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Hepatic purinergic signaling gene network expression and its relationship with inflammation and oxidative stress biomarkers in blood from peripartal dairy cattle

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ABSTRACT

The liver plays a central role in allowing dairy cattle to make a successful transition into lactation. In liver, as in other tissues, extracellular nucleotides and nucleosides trigger cellular responses through adenosine and ATP receptors. Adenosine triphosphate and certain nucleotides serve as signals that can heighten purinergic receptor activation in several pathologic processes. We evaluated the mRNA expression of genes associated with the purinergic signaling network in liver tissue during the peripartal period. Seven multiparous Holstein cows were dried off at d −50 relative to expected parturition and fed a controlled-energy diet (net energy for lactation = 1.24 Mcal/kg of DM) for ad libitum intake during the entire dry period. After calving, all cows were fed a common lactation diet (net energy for lactation = 1.65 Mcal/kg of DM) until 30 DIM. Biopsies of liver were harvested at d −10, 7, and 21 for mRNA expression of 9 purinergic receptors, 7 ATP and adenosine transport channels, and 10 enzymes associated with ATP hydrolysis. Blood collected at d −21, −10, 7, 14, and 21 was used to measure concentrations of inflammation and oxidative stress biomarkers. The expression of type 1 purinergic receptors (*ADORA2A* and *ADORA3*), several nucleoside hydrolases [ectonucleoside triphosphate diphosphohydrolase 7 (*ENTPD7*), ectonucleotide pyrophosphatase/phosphodiesterase 2 (*ENPP2*), *ENPP3*, and adenosine deaminase (*ADA*)], and a type 2 purinergic receptor (*P2RX7*) was downregulated after calving. In contrast, the expression of type 2 purinergic receptors (*P2RX4* and *PR2Y11*), an ATP release channel (gap junction hemichannel *GJB1*), and an adenosine uptake protein (*SLC29A1*) followed the opposite response, increasing after calving and remaining elevated through 21 d. Haptoglobin, ceruloplasmin, and reactive oxygen metabolite concentrations increased gradually from d −21 d through at least d 7. The opposite response was observed for albumin, paraoxonase, α-tocopherol, and nitric oxide, which decreased gradually to a nadir at 7 and 14 d. Our results suggest that alterations after calving of the expression of hepatic purinergic signaling genes could be functionally important because in nonruminants, they play roles in bile formation, glucose metabolism, cholesterol uptake, inflammation, and steatosis. The correlation analysis provided evidence of a link between purinergic signaling genes and biomarkers of inflammation and oxidative stress.
Key words: liver metabolism, transition cow, adenosine, adenosine triphosphate

INTRODUCTION

In the 1970s, the mechanism of purinergic signaling and the function of extracellular ATP as a signaling molecule were demonstrated by Burnstock (1972). Since then, work in nonruminants revealed that various metabolic tissues such as liver, adipose, and skeletal muscle express purinergic receptors and ATP-hydrolysis ectoenzymes (Fausther et al., 2012). The P1 (or **ADORA**) and P2 (or **P2RX/Y**) purinergic receptors are activated by extracellular nucleotides [e.g., ATP, adenosine diphosphate (**ADP**), uridine triphosphate uridine diphosphate (UTP), and (UDP)] and adenosine (also inosine) to modulate liver functions such as protein synthesis, cell proliferation, glucose metabolism, and immune responses (Beldi et al., 2008a). Besides their role within tissues, various nucleosides (e.g., adenosine, adenine, and 5-guanosine monophosphate)

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released by bacterial pathogens serve as signals to suppress components of the oxidative killing mechanisms of immune cells (e.g., neutrophils, macrophages; Canning et al., 1985, 1986).

The P1 receptors are classified as A1 (*ADORA1*), A2a (*ADORA2A*), A2b (*ADORA2B*), and A3 (*ADORA3*; Barletta et al., 2012). The P2 receptors are classified as ligand ion-gated channels (*P2RX1* to *P2RX7*) or G-protein coupled channels (*P2RY1*, *P2RY2*, *P2RY4*, *P2RY6*, and *P2RY11* to *P2RY14*). The former modulate extracellular ion fluxes (e.g., Na^+, K^+, Ca^{2+}, and Cl^-) and the latter modulate cytosolic cyclic AMP (**cAMP**) concentration (Beldi et al., 2008a; Junger, 2008), glycogen phosphorylase activity, bile formation, cytokine secretion, and cholesterol metabolism (Fausther and Sévigny, 2011; Fausther et al., 2012).

