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Serotonin receptor expression is dynamic in the liver during the transition period in Holstein dairy cows

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ABSTRACT

Nonneuronal serotonin (5-HT) participates in glucose metabolism, but little is known regarding the actions of 5-HT in the liver during the transition period in dairy cattle. Here, we explore circulating patterns of 5-HT and characterize the hepatic 5-HT receptor and glucose transporter profiles around calving in multiparous Holstein dairy cows (n = 6, average lactation = 4 ± 1.9). Concentrations of serum 5-HT decreased on day −3 compared with −5 and −7 precalving (167.7 ± 80 vs 1511.1 ± 602 ng/mL). 5-HT nadir was on day −1 precalving and remained low postcalving (481.4 ± 49 ng/mL). Plasma glucose concentrations decreased precalving ($P = 0.008$) and were positively correlated with 5-HT during the precalving period (r = 0.55, $P = 0.043$). On day 1, postcalving hepatic messenger RNA expression of *5-HT*$_{1D,\ 2B,\ 3C,\ 6,\ \text{and}\ 7}$ receptors were decreased compared with day −7 ($P < 0.048$). The *5-HT*$_{3A}$ and *5-HT*$_{3B}$ decreased on day 7. The *5-HT*$_{2A}$ increased on days 1 and 7 compared with −7 ($P < 0.05$). The *5-HT*$_{1F}$ and *5-HT*$_{1A}$ receptors were increased 2.5- and 3.8-fold on day 7, respectively, compared with days −7 and 1 ($P < 0.046$). The *5-HT*$_{5A}$ was not detected, and *5-HT*$_{4}$ was detected on days −7 and 1 only. Expression of Glut-2,-5 and SGLT1 were decreased on days 1 and 7 compared with −7 ($P < 0.05$), whereas Glut-1 was increased on day 7 compared with −7 ($P < 0.05$). These results indicate that 5-HT could be important for liver glucose homeostasis possibly through receptor mediated signaling at specific times. Additional research is needed to further explore the functional role of these receptors in the liver during the transition from pregnancy to lactation.

1. Introduction

The transition from pregnancy to lactation is an extremely challenging time period for the homeostatic regulatory mechanisms of a dairy cow [1]. This is the result of a drastic increase in nutrient requirements for milk production, particularly glucose, which is an important source of energy for the neonate and a lactose precursor for milk synthesis. The onset of lactation is characterized by the most substantial endocrine and metabolic changes at any point in time during the lactation cycle [2]. As a consequence, all dairy cows experience some degree of negative energy balance (NEB) which is characterized by increased nonesterified fatty acids (NEFA) and decreased circulating glucose levels [3]. The ability of the cow to overcome this NEB is critical for a successful lactation. Furthermore, the liver is crucial for the metabolism and partitioning of nutrients to the mammary gland during lactation [1].

Recent studies support a physiological role for the monoamine 5-hydroxytryptamine (serotonin, 5-HT) in glucose and energy metabolism [4–6]. Serotonin is a monoamine synthesized from the amino acid L-tryptophan, with the rate-limiting step catalyzed by tryptophan hydroxylase (TPH) to form 5-hydroxytryptophan, which is then converted to 5-HT. Two independent 5-HT systems exist that contribute to the overall available 5-HT in the body, with separate genes encoding TPH resulting in the

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production of 5-HT from L-tryptophan: one in the brain, where 5% of 5-HT is located and synthesized by the TPH2 enzyme and one in the nonneuronal tissues, primarily stored in platelets, where 95% of 5-HT is located in the systemic circulation and various tissues are synthesized by TPH1 [7]. Research has been traditionally focused on the neuronal aspects of 5-HT and its role as a neurotransmitter in aspects of depression, behavior, and anxiety [8]. However, most peripheral tissues and organs, including the liver, have the machinery required to synthesize and metabolize 5-HT, as well as express unique patterns of 5-HT receptors [6,9]. Indeed, 5-HT signals through more than 15 receptor subtypes that include 7 classes (5-HT_1 to 5-HT_7), which signal through various G-protein coupled receptor pathways, and ligand-gated ion channels (5-HT_3), allowing 5-HT to regulate a multitude of physiological functions in a variety of tissues.

