Regulatory Effects of Butyrate on Gene Expression in Mammary Tissue and Its Mechanism: Postprint

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Abstract

Butyric acid is an important short-chain fatty acid that functions as a signaling molecule by binding to its receptors to exert important physiological roles in the organism, such as regulating lipid metabolism in mammary gland, liver, and adipose tissues. The mechanisms underlying butyric acid' s actions are multifaceted, many of which are associated with its involvement in regulating gene expression. Butyric acid not only modulates the expression of individual genes but also participates in the regulation of signaling pathways and gene networks. This review summarizes the regulatory effects of butyric acid on gene expression in mammary tissue and the associated mechanisms, focusing on its roles as an important precursor for milk component synthesis, a histone deacetylase (DHAC) inhibitor, and a ligand for G protein-coupled receptors.

Full Text

Regulatory Functions and Mechanism of Butyrate on Gene Expression in Mammary Tissue

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Abstract

Butyrate is an important short-chain fatty acid that serves as a signaling molecule binding to its receptors to exert crucial physiological functions in the body, such as regulating lipid metabolism in mammary, hepatic, and adipose tissues. The mechanisms of butyrate action are multifaceted, many of which involve its participation in the regulation of gene expression. Butyrate not

only modulates the expression of individual genes but also participates in the regulation of signaling pathways and gene networks. This review summarizes the regulatory effects of butyrate on gene expression in mammary tissue and the underlying mechanisms, focusing on its roles as a key precursor for milk component synthesis, a histone deacetylase (HDAC) inhibitor, and a ligand for G protein-coupled receptors.

Keywords: butyrate; milk fat synthesis precursor; histone deacetylase inhibitor; G protein-coupled receptor ligand; mechanism

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Butyrate is an important short-chain fatty acid (SCFA) produced by microbial fermentation of dietary fiber in the digestive tract. It functions as a signaling molecule that binds to its receptors to exert physiological effects, thereby regulating lipid metabolism in mammary, hepatic, and adipose tissues. As a milk fat synthesis precursor, butyrate has been widely used in infusion studies to investigate its effects on milk fat synthesis, yielding significant findings. Storry et al. [1] reported that intravenous infusion of β-hydroxybutyrate in dairy cows significantly increased the synthesis of myristic, palmitic, stearic, and oleic acids in milk fat while decreasing milk fat yield compared to the control group. Maxin et al. [2] found that ruminal infusion of β-hydroxybutyrate increased both milk fat content and yield. Jenkins et al. [3] demonstrated that supplemental dietary fatty acids enhanced milk production and fat percentage but concurrently decreased milk protein percentage.

Additionally, butyrate provides energy for gastrointestinal epithelial cell metabolism, maintains intestinal mucosal integrity [4-5], improves gastrointestinal morphology and function, promotes proliferation of mucosal immune-related cells [6], and enhances immunity and disease resistance. Studies have shown that adding 0.3%, 1.0%, and 3.0% sodium butyrate to the diet of weaned calves improved body weight and average daily gain both before and after weaning [7-8], thereby promoting growth and development. Guilloteau et al. [9] reported that dietary supplementation with 3 g/kg sodium butyrate increased growth rate and body weight gain in calves. In vitro studies have demonstrated that butyrate plays a critical role in regulating the utilization of nutrients for milk fat and protein synthesis in bovine mammary epithelial cells (BMECs), affecting both gene transcription and protein translation stages.

Yonezawa et al. [10] observed that butyrate promoted triglyceride accumulation in dairy cow mammary epithelial cells but failed to induce lipid droplet formation. Research examining the effects of short-chain fatty acids (acetate and butyrate) added to BMEC culture medium revealed that these fatty acids stimulated triglyceride accumulation in a concentration-dependent manner and increased mRNA expression of transport genes such as cluster of differentiation 36 (CD36) and lipoprotein lipase (LPL) [11]. Regarding mechanisms, butyrate acts as a regulator of gene expression, directly modulating genes encoding key metabolic enzymes. Comprehensive studies have shown that butyrate upregu-

lates the expression of genes involved in de novo fatty acid synthesis [12-15] and regulates the expression of important transcription factors controlling milk fat synthesis, including sterol regulatory element-binding protein 1 (SREBP1) and peroxisome proliferator-activated receptor γ (PPARG) [13,15-16].

This review primarily discusses the major functions and related mechanisms of butyrate in dairy cow mammary tissue as an important milk fat synthesis precursor, an HDAC inhibitor, and a G protein-coupled receptor ligand, providing a reference for further investigation into the regulatory mechanisms of butyrate on milk component synthesis within the mammary gland.

