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Propionate induces mRNA expression of gluconeogenic genes in bovine calf hepatocytes

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ABSTRACT

Hepatocytes monolayers from neonatal calves were used to determine the responses of the cytosolic phosphoenolpyruvate carboxykinase (*PCK1*) mRNA expression to propionate and direct hormonal cues including cyclic AMP (cAMP), dexamethasone, and insulin. The responses of other key gluconeogenic genes, including mitochondrial phosphoenolpyruvate carboxykinase (*PCK2*), pyruvate carboxylase (*PC*), and glucose-6-phosphotase (*G6PC*), were also measured. Expression of *PCK1* was linearly induced with increasing propionate concentrations in media and 2.5 m*M* propionate increased *PCK1* mRNA at 3 and 6 h of incubation; however, the induction disappeared at 12 and 24 h. The induction of *PCK1* mRNA by propionate was mimicked by 1 m*M* cAMP, or in combination with 5 μ*M* dexamethasone, but not by dexamethasone alone. The induction of *PCK1* mRNA by propionate or cAMP was eliminated by addition of 100 n*M* insulin. Additionally, expression of *PCK2* and *PC* mRNA was also induced by propionate in a concentration-dependent manner. Consistent with *PCK1*, propionate-stimulated *PCK2* and *PC* mRNA expression was inhibited by insulin. Expression of *G6PC* mRNA was neither affected by propionate nor cAMP, dexamethasone, insulin, or their combinations. These findings demonstrate that propionate can directly regulate its own metabolism in bovine calf hepatocytes through upregulation of *PCK1*, *PCK2*, and *PC* mRNA expression.
Key words: *PCK1*, hormonal regulation, gluconeogenesis

INTRODUCTION

The importance of gluconeogenesis in ruminants is highlighted by the extensive fermentation of simple sugars and starch in the rumen, despite extensive metabolic needs for glucose by tissues and as a precursor for milk lactose synthesis (Aschenbach et al., 2010). Hypoglycemia, ketosis, and related metabolic disorders are often observed when gluconeogenic capacity in the liver fails to adapt to the increased tissue demands for glucose in dairy cattle (Aschenbach et al., 2010). The prevalence of this insufficiency is highlighted by reports that the incidence of ketosis in commercial dairy herds may be as high as 17% (Dohoo and Martin, 1984). Because propionate contributes approximately 50% of the carbon for gluconeogenesis (Huntington, 1990), strategies to increase propionate supply, including monensin feeding, have successfully reduced the incidence of ketosis in transition dairy cows (Duffield et al., 1998). In addition to the documented effect of monensin on feed efficiency and increased ruminal propionate production (Sauer et al., 1989), a portion of the effect of monensin in transition cows may be linked to increased capacity for gluconeogenesis through enhanced expression of cytosolic phosphoenolpyruvate carboxykinase (*PCK1*), a key gene for glucose synthesis (Karcher et al., 2007).

Control of *PCK1* enzyme activity has been studied extensively in nonruminants and is primarily exerted through transcription of the gene via activation of basal, tissue-specific, and hormone-dependent promoter elements within the 5′-flanking region of the *PCK1* gene (Hanson and Reshef, 1997). Expression of *PCK1* mRNA in nonruminants is activated by glucagon and glucocorticoids and insulin counteracts these effects (O′Brien and Granner, 1990). A lack of response for *PCK1* to feed restriction in dairy cows (Velez and Donkin, 2005) and sheep (Filsell et al., 1969; Taylor et al., 1971; Smith et al., 1982) is in direct contrast with data from nonruminants demonstrating that feed restriction induces the *PCK1* gene (Hanson and Reshef, 1997). Although in vivo administration of glucagon in dairy cattle suggests *PCK1* induction in dairy cattle (Bobe et al., 2009), these data are equivocal perhaps due to counter-regulatory release of insulin and other hormones (Bobe et al., 2009) in the experimental models used.

Regulation of *PCK1* in response to glucagon and other hormones has been extensively studied in rat hepatocytes and hepatoma cells. The mRNA for *PCK1*

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is maximally induced by cyclic AMP (**cAMP**) in presence of glucocorticoids within 3 h (Christ et al., 1988). Likewise, short-chain fatty acids, including propionate, induce *PCK1* mRNA in rat hepatocytes (Massillon et al., 2003), indicating the potential for propionate to control its own metabolism. Effects of glucagon on propionate and lactate metabolism in hepatocytes from neonatal calves indicate increased gluconeogenesis from propionate and lactate, but not glycerol (Donkin and Armentano, 1994), suggesting a regulation point of *PCK1* by glucagon. However, the direct effects of propionate and hormones on *PCK1* mRNA in bovine hepatocytes have not been determined. We hypothesized that propionate and cAMP act to induce *PCK1* mRNA expression and insulin opposes these effects on *PCK1* mRNA in liver hepatocytes.