Studies have shown that 5-HT regulates insulin secretion [10–12], suggesting a role for 5-HT in glucose uptake. Serotonin induces both hyperinsulinemia and hyperglycemia simultaneously [4] and participates in liver regeneration [9]. Moore et al [13,14] demonstrated that infusion of 5-HT or 5-hydroxytryptophan enhances hepatic glucose uptake in dogs. Our laboratory has shown that 5-HT is responsible for hepatic upregulation of gluconeogenic enzymes as well as mammary gland and liver glucose transporters, as well as impacts mammary gland energy metabolism [5]. In addition, the severity of ketosis, one of the major metabolic disorders associated with glucose demands at the onset of lactation, is negatively correlated with circulating 5-HT in Holstein dairy cows [15]. Furthermore, 5-HT is increased during fasting in adipose tissue favoring lipolysis and in hepatocytes favoring liver gluconeogenesis [16]. It is plausible that the diverse actions regulated by 5-HT might be modulated through independent receptors and mechanisms of action.

This research presents new opportunities to explore the serotonergic axis in dairy cows during the transition from pregnancy to lactation with specific interest on its implication for the regulation of maternal glucose metabolism. To date, little is known regarding the actions of 5-HT and the receptor expression profile in the liver during the transition period in dairy cattle. Therefore, we performed an observational study to characterize the dynamics of circulating and liver 5-HT during the transition from pregnancy to lactation as well as the expression pattern of the major 5-HT receptors and glucose transporters in the liver of Holstein dairy cows.

2. Materials and methods

2.1. Animals

All experiments were performed under protocols approved by the Animal Care and Use Committee at the University of Wisconsin-Madison. Pregnant multiparous Holstein dairy cows were used in an observational time course experiment (n = 6, average lactation = 4 ± 1.9). Cows were fed a standard prefresh diet and given a Prominerall mix (VitaPlus, Madison, WI). Pregnant cows were enrolled on the experiment 2 weeks before the initiation of sample collection. All cows calved within a 10-day period (April 30, 2013 to May 7, 2013). Cows enrolled in the study remained mostly healthy during the experiment; however, we did observe retained placentas in 75% of the animals.

2.2. Sample and data collection

Whole blood samples were collected from coccygeal vessels every 2 days beginning 7 d precalving (−7, −5, −3, and −1) through 7 d postcalving (1, 3, 5, and 7). To harvest the serum and plasma fractions 10 mL BD Vacutainer Serum Plus (367820, BD, Franklin Lakes, NJ, USA) and lithium heparin 158 USP Units Plus Blood Collection Tubes (367880, BD) were used, respectively. Samples were centrifuged at 3000*g* for 20 min at 4°C, and the serum and plasma fractions were collected and stored at −80°C until analysis.

Liver biopsies were performed on day −7 ± 1.9 d precalving, and days 1 and 7 postcalving. Liver tissue was snap frozen in liquid nitrogen and stored at −80°C until analysis. Milk yield was recorded daily (in the morning and evening) for all cows the first week postcalving (days 1 to 7).

2.3. Blood measurements

Blood serum 5-HT was measured using the Serotonin Enzyme Immunoassay (EIA) Kit (IM1749, Immunotech, Beckman Coulter, Marseille Cedex 9, France). Samples were diluted 1:100 to fall within the range of the standard curve of the assay. The intra- and inter-assay coefficient of variation was 6.3% and 7.6%, respectively. Glucose concentrations were measured in plasma using a glucose oxidase-peroxidase assay specific for glucose [17]. The intra- and inter-assay coefficient of variation was 5.6% and 6.4%, respectively.

2.4. RNA isolation and gene expression assessment

Total RNA was isolated from liver tissue using TRI-Reagent (Molecular Research) according to manufacturer's instructions. RNA concentration and absorbance ratios were quantified using a Nanodrop spectrophotometer (ND-1000; Nanodrop Technologies). One μg of total RNA was reversed transcribed using iScript reverse transcription supermix for RT-qPCR Kit (BioRad #1708841) and diluted (1:5) in deionized water. Quantitative PCR was conducted using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using SSoFast EvaGreen Supermix (Bio-Rad # 1725203) as described previously [5]. We evaluated the hepatic expression of 5-HT receptors (5-HTR, isoforms 1a, 1b, 1d, 1f, 2a, 2b, 2c, 5a, and 7) and glucose transporters (SGLT-1, Glut-1, Glut-2, Glut-5, Glut-8, Glut-9 and Glut-10). Primer sequences used are located in Table 1.

2.5. Protein isolation and analysis

Protein was isolated from the liver tissue using radioimmunoprecipitation assay buffer plus 10 μL/mL of Halt Protease and Phosphatase Inhibitors Cocktail (Thermo scientific #78441). Protein concentrations were determined with the bicinchoninic acid assay (Pierce Chemicals