal growth and development, intestinal mucosal barrier function, antioxidant activity, and metabolism. However, the mechanism underlying the regulation of intestinal homeostasis by polyamines remains unclear. This article reviews the regulatory effects of polyamines on intestinal homeostasis, analyzes the possible mechanisms of action, and aims to provide a reference for the further application of polyamines.

Full Text

Polyamines: Regulation on Intestinal Homeostasis and Possible Mechanisms

FANG Tingting[1], LIU Guangmang*, JIA Gang, ZHAO Hua, CHEN Xiaoling, WU Caimei, CAI Jingyi

(Institute of Animal Nutrition, Sichuan Agricultural University, Key Laboratory for Animal Disease-Resistance Nutrition of Ministry of Education, Chengdu 611130, China)

Abstract: Polyamines (putrescine, spermidine, spermine, etc.) are a class of low-molecular-weight aliphatic compounds with biological activity in animals. Polyamines can regulate intestinal homeostasis and participate in various physiological processes including intestinal growth and development, intestinal mucosal barrier function, antioxidant capacity, and metabolism. However, the

mechanisms by which polyamines regulate intestinal homeostasis remain unclear. This review summarizes the regulatory effects of polyamines on intestinal homeostasis and analyzes their possible mechanisms, aiming to provide a reference for further application of polyamines.

Keywords: polyamine; intestinal homeostasis; regulatory effects; mechanism

*Corresponding author, associate professor, Email: liugm@sicau.edu.cn

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Gut homeostasis is a dynamic equilibrium state formed by the interaction of host intestinal mucosal and immune barriers, intestinal microbiota, nutrients, and metabolites [1]. The proliferation, differentiation, migration, and apoptosis of intestinal epithelial cells are regulated by numerous intracellular and extracellular factors, including polyamines. Polyamines participate in regulating many biological processes in animals, such as DNA, RNA, and protein synthesis, cell signal transduction, cell cycle progression, and cell proliferation and differentiation [2-3], and are particularly crucial for maintaining intestinal homeostasis. Recent studies have shown that rapidly proliferating and developing intestinal cells are often accompanied by increased polyamine concentrations, and polyamines play important regulatory roles in intestinal development, mucosal barrier function, antioxidant capacity, and metabolism. Therefore, elucidating the mechanisms by which polyamines regulate intestinal homeostasis holds significant theoretical and practical importance. This review summarizes the regulatory functions of polyamines on animal intestinal homeostasis, aiming to establish a theoretical foundation for research on polyamines in maintaining intestinal homeostasis and their regulatory mechanisms.

1.1 Physicochemical Properties of Polyamines

Polyamines are important polycationic aliphatic amines in animals, mainly including putrescine (PUT), spermidine (SPD), and spermine (SPM) [4]. Putrescine, chemically known as butanediamine with the molecular formula $H_2N(CH_2)_4NH_2$, is formed from ornithine through decarboxylation by ornithine decarboxylase (ODC). Spermidine, chemically known as N-(3-aminopropyl)-1,4-butanediamine with the molecular formula $H_2N(CH_2)_3NH(CH_2)_4NH_2$, is generated by combining putrescine with an aminopropyl group. Spermine, chemically known as N,N' -bis(3-aminopropyl)-1,4-butanediamine with the molecular formula $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, is synthesized by further combining spermidine with an aminopropyl group. Among these, putrescine is the shortest-chain polyamine in the body and serves as the precursor for spermidine and spermine synthesis. Spermine is the longest-chain aliphatic polyamine bearing four positive charges [4].

