Peroxisome Proliferator-Activated Receptor Regulates Rumen Ketogenesis in Young Ruminants and Its Mechanism: Postprint

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Date: 2018-12-24T00:00:00+00:00

Abstract

A well-developed rumen is essential for young ruminants, being critical not only for their immediate health but also for their future productive performance in adulthood. The rumen of newborn ruminants lacks ketogenic function; however, with increasing age, both rumen morphology and function gradually mature, eventually acquiring this capability. Ketogenesis serves as a key factor in rumen maturation, with -hydroxybutyrate (BHBA) considered a marker of rumen development. Over the past decade, numerous studies have investigated factors influencing rumen ketogenesis, revealing that peroxisome proliferator-activated receptors (PPARs) play an important role in rumen ketogenesis and epithelial cell proliferation, and that the transcription factor PPARs can affect the expression of the key ketogenic enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). Nevertheless, current understanding of the molecular mechanisms underlying PPARs regulation of rumen ketogenesis remains very limited; therefore, this review summarizes the research progress on PPARs regulation of rumen development in young ruminants.

Full Text

Peroxisome Proliferator-Activated Receptors: Regulation of Ketogenesis in the Rumen of Young Ruminants and Its Mechanisms

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Abstract: A well-developed rumen is critical for young ruminants, not only for their health but also for their production performance in adulthood. Newborn ruminants lack ketogenic function in the rumen, but as age increases, rumen morphology and function gradually mature, eventually acquiring this capacity. Ketogenesis is a key factor in rumen development and maturation, with -hydroxybutyric acid (BHBA) considered a marker of rumen development. Over the past decade, numerous studies have investigated factors affecting rumen ketogenesis, revealing that peroxisome proliferator-activated receptors (PPARs) are important for both rumen ketogenesis and epithelial cell proliferation. The transcription factor PPARs can influence expression of 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (HMGCS2), a key enzyme in ketogenesis. However, understanding of the molecular mechanisms by which PPARs regulate rumen ketogenesis remains limited. This review summarizes research progress on PPARs regulation of rumen development in young ruminants.

Keywords: peroxisome proliferator-activated receptors; young ruminants; rumen; ketogenesis; molecular mechanism

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Rumen functional development is crucial for young ruminants, and rumen ketogenesis is a key factor promoting rumen maturation. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcription factors that, in monogastric animals, primarily participate in mitochondrial fatty acid oxidation and energy metabolism [1-2]. In recent years, only a few studies have reported on the role of PPARs in ketogenesis [3-4], identifying some target genes such as 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (HMGCS2) and cholesterol acyltransferase 1 (ACAT1). While some ketogenesis-related enzymes regulated by PPARs have been identified, studies in mice have shown that butyrate can activate PPARs [5], but whether butyrate can activate PPARs in the rumen of ruminants remains unknown. We hypothesize that ruminal butyrate likely acts as a ligand to activate PPARs, thereby regulating downstream target gene expression. In monogastric animals, few studies have reported epigenetic regulation mechanisms of PPARs gene expression. For example, hypomethylation of the PPAR- promoter region in mice leads to increased mRNA expression of PPAR- and its target genes [6]. Since butyrate can cause epigenetic changes, we speculate that ruminal butyrate may induce epigenetic modifications of PPARs, thereby affecting their expression. Nevertheless, understanding of the epigenetic mechanisms affecting PPARs expression remains very limited. This review summarizes research progress on PPARs regulation of rumen ketogenesis in young ruminants, aiming to provide clues for further exploration of the molecular mechanisms underlying PPARs regulation of rumen ketogenesis, which is important for deeper understanding of rumen development and establishing optimal nutritional strategies for ruminants.

1 Developmental Characteristics of Rumen Ketogenesis

Rumen functional development is essential for healthy growth of ruminants after weaning [7]. Previous studies have shown that rumen development in lambs occurs in three stages: the non-ruminant stage (1-3 weeks), the transitional stage (4-8 weeks), and the ruminant stage (after 9 weeks). Newborn ruminants primarily depend on milk digestion for survival, and their rumen lacks the functions of adult ruminants. At this stage, glucose is the main energy source [1]. Through continuous exposure to the external environment, they gradually transition to plant-based feed digestion. During this shift in digestive mode, the rumen undergoes continuous changes, including extensive keratinization of the rumen wall, enabling rumen epithelial cells to absorb volatile fatty acids (VFA) and metabolize them to provide energy for the body, ensuring adequate nutrient absorption to meet metabolic needs. From 2 days to 6 months of age, glucose utilization in lambs decreases by approximately 90%, while rumen epithelial cell keratinization increases continuously, VFA metabolism by epithelial cells rises, and the utilization efficiency of butyrate and lactate gradually increases, along with the efficiency of butyrate conversion to ketone bodies.