1. Butyrate as a Precursor for Milk Fat Synthesis in Ruminant Mammary Tissue

The formation and utilization of milk component precursors (MCPs) are critical for controlling milk nutritional quality, as their content and composition directly affect the synthesis of milk components such as milk fat and protein, thereby influencing milk quality [17]. McCarthy et al. [18] added radioisotope-labeled β-hydroxybutyrate to bovine mammary tissue homogenate culture medium and analyzed the fatty acids, demonstrating that β-hydroxybutyrate serves as a milk fat synthesis precursor for fatty acid synthesis.

The primary component of milk fat is triglycerides, which contain essential fatty acids and phosphates with high nutritional value. The fatty acids comprising triglycerides originate from different sources: 50% of C16:0 and all long-chain fatty acids are derived from blood, whereas medium- and short-chain fatty acids and the remaining 50% of C16:0 are synthesized de novo in mammary epithelial cells from acetate and β-hydroxybutyrate. Both acetate and β-hydroxybutyrate originate from volatile fatty acids (acetic acid and butyric acid) produced by rumen fermentation. While acetate contains two carbon atoms, β-hydroxybutyrate contains four. Medium- and short-chain fatty acids are synthesized by sequentially adding acetic acid molecules, increasing chain length by two carbons each time. β-hydroxybutyrate is utilized by splitting the four-carbon unit into two-carbon units that function as acetate. Another pathway involves conversion of β-hydroxybutyrate back to its original volatile fatty acid—butyric acid—within mammary epithelial cells, followed by sequential addition of two-carbon units to gradually form fatty acids of varying lengths [18].

The precise mechanisms by which exogenous butyrate supplementation affects milk fat synthesis remain incompletely understood. Based on previous research, two primary aspects may be involved. First, butyrate supplementation increases the supply of milk fat synthesis precursors, thereby promoting milk fat synthesis. Studies have shown [19] that changes in the concentration and composition of milk fat precursors in blood not only directly affect milk fat synthesis but also feedback-regulate lipid metabolism in liver and adipose tissue to accommodate milk fat synthesis. β-hydroxybutyrate and non-essential fatty acids can function

as signaling molecules that bind to their receptors to regulate lipid metabolism in mammary tissue [20].

Second, butyrate supplementation upregulates the expression of genes related to fatty acid synthesis, thereby promoting milk fat synthesis. The expression of key functional genes during lactation forms the basis for regulating mammary growth, development, differentiation, and important physiological processes such as milk synthesis, secretion, and transport. As a milk fat synthesis precursor in ruminants, butyrate regulates milk fat synthesis by modulating the expression of genes involved in de novo milk fat synthesis, thereby enhancing the lactation capacity of mammary epithelial cells. Acetyl-CoA carboxylase (ACC) is the rate-limiting enzyme in fatty acid synthesis, while fatty acid synthase (FASN) is a multifunctional enzyme complex involved in the entire process of de novo fatty acid synthesis [12]. Kong et al. [13] demonstrated that different concentrations of sodium butyrate (0–1.25 mmol/L) significantly increased triglyceride synthesis in dairy cow mammary epithelial cells while upregulating FASN and ACC gene expression. Qi [14] reported that in vitro-cultured BMECs, a β-hydroxybutyrate concentration of 2.32 mmol/L optimally promoted milk fat and protein synthesis, and when the ratio of acetate to β-hydroxybutyrate was 2:1, it optimally promoted triglyceride synthesis and mRNA expression of milk fat synthesis-related genes including FASN, ACC, fatty acid-binding protein 3 (FABP3), LPL, stearoyl-CoA desaturase (SCD), and PPARG.

Butyrate not only regulates the expression of milk fat synthesis-related genes but also controls milk fat synthesis by modulating the expression of transcription factors governing milk fat synthesis. Fatty acids serve as endogenous ligands for peroxisome proliferator-activated receptors (PPARs) and can effectively activate PPARG [13]. PPARG, a member of the ligand-activated nuclear hormone receptor superfamily, is a crucial transcription factor regulating genes related to milk fat synthesis. Kadegowda et al. [21] investigated the effects of PPARG on immortalized bovine mammary epithelial cells (MAC-T cells) and found that PPARG upregulated expression of genes throughout the lipid metabolism process, including de novo fatty acid synthesis genes (ACC, FASN), triglyceride synthesis genes (SCD), and fatty acid transport genes (CD36). Many studies have shown that sodium butyrate inhibits expression of lipogenic genes by suppressing nuclear abundance of sterol regulatory element-binding proteins (SREBPs) [16]. SREBP1 is a key transcription factor controlling early steps in fatty acid synthesis, desaturation, elongation, and triglyceride synthesis. Ta [16] demonstrated that at 0.8 mmol/L, β-hydroxybutyrate significantly promoted PPARG gene expression, with concurrent changes in diacylglycerol acyltransferase (DGAT), ACC, and FASN gene expression mirroring PPARG expression trends.