1.2 Biosynthesis and Metabolism of Polyamines

Polyamines are widely distributed in living organisms. In the animal digestive tract, polyamines can be obtained not only from breast milk and diet [5], but also synthesized by intestinal microorganisms and intestinal cells through metabolic pathways of amino acids such as arginine, methionine, proline, and glutamate [6]. The continuous synthesis and catabolism of polyamines in animals regulate intracellular polyamine content and maintain their dynamic equilibrium in the body [6]. The endogenous synthesis and homeostasis of polyamines are under precise regulation, with the ornithine pathway being the sole route for polyamine synthesis in the body [7-8]. Animal polyamines are derived from L-ornithine decarboxylation, where ornithine is decarboxylated by ODC to generate putrescine, which serves as the precursor for spermidine and spermine synthesis. Putrescine undergoes an aminopropyl transfer reaction catalyzed by spermidine synthase to produce spermidine, which is then further catalyzed by spermine synthase to incorporate another aminopropyl group and ultimately synthesize spermine. In this anabolic pathway, the aminopropyl groups required for spermidine and spermine synthesis are derived from S-adenosylmethionine decarboxylase (AdoMetDC)-processed S-adenosylmethionine. Therefore, in the de novo synthesis pathway, ODC is the first rate-limiting enzyme, while AdoMetDC is a key metabolic enzyme [9-10].

In addition to synthesis, intracellular polyamine content is also regulated by catabolism. Spermine and spermidine are progressively converted back to putrescine through the action of spermine/spermidine N^1-acetyltransferase and polyamine oxidase. Putrescine is then degraded to succinate by diamine oxidase and excreted from the cell, thereby maintaining normal intracellular polyamine levels [11].

2 Effects of Polyamines on Animal Intestinal Homeostasis

The intestinal mucosa occupies a critical position connecting internal and external environments, performing essential functions including digestion, absorption, secretion, and defense. The structural integrity of intestinal mucosal epithelial cells is fundamental to executing these digestive and absorptive functions. The unique molecular structure of polyamines enables them to play important roles in promoting intestinal mucosal growth, development, maturation, environmental adaptation, and damage repair. Polyamines maintain intestinal homeostasis by promoting intestinal development, maintaining mucosal barrier function, enhancing antioxidant capacity, and regulating intestinal metabolism.

2.1.1 Promotion of Intestinal Epithelial Cell Proliferation and Differentiation As the primary site for nutrient digestion and absorption in higher animals, the intestinal mucosa features a crypt-villus structure that serves as the basic functional unit. Intestinal crypt stem cells possess strong proliferative potential and are precursors of mature epithelial cells. The mature epithelial cells on intestinal villi are continuously generated through proliferation, migra-

tion, and differentiation of crypt stem cells. Intestinal mucosal growth and development depend on the proliferation and differentiation of intestinal epithelial cells [12]. A key manifestation of polyamines promoting intestinal growth is their ability to stimulate intestinal epithelial cell proliferation and differentiation. All polyamines can promote cell proliferation, with spermine significantly stimulating cell proliferation at low concentrations, while spermidine and putrescine require higher concentrations to exert significant proliferative effects [13]. Spermine or spermidine can promote intestinal growth by facilitating morphological maturation and functional improvement. Studies in suckling rats have demonstrated that spermine significantly increases the number of columnar and goblet cells in the duodenum and ileum [14], and regulates villus height, villus width, and crypt depth in the jejunum and ileum [15-17]. Additionally, experiments in piglets have shown that spermine linearly increases small intestinal weight, duodenal and jejunal mucosal weight, and mucosal protein, DNA, and RNA content [18], while increasing villus height and crypt depth [19]. Furthermore, feeding spermine for different durations significantly increases villus height, villus width, and villus surface area in the jejunum and ileum of suckling piglets [20]. This villus hyperplasia and increased mucosal surface area enable the intestine to adapt to increasing nutrient supply.