Rumen ketogenesis primarily refers to the process by which butyrate generates acetyl-CoA, which is then converted to the ketone bodies acetoacetate and BHBA. 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) is the rate-limiting enzyme in ketogenesis, existing in two forms: 3-hydroxy-3-methylglutaryl coenzyme A synthase 1 (HMGCS1) and HMGCS2. Since ketogenesis in the rumen occurs mainly in mitochondria, HMGCS2 plays the primary role. Recent studies have found that both HMGCS2 and HMGCS1 participate in rumen ketogenesis [4], with HMGCS2 expression regulated by PPARs [3]. Therefore, PPARs play an important regulatory role in rumen ketogenesis.

2 Structure and Function of PPARs

PPARs are a class of ligand-activated nuclear transcription factors comprising three members: PPAR- , PPAR- , and PPAR- , belonging to the receptor superfamily [8]. PPARs contain four functional domains, which researchers have divided into six regions (A-F) for convenience. Domains A and B contain serine residues that can be phosphorylated by mitogen-activated protein kinase (MAPK), affecting PPARs activity. Domain C is the DNA-binding domain (DBD), through which PPARs bind to response elements to regulate gene transcription. Domain D is a transcriptional activity regulatory domain that binds nuclear cofactors to modulate PPARs activity. Domains E and F constitute the ligand-binding domain [Figure 1: see original paper] [9].

PPARs first bind to ligands, then form heterodimers with retinoid X receptor (RXR), causing changes in covalent bond properties. Subsequently, they recruit various coactivators and, after recognizing and binding to peroxisome proliferator response elements (PPRE) in the upstream promoters of target genes, exert regulatory effects [Figure 2: see original paper] [10-11]. PPRE is a hexanu-

cleotide repeat (AGGTCA).

Numerous studies have investigated PPARs functions in humans and monogastric animals. The three PPAR subtypes show different tissue distributions and physiological functions. PPAR- was the first subtype studied in mammals, being highly expressed in liver, heart, and kidney, and primarily participates in mitochondrial fatty acid transport and oxidation, energy metabolism, and oxidative stress [12]. PPAR- is highly expressed in skeletal cells and mainly participates in fatty acid metabolism. PPAR- has similar functions to PPAR- and also regulates rumen epithelial proliferation and differentiation, with expression levels significantly higher than PPAR- in the rumen [13], suggesting it may play a more important role in regulating rumen epithelial cell proliferation, though further research is needed. In human cancer studies, PPAR- expression is significantly elevated in renal cell carcinoma compared to normal cells [14], indicating a potential role in cell proliferation regulation. PPAR- is also detected in ruminant rumen with higher expression than PPAR- [15], but whether it regulates epithelial cell proliferation in ruminants requires further investigation.

In recent years, PPARs functions in ruminants have attracted considerable attention, particularly their roles in lipid metabolism. Fatty acids and their derivatives can serve as PPAR ligands to activate the receptors. PPARs play crucial roles in lipid and carbohydrate metabolism [16], promoting adipocyte proliferation upon binding to specific ligands [17]. PPAR- has been relatively less studied in ruminants, being highly expressed in kidney and liver but at low levels in rumen [15]. Fifteen years ago, studies in goats found that PPAR- participated in hepatic fatty acid oxidation [18]. Subsequently, Schlegel et al. [19] further demonstrated that PPAR- regulates fatty acid metabolism by controlling carnitine palmitoyltransferase 1A (CPT1A) expression. Compared to PPAR- , although some studies suggest PPAR- also participates in fatty acid oxidation [20], this requires further verification. PPAR- has been most extensively studied in ruminants, being highly expressed in adipose tissue and associated with fat generation [21] and long-chain fatty acid oxidation [22].

3 Molecular Mechanisms of PPARs Regulation of Rumen Ketogenesis

During rumen development in ruminants, ketone body production continuously increases. After activation by ligands, PPARs regulate downstream target gene expression, promoting rumen ketogenesis and epithelial cell proliferation and differentiation. In recent years, gene expression profiling has identified numerous differentially expressed genes accompanying accelerated rumen papillae growth. The promoter regions of HMGCS2, ACAT1, and fatty acid binding protein 3 (FABP3) all contain PPRE elements, and their expression is regulated by PPAR- [22]. Therefore, PPAR- plays a crucial regulatory role in rumen development. Kinoshita et al. [5] found that VFA can affect rumen ketogenesis and ketogenic gene expression, leading Penner et al. [23] to propose that VFA influence PPAR- expression to regulate ketogenesis during the transition from

glucose to butyrate utilization in the rumen. Connor et al. [24] identified PPAR- as associated with rumen epithelial cell proliferation and differentiation while exploring rumen developmental mechanisms. Additionally, fibroblast growth factor 21 (FGF21), another PPAR- target gene, plays an important role in energy metabolism. Mouse studies have shown that treatment with PPAR- agonists increases FGF21 expression, which in turn elevates hepatic CPT1A and HMGCS2 expression to promote ketogenesis [25]. However, whether PPAR- can induce FGF21 expression in young ruminant rumen and whether FGF21 promotes HMGCS2 expression to enhance rumen ketogenesis remain unknown.