Furthermore, butyrate can regulate milk protein synthesis by modulating expression of related genes. Previous research suggests that butyrate' s effects on milk protein synthesis may be related to its regulatory role on leptin transcription. This regulation may occur through two pathways: via short-chain fatty acid-specific G protein-coupled receptors and through mitogen-activated protein

kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) signaling pathways. Leptin, a protein hormone secreted by adipose tissue, promotes fatty acid synthesis in dairy cow mammary tissue and upregulates expression of α-casein and β-lactoglobulin genes [23]. Wang et al. [22] reported that β-hydroxybutyrate at concentrations of 2.32–9.28 mmol/L upregulated αs1-casein (CSN1S1) gene expression, potentially promoting milk protein synthesis. CSN1S1 is the most abundant protein in milk, and its gene expression is closely correlated with protein synthesis. Yonezawa et al. [24] found that adding 10 mmol/L acetate or butyrate to BMEC culture medium significantly downregulated leptin gene expression. Conversely, Soliman et al. [25] demonstrated in vitro studies using bovine adipocytes that supplementation with 0.1 or 0.5 mmol/L acetate, propionate, and butyrate all upregulated leptin gene expression.

2. Butyrate as a Histone Deacetylase (HDAC) Inhibitor in Mammary Tissue Gene Expression Regulation

Butyrate regulates gene expression by inhibiting histone deacetylation, leading to histone hyperacetylation. Acetylated histones bind loosely to DNA, and this relaxed structure facilitates contact between transcription factors and co-regulators with DNA molecules, thereby modulating gene expression [26]. Studies have shown that milligram doses of butyrate cause accumulation of acetylated histones and induce synthesis of non-histone chromosomal proteins in various vertebrate cell lines cultured in vitro [27]. In mammary tissue, butyrate plays an important role in gene expression as an HDAC inhibitor. Tsubaki et al. [28] investigated the effects of butyrate on insulin-like growth factor-binding protein (IGFBP)-3 and IGFBP-rp2 gene expression in normal mammary epithelial cells and breast cancer cells. The results showed that butyrate inhibited proliferation of breast cancer cells by upregulating IGFBP-3 and IGFBP-rp2 mRNA and protein expression, while normal mammary epithelial cell proliferation was also affected due to increased IGFBP-rp2 mRNA and protein expression.

Histone acetylation is associated with gene activation, whereas deacetylation is linked to gene silencing [29]. Histone acetyltransferases transfer the acetyl moiety from acetyl-CoA to specific lysine residues at the -amino group on the amino terminus of core histones. This neutralizes the positive charge, and the negative charge of DNA molecules facilitates DNA conformational unfolding. The relaxed nucleosome structure promotes contact between transcription factors and co-regulators with DNA, thereby activating transcription of specific genes. Conversely, histone deacetylases remove acetyl groups from lysine residues, restoring the positive charge of histones. The positively charged lysine residues create electrostatic attraction with DNA, increasing histone-DNA affinity and making promoters less accessible to transcriptional regulatory elements, thus inhibiting transcription [30].

All HDAC inhibitors share common structural features: a surface recognition moiety, a linker moiety that occupies the HDAC active site channel, and a zinc-binding group (metal-binding region) [31]. For fatty acid inhibitors, the metal-

binding region is the carboxylic acid group [32]. As an HDAC inhibitor, butyrate modulates deacetylation of lysine residues at histone N-termini, inhibits HDAC activity, prevents excessive histone hypoacetylation, and induces a state of moderate histone acetylation. This opens the highly ordered chromatin into a relaxed state, promoting transcription factor binding to DNA and activating transcription of milk fat and protein synthesis genes. The precise mechanisms by which histone acetylation and deacetylation regulate gene expression remain unclear. Previous research suggested three primary mechanisms: (1) altering the nucleosome environment to strengthen or weaken interactions between gene expression-related proteins and DNA; (2) participating in chromatin conformational changes that affect protein-protein and protein-DNA interactions; and (3) serving as specific signals recognized by other protein factors to influence their activities, thereby regulating gene expression [33].