The primary objective of the current research was to determine the direct effects of propionate, cAMP, dexamethasone, and insulin on mRNA expression of *PCK1* in the neonatal calf hepatocytes. The secondary objective was to evaluate the relative effects of propionate and these hormonal cues on mRNA expression of other key gluconeogenic genes, including mitochondrial phosphoenolpyruvate carboxykinase (*PCK2*), pyruvate carboxylase (*PC*), and glucose-6-phosphatase (*G6PC*).

MATERIALS AND METHODS

Care and Use of Hepatocyte Donor Animals

All experimental procedures involving animals were approved by the Purdue University Animal Care and Use Committee. Three intact male dairy calves were used as hepatocyte donor animals. All calves received 3.8 L of superior colostrum for the first feeding postpartum and 1.9 L of superior colostrum during the first 48 h of life, for a total of 5.7 L. Calves subsequently received milk at 10% of BW in 2 equal feedings per day. Calves were housed in a drying pen for the first 24 h, then moved to individual calf hutches. At less than 7 d of age, the calves (47 ± 3 kg of BW) were given heparin (1,000 USP, Sagent Pharmaceuticals, Schaumburg, IL) intravenously and anesthetized with 1% propofol (6 mg/kg of calf BW, Hospira Inc., Lake Forest, IL) via intravenous injection. The caudate process was excised and the animals were euthanized immediately by intravenous injection of saturated potassium chloride (150 mg/kg of calf BW, Sigma-Aldrich, St. Louis, MO).

Hepatocyte Monolayer Preparation and Culture

Liver cells were dispersed via collagenase perfusion as described previously (Donkin and Armentano, 1993). The isolated hepatocytes were plated on 35-mm Falcon Primaria Easy Grip tissue culture dish (Becton Dickinson, Lincoln Park, NJ) at an approximate density of 5.3×10^4 cells/cm^2 in Dulbecco's Modified Eagles Medium (**DMEM**) containing 20% fetal bovine serum and 1% antibiotic, antimycotic solution (Sigma-Aldrich). After 4 h, the plating medium and unattached cells were removed by aspiration and media was replaced by DMEM containing 10% fetal bovine serum and 1% antibiotic, antimycotic solution.

Twenty hours after seeding, the media was removed by aspiration and replaced with DMEM supplemented with 1% BSA (Merck Millipore, Billerica, MA) and 1% antibiotic, antimycotic solution, and the respective treatment additions. Time-dependent effect of propionate exposure was determined in the presence of either 0 or 2.5 m*M* propionate for 3, 6, 12, and 24 h. Concentration dependence to propionate was determined in the presence of 0, 0.625, 1.25, 2.5, and 5.0 m*M* propionate for 6 h. The effects of hormone additions and their relationship with propionate were tested using 1 m*M* 8-Br-cAMP, 5 μ*M* dexamethasone, 100 n*M* insulin, 2.5 m*M* propionate, or their designated combinations over a period of 6 h.

RNA Extraction and PCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen, Inc., Thousand Oaks, CA) and quantified by absorbance at 260 nm using a ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE). A total of 2 μg of purified sample was reverse transcribed to cDNA using an Omniscript reverse transcriptase kit (Qiagen, Inc.), random decamers (Ambion, Foster City, CA), and oligo-dT (Qiagen, Inc.). Abundance of cDNA product for each RNA sample was quantified using real-time PCR, Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies, Inc., Santa Clara, CA) with primers described below. A cDNA pool was generated from an equivalent quantity of cDNA from each sample. A 1:4 dilution series of the cDNA pool was used to generate the standard curve. No-template control (using water as template) and no-reverse transcription control (using RNA pool as template) were included in the real-time PCR analysis. The forward and reverse primers, respectively, for *PCK1*, *PCK2*, *PC*, *G6PC,* GAPDH, and *18S* were: bovine *PCK1* (GenBank accession: NM_174737.2), AGGGAAATAGCAGGCTCCAGGAAA, CACACGCATGTGCACACACACATA; bovine *PCK2* (GenBank accession: NM_001205594.1), TGACTGGGCAAGGGGAGCCG, GGGGCCACCCCAAAGAAGCC; bovine *PC* (GenBank accession: NM_177946.4), CCACGAGT TCTCCAACACCT, TTCTCCTCCAGCTCCTCGTA; bovine *G6PC* (GenBank accession: NM_001076124.2),