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Propionate induces the bovine cytosolic phosphoenolpyruvate carboxykinase promoter activity

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ABSTRACT

Cytosolic phosphoenolpyruvate carboxykinase (*PCK1*) is a critical enzyme within the metabolic networks for gluconeogenesis, hepatic energy metabolism, and tricarboxylic acid cycle function, and is controlled by several transcription factors including hepatic nuclear factor 4α (HNF4α). The primary objective of the present study was to determine whether propionate regulates bovine *PCK1* transcription. The second objective was to determine the action of cyclic AMP (cAMP), glucocorticoids, and insulin, hormonal cues known to modulate glucose metabolism, on bovine *PCK1* transcriptional activity. The proximal promoter of the bovine *PCK1* gene was ligated to a Firefly luciferase reporter and transfected into H4IIE hepatoma cells. Cells were exposed to treatments for 23 h and luciferase activity was determined in cell lysates. Activity of the *PCK1* promoter was linearly induced by propionate, and maximally increased 7-fold with 2.5 m*M* propionate, which was not muted by 100 n*M* insulin. Activity of the *PCK1* promoter was increased 1-fold by either 1.0 m*M* cAMP or 5.0 μ*M* dexamethasone, and 2.2-fold by their combination. Induction by cAMP and dexamethasone was repressed 50% by 100 n*M* insulin. Propionate, cAMP, and dexamethasone acted synergistically to induce the *PCK1* promoter activity. Propionate-responsive regions, identified by 5′ deletion analysis, were located between −1,238 and −409 bp and between −85 and +221 bp. Deletions of the core sequences of the 2 putative HNF4α sites decreased the responsiveness to propionate by approximately 40%. These data indicate that propionate regulates its own metabolism through transcriptional stimulation of the bovine *PCK1* gene. This induction is mediated, in part, by the 2 putative HNF4α binding sites in the bovine *PCK1* promoter.

Key words: hormone, short-chain fatty acid, *PCK1*, promoter

INTRODUCTION

Phosphoenolpyruvate carboxykinase (**PCK**; EC 4.1.1.32), a key enzyme of gluconeogenesis, tricarboxylic acid (TCA) cycle function, and several metabolic networks that affect energy metabolism in the liver and kidney, catalyzes the irreversible formation of phosphoenolpyruvate from oxaloacetate (Rognstad, 1979; She et al., 2000; Burgess et al., 2007). It is well documented in nonruminant species that the gene for the cytosolic form of PCK (*PCK1*) is actively regulated by hormones and nutrients at the transcriptional level, most notably through induction by glucagon and glucocorticoids, effects that are repressed by insulin action (Hanson and Reshef, 1997). Accordingly, expression of *PCK1* is induced by starvation and reduced during feeding (Hanson and Reshef, 1997). However, in ruminants, the expression of *PCK1* is not altered during feed restriction (Velez and Donkin, 2005), but is induced in response to increased feed intake (Greenfield et al., 2000) and monensin feeding (Karcher et al., 2007), conditions that are linked to increased ruminal propionate production. Expression of *PCK1* mRNA is increased in neonatal calves and at least maintained with postruminal propionate infusions in lactating dairy cows despite elevated insulin concentrations (Zhang et al., 2015), a hormone that potently and rapidly represses *PCK1* mRNA expression (Granner et al., 1983; Chakravarty and Hanson, 2007).

Studies using primary rat hepatocytes and H4IIE rat hepatoma cells indicate that short-chain fatty acids (**SCFA**), including propionate and butyrate, modulate expression of both *PCK1* mRNA and *G6PC* mRNA (Massillon et al., 2003). Binding of hepatic nuclear factor 4α (**HNF4α**) to mouse *G6PC* promoter is required for stimulation of transcription by propionate and butyrate (Massillon et al., 2003). Hepatic nuclear factor 4α is a member of the nuclear receptor superfamily abundantly expressed in the liver and required for liver-specific gene expression in response to hormones and nutrients (Sladek et al., 1990; Babeu and Boudreau, 2014). Upon binding to its recognition sites in a gene promoter, HNF4α is able to recruit coactivators of

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transcription factors, such as peroxisome proliferator-activated receptor γ coactivator-1α, to stimulate transcriptional activity (Gonzalez, 2008). The critical role of HNF4α in activating transcription of the *PCK1* and *G6PC* gene has also been confirmed using hepatocytes from mice lacking hepatic HNF4α (Rhee et al., 2003).