In monogastric animals, the synthetic compound WY-14643 is a specific PPAR- ligand [26], L165041 is specific for PPAR- [27], and TZD is specific for PPAR- [28]; all can bind to and activate PPARs to exert gene regulatory effects. In ruminants, endogenous PPAR ligands may exist. Glucose in the rumen and fermentation products such as long-chain fatty acids (LCFA), propionate, and butyrate may serve as specific PPAR ligands to activate PPARs and regulate target gene expression, thereby promoting rumen ketogenesis and papillae development [Figure 3: see original paper] [15,29-33], though this requires experimental verification. Whether LCFA are true PPAR ligands in ruminants remains uncertain, as mouse studies indicate that hepatic LCFA exert transcriptional regulation by activating other transcription factors besides PPARs, including hepatic nuclear factor-4 (HNF4) and liver X receptor (LXR) [31]. Additionally, estrogen-related receptors (ESRRA) can directly activate PPAR- gene expression [32]. Connor et al. [24] demonstrated in calves that activated ESRRA further activates PPAR- to regulate rumen ketogenesis. Both PPAR- and PPAR- are regulators of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1), which in turn regulates ESRRA. Therefore, PPAR- and PPAR- activate ESRRA through interaction with PGC-1 [Figure 3: see original paper] [33]. Elucidating the interrelationships among PPARs, ESRRA, and PGC-1 is crucial for understanding PPARs and rumen ketogenesis.

4 Epigenetic Regulation Mechanisms of PPARs Gene Expression

Epigenetics refers to molecular mechanisms causing phenotypic and gene expression changes through modifications other than DNA sequence alterations, including DNA methylation, histone modification, non-coding RNA, and chromatin remodeling [34]. Mouse studies have shown that butyrate can activate PPAR- [5], and PPAR- can be silenced or activated through DNA hyper- or hypomethylation [6]. High fructose feeding in mice caused hypermethylation of PPAR- and CPT1A promoter regions, reducing their expression, while betaine supplementation significantly decreased PPAR- promoter methylation and upregulated PPAR- and target gene expression. Similarly, low PPAR- promoter methylation in rats increased PPAR- expression and enhanced hepatic fatty acid -oxidation [35]. Studies in humans [36] and mice [37] have demonstrated that lipid metabolism-related gene expression is affected by DNA methylation.

Supplementing methionine in dairy cow diets caused global DNA hypomethylation and specific region hypermethylation of PPAR- , upregulating its expression [38]. Therefore, nutritional regulation of young ruminants can affect lipid metabolism and ketogenesis-related genes through DNA methylation. DNA methylation levels influence PPARs activation and subsequent target gene expression, and butyrate plays an important role in rumen development. Based on this, we hypothesize that butyrate regulates rumen PPARs DNA methylation, with methylation changes altering PPARs and target gene expression, thereby modifying rumen ketogenesis [Figure 4: see original paper] [35-37].

Chromatin remodeling through histone modification plays an important role in eukaryotic gene expression regulation. Histone acetylation is catalyzed by histone acetylases (HATs) and histone deacetylases (HDAC) [39]. Histone acetylation activates gene transcription, while deacetylation inhibits it. Studies have shown that butyrate causes histone hyperacetylation and acts as an HDAC inhibitor [40]. Therefore, butyrate produced through rumen fermentation likely affects PPARs transcription as an HDAC inhibitor, influencing rumen ketogenesis and development [Figure 4: see original paper], though this requires verification. Currently, research on epigenetic mechanisms during rumen development is scarce, and no studies have reported on epigenetic mechanisms regulating PPARs expression in the rumen.

5 Summary

Ketogenesis is a hallmark of rumen maturation, and PPARs are important transcriptional regulators controlling ketogenesis and rumen development. To date, only some enzymes related to ketogenesis and regulated by PPARs have been identified. While mouse studies have shown that butyrate can activate PPARs, whether butyrate activates PPARs in the ruminant rumen remains unreported. The pathways through which PPARs regulate rumen ketogenesis and the factors activating PPARs are not yet clear. The possible molecular mechanism is that glucose and fermentation products (especially propionate and butyrate) in the rumen act as ligands to activate PPARs, thereby regulating target gene expression. VFA (especially butyrate) affect rumen DNA methylation levels, regulating PPARs and related target gene expression, while butyrate acts as an HDAC inhibitor to influence PPARs transcription, thereby affecting rumen ketogenesis and development. However, the pathways of PPARs regulation of rumen ketogenesis and factors activating PPARs remain unclear. The relative expression levels of the three PPAR subtypes in the ruminant rumen require further investigation, and the relative importance of their functions warrants significant exploration.

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Note: Figure translations are in progress. See original paper for figures.