2.1.2 Enhancement of Digestive Enzyme Activity in Animal Intestine After migrating out of crypts, intestinal mucosal cells gradually differentiate and synthesize digestive and absorptive enzymes such as alkaline phosphatase, lactase, sucrase, and maltase. Lactase is the primary digestive enzyme during the lactation period, while sucrase and maltase become dominant after weaning. The reduction of brush border enzyme profiles, particularly lactase activity, and the increase in maltase and sucrase activities are important markers of intestinal functional maturation in mammals after weaning [21]. Research has shown that polyamines significantly increase maltase and sucrase activities while decreasing lactase activity in mouse intestine [22]. Similarly, feeding spermine significantly increases sucrase and maltase activities while decreasing lactase activity in the jejunum and ileum of suckling piglets [20]. In addition to disaccharidases, alkaline phosphatase and diamine oxidase also play important roles in intestinal development. Exogenous spermine increases alkaline phosphatase activity in mouse jejunum while decreasing it in the ileum [23], and significantly enhances diamine oxidase activity in the jejunum and ileum of suckling piglets [20]. These findings demonstrate that polyamines promote intestinal development and maturation by regulating different digestive enzyme activities, which is crucial for young animals to adapt to substantial dietary changes before and after weaning.

2.1.3 Possible Pathways Regulating Intestinal Development Currently, few studies have reported on the pathways through which polyamines regulate intestinal cell proliferation and development. Only the regulation of gene expression through human antigen R (HuR) and Jun homolog gene family member D (JunD) pathways has been identified (Figure 1 [Figure 1: see

original paper][24-25]). Polyamine depletion approaches are commonly used to investigate these regulatory mechanisms, with the most representative inhibitor being difluoromethylornithine (DFMO), a specific ODC-targeting inhibitor that irreversibly inhibits ODC activity and depletes intracellular polyamines.

2.1.3.1 HuR Pathway HuR is a key post-transcriptional regulator that exhibits high affinity and binding capacity for U- and AU-rich mRNAs, and can stabilize and regulate mRNA translation [26]. When polyamines are depleted, HuR translocates to the cytoplasm in intestinal epithelial cells, significantly increasing cytoplasmic HuR accumulation. However, adding putrescine to DFMO-treated cells reduces this cytoplasmic HuR accumulation [27-28]. HuR can bind to mRNAs of nucleophosmin (NPM) and tumor suppressor protein 53 (p53) through their 3' -untranslated regions or coding regions, thereby affecting their expression [24,29]. p53 expression is closely related to cell growth status [3]. DFMO treatment of IEC-6 intestinal epithelial cells significantly increases p53 expression [30-32]. Additionally, Zou et al. [33] found that polyamine depletion-induced p53 expression increase is associated with NPM. NPM is a multifunctional protein that regulates p53 activity. Polyamine depletion increases NPM gene expression and NPM protein nuclear translocation, forming NPM/p53 complexes in cells [33]. Under polyamine depletion conditions, HuR can inhibit intestinal epithelial cell proliferation by increasing the expression and stability of this complex. Furthermore, studies have shown that treatment of IEC-6 cells with the polyamine analogue DENSPM reduces polyamine content while increasing p53 and p21 expression [9]. These results indicate that reduced cellular polyamine levels can stabilize growth-inhibitory gene mRNAs by increasing cytoplasmic levels of the RNA-binding protein HuR, leading to protein accumulation. The increased levels of growth-inhibitory proteins such as p53 and NPM subsequently enhance transcription of cell cycle arrest genes like p21, thereby inhibiting cell proliferation and intestinal mucosal growth.

2.1.3.2 JunD Pathway JunD is a member of the Jun family and a major component of the activator protein-1 (AP-1) transcription factor [34]. While AP-1 plays important roles in cell proliferation, differentiation, and apoptosis, JunD is an atypical AP-1 factor whose increased expression reduces cell proliferation and differentiation, increasing the proportion of cells in the G0/G1 phase [35]. Polyamine depletion significantly increases JunD/AP-1 activity in the small intestine, primarily due to increased JunD expression, as both JunD mRNA and protein levels significantly increase after polyamine depletion in IEC-6 cells [36]. Li et al. [25] demonstrated that DFMO-induced polyamine inhibition does not increase JunD transcription but extends JunD mRNA half-life to 4 hours. However, adding spermidine during DFMO treatment reduces JunD mRNA half-life to 60 minutes, decreasing its stability. Additionally, DFMO-induced polyamine depletion increases p21 mRNA levels in IEC-6 cells, and reducing JunD protein levels with JunD antisense oligonucleotides significantly decreases p21 protein levels [25]. These findings indicate that polyamines primarily af-