These observations led us to question if the induction of *PCK1* mRNA expression by propionate in liver of dairy cattle might be due to direct activation of the bovine *PCK1* promoter. We were likewise interested in determining the relationship between propionate, glucagon, glucocorticoids, and insulin on bovine *PCK1* gene transcription. In addition, the bovine *PCK1* promoter was analyzed to reveal putative transcription factor binding sites and the functionality of the putative HNF4α binding sites in mediating responses to propionate and butyrate were tested. Here, we show that propionate has an inductive effect on the transcriptional activity of the bovine *PCK1* promoter, which is dominant to the repressive effect of insulin and synergistic to the inductive effect of the combination of cyclic AMP (**cAMP**) and dexamethasone. We also demonstrate that 2 putative HNF4α-binding sites within the bovine *PCK1* promoter are responsible, in part, for propionate-induced *PCK1* transcription.

MATERIALS AND METHODS

Promoter-Luciferase Reporter Constructs

The bovine *PCK1* proximal promoter sequence was obtained from National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/). The DNA sequence containing the proximal promoter region from −1,238 through +221 bp relative to the transcription start site (**TSS**) of the bovine *PCK1* gene (NCBI Gene ID: 282855) was linked to a Firefly luciferase reporter. The promoter region corresponds to bases 59,151,952 through 59,150,494 of the bovine chromosome 13. To determine the propionate-responsive elements within the bovine *PCK1* promoter, 5′ nested deletions of the proximal promoter region were individually linked to the Firefly luciferase reporter. The target promoter sequences were amplified from bovine genomic DNA by PCR using the primers presented in Table 1. The amplified PCR products were purified using agarose gel electrophoresis and the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and each was ligated into the pDrive cloning vector (Qiagen), which was used to transform Qiagen EZ Competent Cells (Qiagen). Positive clones were grown individually overnight for plasmid DNA isolation using the Wizard Plus SV Miniprep kit (Promega, Madison, WI). The cloned sequences were then excised from pDrive and ligated separately into the pGL3-Basic vector (Promega). Each *PCK1* promoter-luciferase plasmid was used to transform competent JM109 *Escherichia coli*, and positive clones were grown individually overnight for plasmid DNA isolation using the Wizard Plus SV Miniprep kit (Promega). Insert sequence and orientation of the promoter regions were verified by sequencing at the DNA Sequencing Low Throughput Laboratory of Purdue Genomics Core Facility (Purdue University, West Lafayette, IN) using the ABI 3700 sequencer (Amersham Biosciences, Piscataway, NJ).

In Silico Promoter Sequence Analysis

The bovine *PCK1* proximal promoter sequence (−1,238/+221) was analyzed using the TRANSFAC transcription factor analysis tool (Biobase, Beverly, MA). The search parameters were limited to liver-specific factors, high-quality matrices, and set to minimize false positives.

Site-Directed Mutagenesis

The essentiality of the putative HNF4α binding sites within the bovine *PCK1* gene located at +68 through

Table 1. Primer sequences used to amplify the promoter regions of the bovine *PCK1* gene and for the site-directed mutagenesis

Promoter construct	Primer sequence (5′–3′)
PCK1 promoter (−1,238/+221)	Forward: GGTCTCATTGCTCAAGTGTAATCG
PCK1 promoter (−815/+221)	Forward: AATCCACAAGGCAGGTGTGACTGA
PCK1 promoter (−409/+221)	Forward: ACCACTGCTCTATTCTGGCAACCA
PCK1 promoter (−251/+221)	Forward: CAGAAGTTGTGTAAAGGCCTGCCA
PCK1 promoter (−85/+221)	Forward: AGTCGAGCCTCTCTGGGTGTG
All promoter regions of *PCK1*	Reverse: AGAGTTGAGGGTGTCCATGGTTGT
Mutant HNF4α$_{(+68_+72)^-}$	Forward: AAGGGACCCTTTGGCTGACCTGATCGTCCA
	Reverse: TGGACGATCAGGTCAGCCAAAGGGTCCCTT
Mutant HNF4α$_{(-1,078_-1,074)^-}$	Forward: AGGTTCCAGGAAGGGGTCCCTTACAAGAGG
	Reverse: CCTCTTGTAAGGGACCCCTTCCTGGAACCT
Double mutant HNF4α$_{(+68_+72)^-}$/HNF4α$_{(-1,078_-1,074)^-}$	Forward: AAGGGACCCTTTGGCTGACCTGATCGTCCA
	Reverse: TGGACGATCAGGTCAGCCAAAGGGTCCCTT