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Concentrations of hormones and metabolites in cerebrospinal fluid and plasma of dairy cows during the periparturient period

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ABSTRACT

During early lactation, high-yielding dairy cows often show insufficient feed intake (FI) and, as a consequence, they enter into a negative energy balance associated with an altered pattern of plasma metabolites and hormones. These act as short- and long-term hunger or satiety signals in the brain and play an important role in the control of FI. Metabolites and hormones also occur in cerebrospinal fluid (CSF), which surrounds the hypothalamus and brainstem, 2 major centers of FI regulation. The CSF hormone and metabolite concentrations are mainly under control of the blood-brain barrier. Consequently, CSF hormone and metabolite concentrations differ from those in blood. However, the contribution of putative orexigenic and anorexigenic CSF signals possibly leading to insufficient FI of high-yielding dairy cows during early lactation has not been studied so far. Therefore, the aim of this study was to elucidate associations existing between both plasma and CSF hormones and metabolites during the periparturient period. Ten multiparous German Holstein dairy cows were fed ad libitum and samples of CSF from the spinal cord and blood from the jugular vein were withdrawn before morning feeding on d −20, −10, +1, +10, +20, and +40 relative to calving. Feed intake started to decrease from d 5 before calving and increased thereafter. Glucose, β-hydroxybutyrate (BHBA), cholesterol, nonesterified fatty acids, urea (all enzymatic), lactate (colorimetric), amino acids (HPLC), osmolality (osmometer), ghrelin (RIA), leptin (ELISA), and resistin (Western immunoblot) were measured in both CSF and plasma, whereas free fatty acids (gas chromatography-mass spectrometry) and volatile fatty acids (gas chromatography-flame-ionization detector) were determined in plasma only. Whereas leptin concentrations decreased after calving in both plasma and CSF, ghrelin concentrations were not altered, and abundances of total resistin and its hexamers decreased only in plasma. Although plasma concentrations of cholesterol and nonesterified fatty acids changed during the periparturient period, their concentrations were not affected in CSF. In contrast, CSF Gln concentration tended to increase until calving, whereas CSF concentrations of BHBA, α-aminobutyric acid, Cit, Gly, Ile, Val, and Leu were increased in early lactation compared with the preparturient period. Because Gln is known to serve as neuronal substrate generating ATP, Gln is suggested to act as a central anorexigenic signal shortly before parturition. Moreover, due to their known anorexic effect, BHBA and Leu may potentially act as central signals and thereby suppress a sufficient increase in FI during early lactation.
Key words: cerebrospinal fluid, dairy cow, hormone, metabolite

INTRODUCTION

High-yielding dairy cows often show increased but inadequate feed intake (**FI**) during early lactation and, as a consequence, enter into a negative energy balance (**EB**). This period is characterized by an altered pattern of plasma metabolites, driven by an increased release of nutrients derived from body reserves and an efflux of metabolites supporting high milk production. Such an imbalanced metabolic profile also elicits moderate changes in the production and release of adipokines, which have been suggested to be essential in the prevention of sufficient FI (Ingvartsen, 2006). Both metabolites and hormones may act as satiety signals in the brain and play an important role in the control of FI. Due to the blood-brain barrier (**BBB**) and the blood-cerebrospinal fluid (**CSF**) barrier (choroid plexus epithelium), the passage of molecules between blood and neural tissue or its fluid spaces is limited

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and regulated. The transport of small molecules such as AA across the BBB may be limited by the competition among AA sharing a common transporter for the uptake into the brain (Harper and Peters, 1989). In this regard, it was recently demonstrated that, for example, concentrations of NEFA, Arg, and Leu were altered in plasma but not in CSF after a 4-d feed restriction period in dairy cows between 87 and 96 DIM (Laeger et al., 2012). Intravenous administration of metabolites such as NEFA, lactate, and Lys may reduce FI, whereas glucose is believed to play a minor role in the control of FI in ruminants (Baile and Forbes, 1974). However, when an isotonic glucose solution was injected into CSF of the third brain ventricle of sheep, FI decreased within the first 2 h (Seoane and Baile, 1972). Moreover, the osmolality affects FI in ruminants and tended to decrease in CSF of restrictive feed cows, whereas the osmolality is unaffected after feed restriction in plasma (Laeger et al., 2012). These findings suggest that central, rather than peripheral, mechanisms are important for the control of FI in ruminants.