Butyrate not only induces histone H4 acetylation but also enhances RNA polymerase synthetic capacity, thereby regulating alternative splicing processes [34]. HDAC activity affects splice site selection, and as an HDAC inhibitor, butyrate can modulate alternative splice sites to regulate gene expression. The splicing process of precursor RNA is crucial for gene expression in eukaryotes and represents an indispensable biological process during cell differentiation and development. Hnilicová et al. [35] found in studies of alternative splicing in HeLa cell lines that butyrate could regulate alternative splicing, with HDAC activity influencing splice site selection. Faddy et al. [36] demonstrated that treating human breast cancer MCF-7 cells with butyrate for 4 hours promoted PPARG receptor gene expression. Estrogen regulates PPARG gene expression, while HDAC inhibitors can modulate estrogen receptor expression. In cultured ovarian cancer cells, butyrate significantly promoted proto-oncogene mRNA degradation and inhibited splicing to reduce proto-oncogene expression.

As an HDAC inhibitor, butyrate regulates gene expression through multiple pathways. It not only modulates expression of genes related to proliferation and differentiation but also regulates immune-related genes. Without cytotoxic effects on dairy cow mammary epithelial cells, butyrate supplementation reduced Staphylococcus aureus numbers and upregulated expression of antimicrobial peptides, β-defensins, and inducible nitric oxide synthase genes [37]. Concurrently, both butyrate treatment and S. aureus infection increased histone H3 acetylation in BMECs. These results indicate that butyrate effectively regulates immune gene expression in mammary tissue and enhances anti-infective capacity. Butyrate also prevents Campylobacter invasion of cultured intestinal cells, which can cause enteritis [38]. Butyrate regulates not only individual gene expression but also participates in signaling pathway and gene network regulation. Microarray data analysis revealed that butyrate interferes with four classic signaling pathways: G2/M DNA damage checkpoint, pyrimidine metabolism, G1/S checkpoint control, and purine metabolism, thereby affecting intracellular metabolic processes [39]. Butyrate inhibits dimethylbenzanthracene-induced mammary tumor expansion [30] and can regulate chromatin architecture, suppress oncogenes, tumor-promoting genes, and inflammatory genes as an HDAC

inhibitor, playing important roles in cancer and inflammatory disease treatment.

3. Butyrate as a Ligand for G Protein-Coupled Receptors

Butyrate regulates gene expression by altering histone acetylation status and participates in controlling biological functions including cell proliferation, differentiation, apoptosis, and cell cycle arrest. Simultaneously, butyrate acts as an activator of G protein-coupled receptors (GPCRs). Short-chain fatty acids play important roles in mammary epithelial cells through GPCRs. G protein-coupled receptor 41 (GPR41) and GPR43 are members of the GPR40 family within the GPCR superfamily and were classified as orphan receptors until 2003, when Brown et al. [20] discovered that butyrate could serve as a ligand for GPR41 and GPR43. GPCRs regulate various biochemical reactions within cells by modulating cyclic adenosine monophosphate (cAMP) levels. GPR41 and GPR43 are members of the GPCR superfamily, with GPR41 expressed primarily in adipose tissue [20].

Yonezawa et al. [40] reported that short-chain fatty acid treatment of BMECs caused a rapid increase in intracellular calcium ions, activated mitogen-activated protein kinase (MAPK), increased phosphorylation of MAPK p38 and heat shock proteins, decreased cAMP levels, and triggered MAPK activation with increased intracellular MAPK phosphorylation. Xiong et al. [41] used in vivo and in vitro models to demonstrate that short-chain fatty acids could function as signaling molecules to regulate leptin content in adipocytes through GPR41. Wu [42] showed that GPR41 activation could counteract butyrate-induced reduction in cell viability and apoptosis and affect cell cycle distribution. Activated GPR41 participates in the G1-to-S phase transition, inhibiting expression of cyclin-dependent kinase inhibitor p21WAF1/CIP1, which represents an important target gene of HDAC inhibitors [43].

In summary, butyrate, as a precursor for milk component synthesis in ruminants, regulates milk component synthesis and influences milk quality by modulating expression of genes related to milk fat and protein synthesis. Additionally, as an HDAC inhibitor, butyrate regulates gene expression by altering histone acetylation status, suppressing expression of oncogenes, tumor-promoting genes, and inflammatory genes, thereby playing important roles in cancer and inflammatory disease treatment. Simultaneously, butyrate serves as an activator of G protein-coupled receptors, participating in the regulation of cell proliferation, differentiation, apoptosis, and cell cycle arrest. Current research has focused primarily on butyrate' s regulation of milk fat and protein synthesis, while the regulatory effects of exogenous butyrate supplementation on milk component synthesis and the precise mechanisms by which butyrate participates in gene expression regulation as an HDAC inhibitor remain incompletely understood and require further investigation.

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