fect post-transcriptional processes of JunD by modulating mRNA stability to induce increased JunD mRNA and protein levels. The elevated JunD protein subsequently increases p21 expression by activating the p21 promoter, thereby inhibiting cyclin activity and suppressing intestinal epithelial cell proliferation.

HuR: human antigen R; **JunD:** Jun homolog gene family member D; **NPM:** nucleophosmin; **p53:** tumor suppressor protein 53; **AP-1:** activator protein-1; **p21:** Ras homolog gene family member 21.

Figure 1 Signaling pathway following polyamine depletion in growth inhibition of intestinal cell proliferation[24-25]

2.2 Polyamines and Intestinal Mucosal Barrier Function Intestinal mucosal barrier function refers to the ability of intestinal epithelium to separate intraluminal contents and prevent invasion by pathogenic antigens. Normal barrier function maintenance primarily depends on physical, chemical, immune, and microbial barriers. Polyamines maintain intestinal barrier function by regulating the physical, immune, and microbial barriers.

2.2.1 Regulation of the Intestinal Mucosal Physical Barrier The intestinal mucosal physical barrier primarily comprises tight junctions, adherens junctions, gap junctions, and desmosomes. Tight junctions consist mainly of cytoplasmic proteins and transmembrane proteins. The cytoplasmic proteins primarily include zonula occludens (ZO) family proteins ZO-1, ZO-2, and ZO-3, while transmembrane proteins mainly include occludin and claudin. Adherens junctions are primarily composed of E-cadherin (E-cad), a homophilic, calcium-dependent transmembrane glycoprotein involved in maintaining epithelial cell polarity, tissue development, and damage repair. Polyamines regulate the intestinal epithelial barrier primarily by modulating tight junction-related proteins and E-cad expression. In CDX2-transfected IEC-6 cells, polyamine depletion significantly reduces ZO-1 and ZO-2 levels, but barrier damage is ameliorated when spermidine is added to DFMO-treated cells [37]. Polyamine depletion also significantly decreases occludin content without affecting its mRNA expression, and this damage is similarly improved by spermidine supplementation [37]. These results demonstrate that polyamines play an important role in promoting synthesis and maintaining stability of ZO-1, ZO-2, and occludin. Additionally, polyamine depletion in normal IEC-6 cells reduces intracellular free Ca^{2+} concentration and E-cad expression, both of which are restored by polyamine supplementation. Moreover, increasing intracellular free Ca^{2+} concentration through Ca^{2+} channels enhances E-cad expression in polyamine-deficient cells [38]. Thus, polyamines can regulate the intestinal mucosal physical barrier by modulating both cytoplasmic and transmembrane proteins affecting tight junctions, as well as by altering E-cad-dependent adherens junctions.

2.2.2 Regulation of the Intestinal Mucosal Immune Barrier The intestine is not only the largest digestive and absorptive organ but also the largest

immune organ in animals. As components of living matter, polyamines play important roles in the intestinal immune system through multiple pathways. First, polyamines can promote premature maturation of the intestinal immune system in young animals. Pérez-Cano et al. [39] found that adding spermine and spermidine to breast milk of suckling rats improved the immune system by promoting maturation of $CD8^+$ intraepithelial lymphocytes and increasing the proportion of mature $CD4^+$ lamina propria lymphocytes. Additionally, as endogenous immune regulators, polyamines exert anti-inflammatory effects by modulating intestinal cytokines. Studies have shown that spermine can inhibit synthesis of pro-inflammatory factors such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, macrophage inflammatory protein (MIP)-1α, and MIP-1β [40], prevent nitric oxide (NO) production in macrophages induced by bacterial endotoxin [41-42], and alleviate lipopolysaccharide-induced increases in IL-10 content [43]. These studies demonstrate that polyamines exert immunomodulatory effects by promoting intestinal immune system maturation and regulating immune factors, though their molecular mechanisms require further investigation.