Several mechanisms of cellular ATP release have been proposed thus far, including the gap junction (**GJ**) hemichannels (*GJA1*, *GJB1*, and *GJB2*), and pannexin 1 (***PANX1***; Fausther and Sévigny, 2011). Once ATP is released from the cell, it is rapidly converted to ADP, AMP, adenosine, and inosine in a stepwise fashion (Fitz, 2007). Various nucleotide hydrolyzing enzymes take part in the extracellular ATP metabolism cycle, including several isotypes of ectonucleoside triphosphate diphosphohydrolase (***ENTPD***) and ectonucleotide pyrophosphatase/phosphodiesterase (***ENPP***), ecto-5′-nucleotidase (***NT5E***), and adenosine deaminase (***ADA***; Beldi et al., 2008b; Fausther and Sévigny, 2011; Junger, 2011). The ENTPD enzyme converts ATP or ADP to AMP, ENPP converts ATP to AMP, NT5E converts AMP to adenosine, and ADA converts adenosine to inosine. After ATP hydrolysis by ectonucleotidases and other nucleosidases, adenosine is transported into the cytosol primarily via the integral-membrane "equilibrative" nucleoside transporters (e.g., *SLC29A1* and *SLC29A2*; Junger, 2011; Choi and Berdis, 2012).

Strong evidence exists in nonruminants that activation of purinergic receptors in cells of the liver can modulate specific functions, including bile secretion, glucose metabolism, cholesterol metabolism, and inflammation (Fausther and Sévigny, 2011). Furthermore, evidence exists that bacterial pathogens utilize nucleosides as one mechanism to inhibit the host immune response (Canning et al., 1985, 1986). The peripartal period in dairy cattle is characterized by a decrease in plasma cholesterol and glucose concentration along with increases in the concentrations of inflammatory indicators synthesized by the liver, all of which reflect alterations characteristic of the onset of lactation (Drackley et al., 2006; Bertoni et al., 2009). It is currently believed that metabolic stress and an altered immune function are causative factors leading to increased incidence of infectious diseases and chronic inflammatory events (Goff, 2006; Bionaz et al., 2007; Bertoni et al., 2008; Sordillo et al., 2009). However, the cause (or causes) of the so-called immunosuppression (Mallard et al., 1998) around the time of calving is not completely understood.

Alterations in the synthesis of nucleosides or the mRNA expression of purinergic signaling genes, or both, could contribute to metabolic stress and immunosuppression around parturition. For instance, the mRNA expression of *ADA* and *PANX1* (a nucleotide release channel) in blood neutrophils increased almost 2-fold between −10 and 3 d around calving (Seo et al., 2013), suggesting an increase in the local concentration of nucleotides and nucleosides. To our knowledge, no information is available on the plasticity of purinergic signaling gene networks in the liver around parturition. Therefore, our main objective was to evaluate the expression profiles of the purinergic signaling gene network in the liver. Profiles in blood of several indicators of oxidative stress and inflammation also were analyzed.

MATERIALS AND METHODS

Animals and Management

Complete details of these procedures were previously reported by Ji et al. (2012). Briefly, 7 multiparous Holstein cows were used. All cows were dried off at d −50 relative to expected parturition and fed a controlled-energy diet (NE_L = 1.24 Mcal/kg of DM) containing wheat straw at 36% of DM for ad libitum intake for the entire dry period. After calving, all cows were fed a common lactation diet (NE_L = 1.65 Mcal/kg of DM) until 30 DIM.

Liver Biopsy

Liver was sampled via puncture biopsy from cows under local anesthesia at approximately 0700 h on d −10, 7, and 21 relative to parturition, following procedures described previously (Graugnard et al., 2013). Tissue samples were snap-frozen immediately in liquid N and transferred to a −80°C freezer for storage until analyses of lipid composition (Ji et al., 2012) and RNA extraction.

Blood Biomarkers

Blood was sampled from the coccygeal vein every Monday and Thursday before the morning feeding from d −26 to 30 relative to parturition. Samples were collected into evacuated tubes containing Li-heparin or clot activator (BD Vacutainer; BD and Co., Franklin Lakes, NJ). Plasma and serum were obtained by cen-