Furthermore, many peptide hormones or cytokines may cross the BBB, depending on their structural-chemical characteristics by simple diffusion. Other peptide hormones are not able to cross the BBB by diffusion but use a specific saturable transport mechanism to reach the abluminal side (Fry and Ferguson, 2010). Such transport mechanisms exist for the orexigenic ghrelin (Banks et al., 2002) and for the anorexic adipokines leptin and resistin, although the transport proteins for the latter 2 are still unknown (Kos et al., 2007; Fry and Ferguson, 2010). In ruminants, intravenous injection of ghrelin (Iqbal et al., 2006) and leptin (Morrison et al., 2002) did not affect FI, whereas intracerebroventricular (**i.c.v.**) administration of leptin reduced FI (Morrison et al., 2001). Central administration of ghrelin, either increased (Harrison et al., 2008) or did not affect (Iqbal et al., 2006) FI in ruminants. An i.c.v. injection of resistin induces hypophagia in rodents (Cifani et al., 2009), but effects of central or peripheral resistin administrations in ruminants are still unknown.

In a first attempt to understand the interrelationship between peripheral and central metabolite and hormone signals involved in the control of FI of dairy cows, we investigated the concentrations of selected molecules during the periparturient period in both plasma and CSF. Our objective was to generate knowledge about prevailing satiety signals as a prerequisite for developing intervention strategies to counteract insufficient FI increase during early lactation. We expected to identify metabolites and hormones reflecting metabolic processes differentially regulated during the periparturient period as potential signals preventing sufficient FI in early lactation.

MATERIALS AND METHODS

Animals, Husbandry, Feeding, Measurement of Zootechnical Data, and Calculation of Energy Balance

Ten German Holstein dairy cows in second (n = 9) and third (n = 1) parturition were kept in tie-stalls in accordance with the guidelines for the use of animals as experimental subjects of the State Government in Mecklenburg-West Pomerania (Germany; registration no. LALLF M-V/TSD/7221.3-2.1-001/10). All cows were healthy and 44 to 52 mo old. They were fed twice daily (0700 and 1600 h) a TMR ad libitum, adjusted for transition and lactation needs, respectively, consisting of corn and grass silage, grass hay, grain feed, minerals, and vitamins, to meet the energy and nutrient recommendations of dairy cows calculated according to the German Society of Nutrition Physiology (2001) [6.4 MJ of NE_L/kg of DM for the last 25 d of gestation (close-up period) and 7.2 MJ of NE_L/kg of DM for lactation] and sampled for CSF and plasma before morning feeding at d −20, −10, +1, +10, +20, and +40 relative to calving. Cows had free access to water and were milked twice daily (0630 and 1530 h). The daily milk yield and daily FI were measured individually. Body weight was measured once per week (see Table 1). To calculate EB, milk was analyzed for fat, protein, and lactose content by an infrared spectrophotometric method (MilkoScan; Foss GmbH, Rellingen, Germany) at the Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg-Vorpommern e.V. (Güstrow, Germany). Energy-corrected milk was calculated as follows: ECM (kg) = (0.038 × g of fat + 0.024 × g of protein + 0.017 × g of lactose) × kg of milk/3.14. Energy balance [MJ of NE_L/(cow × day)] antepartum (EBap) and postpartum (EBpp) was calculated as EBap = NE_L intake − 0.46 × kg of $BW^{0.75}$, and EBpp = NE_L intake (MJ) − (ECM × 3.14 + 0.293 × kg of $BW^{0.75}$), respectively (Reist et al., 2002). All cows were in positive EB prepartum and in negative EB postcalving until the end of the sampling period (Table 1).

Blood and CSF Sampling

After local anesthesia with 10 mL of Isocaine (20 mg of procainhydrochloride/mL and 0.025 mg of epinephrine/mL; Selectavet Dr. Otto Fischer GmbH, Weyarn-Holzolling, Germany), CSF from spinal cord was obtained by lumbar puncture between the sixth lumbar vertebra and sacrum with a sharpened needle with fitted stylet (120-mm length, 1.2-mm diameter; Walter Veterinär-Instrumente e.K., Baruth/Mark, Germany), and blood was withdrawn into EDTA tubes from the