2.2.3 Regulation of the Intestinal Microecological Barrier As the largest bacterial reservoir in the body, the intestine harbors diverse microorganisms, and animal health and normal intestinal function are closely related to intestinal flora structure. The intestinal microbiota has evolved to form a dynamic equilibrium unified system among microorganisms, host, and environment. Polyamines significantly affect intestinal flora composition and activity. Supplementing formula milk with polyamines in mice significantly increases populations of *Bifidobacterium*, *Lactobacillus*, and *Clostridium*, promoting healthy mucosal status [44]. Polyamines also regulate levels of *Lactobacillus*, *Bifidobacterium*, *Bacteroides-Prevotella*, and *Clostridium* in the intestinal microbiota of neonatal BALB/cOlaHsd mice, promoting intestinal health [45]. Moreover, dietary putrescine supplementation in weaned piglets significantly increases colonic *Lactobacillus* content, decreases *Escherichia coli* populations and diarrhea index, and significantly enhances ODC activity in the middle small intestinal wall [46]. These findings suggest that polyamines may act as growth factors for these microorganisms, stimulating intestinal mucosal cell proliferation and differentiation while regulating relevant microbial communities, though the molecular mechanisms underlying polyamine regulation of intestinal microecology require further study.

2.4 Polyamines and Intestinal Metabolism

Metabolism encompasses all biochemical changes in life activities and represents the essential characteristic and material basis of life. Metabolites are products of biochemical reactions during life processes and can reflect the essence of life processes to some extent. Known phenotypes or overall conditions of organisms are closely related to metabolites, and changes in physiological functions induced by polyamines correspond with alterations in metabolites. Exogenous spermine

affects ileal metabolic processes in suckling rats, including lipid metabolism, energy metabolism, amino acid metabolism, microbial metabolism, and intestinal osmotic pressure, thereby influencing intestinal tissue metabolism [47]. Spermine also alters metabolic processes in the blood of weaned rats, including cell membrane metabolism, lipid metabolism, glucose metabolism, amino acid metabolism, and microbial metabolism, playing an important role in regulating rat blood metabolism [48]. Furthermore, spermine promotes enhanced amino acid metabolism and protein synthesis, phospholipid synthesis to protect cell membrane integrity, and glucose-alanine cycling in weaned rats, affecting microbial growth and metabolism to improve intestinal health during the weaning period [49]. Additionally, under oxidative stress conditions induced by diquat, spermine can partially eliminate changes in amino acid and lipid metabolism, demonstrating its important role in regulating metabolism in oxidative stress conditions [50]. These results indicate that polyamines can adjust overall intestinal metabolism and promote intestinal health by altering relevant metabolic processes under both normal and oxidative stress conditions.

Recent research has confirmed that polyamines maintain intestinal homeostasis by promoting intestinal epithelial cell proliferation and differentiation, enhancing digestive enzyme activity, maintaining intestinal mucosal physical barrier function, regulating intestinal immunity, improving intestinal microecology, enhancing antioxidant capacity, and regulating intestinal metabolism. Although progress has been made in understanding the mechanisms of polyamine regulation of intestinal development, the precise mechanisms underlying other aspects of polyamine-mediated intestinal homeostasis remain unclear. For example, which signaling pathways are involved in polyamine regulation of intestinal barrier function? What are the specific molecular mechanisms by which polyamines improve intestinal microecology and adjust intestinal metabolism? Further in-depth research on polyamines will elucidate their mechanisms in maintaining intestinal homeostasis, providing new insights for enhancing animal intestinal health, improving livestock production performance, and promoting animal husbandry development.

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