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Reduced AgRP activation in the hypothalamus of cows with high extent of fat mobilization after parturition

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ABSTRACT

Agouti-related protein (AgRP), produced by neurons located in the arcuate nucleus of the hypothalamus stimulates feed intake. During early lactation dairy cows increase their feed intake and additionally mobilize their fat reserves leading to increased plasma non-esterified fatty acid (NEFA) concentrations. Since cows with a higher extent of fat mobilization exhibit the lower feed intake, it seems that high NEFA concentrations confine hyperphagia. To test the involvement of AgRP neurons, we investigated 18 cows from parturition until day 40 postpartum (pp) and assigned the cows according to their NEFA concentration on day 40 pp to either group *H* (high NEFA) or *L* (low NEFA). Both groups had comparable feed intake, body weight, milk yield, energy balance, plasma amino acids and leptin concentrations. Studies in respiratory chambers revealed the higher oxygen consumption and the lower respiratory quotient (RQ) in *H* compared to *L* cows. mRNA abundance of neuropeptide Y, peroxisome proliferator-activated receptor-gamma, AMP-activated protein kinase, and leptin receptor in the arcuate nucleus were comparable between groups. Immunohistochemical studies revealed the same number of AgRP neurons in *H* and *L* cows. AgRP neurons were co-localized with phosphorylated adenosine monophosphate-activated kinase without any differences between groups. The percentage of cFOS-activated AgRP neurons per total AgRP cells was lower in *H* cows and correlated negatively with oxygen consumption and NEFA, positively with RQ, but not with feed intake. We conclude that AgRP activation plays a pivotal role in the regulation of substrate utilization and metabolic rate in high NEFA dairy cows during early lactation.

1. Introduction

During the transition from late pregnancy to early lactation, all mammals but particularly high-yielding dairy cows require increased energy and nutrient supply for milk production (Vernon, 2005). Dairy cows meet these requirements by increasing feed intake, mobilization of body reserves, and spare of nutrient utilization in splanchnic and muscle tissue (Ingvartsen, 2006; Kessel et al., 2008). However, the increase of feed intake is often insufficient, resulting in a distinct state of negative energy balance during early lactation (Vernon, 2005; Wade and Schneider, 1992) when primarily adipose tissue reserves are mobilized (Komaragiri et al., 1998). Proportional to the fat mass loss during this time, plasma leptin concentration decreases (Sorensen et al., 2002), whereas non-esterified fatty acid (NEFA) concentrations increase (Bobe et al., 2004; Ingvartsen, 2006; Vernon, 2005). Because often dairy cows with the higher extent of fat mobilization reveal the lower feed intake at the same level of milk yield (Grummer et al., 2004; Hammon et al., 2009), it has been hypothesized that mobilization of fat from adipose tissue suppresses the sufficient increase of feed intake during early lactation (Ingvartsen, 2006).

The hypothalamus plays a pivotal role in the control of feed intake and energy homeostasis in rodents and ruminants (Roche et al., 2008; Sartin et al., 2010; Schwartz et al., 2000). Both, leptin (Ahima et al., 2000) and long-chain fatty acids (Lam et al., 2005) target neurons located in the arcuate nucleus (ARC) of the hypothalamus at least in rodents. In rodents (Ahima et al., 2000; Claret et al., 2007; Ryan et al., 2011; Sarruf et al., 2009) as well as in ruminants (Henry and Clarke, 2008; Kuhla et al., 2011), these neurons express the transmembranal leptin receptor ObRb, adenosine monophosphate activated protein kinase (AMPK; coded by *PRKAA* gene) as the cellular energy sensor, and the peroxisome proliferator-activated receptor-gamma (PPARγ). All are involved in the regulation of the anorexigene proopiomelanocortin and the orexigenes neuropeptide Y (NPY) and agouti-related protein (AgRP) (Claret et al., 2007; Roche et al., 2008; Sartin et al., 2011). During lactation of sheep, the expression of proopiomelanocortin

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decreases while the expression of NPY and AgRP increases in the ARC (Sorensen et al., 2002) as it can also be observed in sheep fasted for 3 days when AgRP producing neurons are cFOS-activated (Wagner et al., 2004). Reduced leptin secretion in ruminants has been attributed to increased expression of AgRP and hyperphagia in early lactation (Sorensen et al., 2002) but on the other hand, intracerebroventricular (icv) administration of long-chain fatty acids inhibited the expression of AgRP in the hypothalamus and exerted hypophagia at least in rodents (Morgan et al., 2004). The latter finding suggest a molecular mechanism for the above mentioned hypothesis (Ingvartsen, 2006) by which fatty acid mobilization from adipose tissue during early lactation may suppress a sufficient increase of feed intake in dairy cows.

Besides increasing feed intake, AgRP can also regulate energy metabolism without influencing feed intake (Makimura et al., 2002; Wortley et al., 2005). For example, AgRP deficient mice did not differ in feed intake from wildtype counterparts but had the lower body weight, respiratory quotient (RQ) and a higher oxygen consumption rate (Makimura et al., 2002; Wortley et al., 2005). On the other hand, icv administration of AgRP in rats increased RQ and tended to decreases oxygen consumption (Semjonous et al., 2009). Moreover, icv administration of AgRP and pair-fed feeding to saline treated rats resulted in increased epididymal fat pad weight and brown adipose tissue (Small et al., 2001).

Little is known about whether fat mobilization interferes with hyperphagia in early lactation. In this study we examined the hypothesis that a high extent of body fat mobilization during early lactation diminishes hypothalamic AgRP expression and feed intake and affects energy expenditure of high-yielding dairy cows.

2. Materials and methods

2.1. Animal experiment

All experimental procedures were approved by an ethical committee of the State Government in Mecklenburg-West Pommerania (Registration No. LALLF M-V/TSD/7221.3-2.1-021/09). Eighteen multiparous high-yielding dairy cows were selected to be heterozygous at a polymorphic locus in acylCoA-diacylglycerol-acyltransferase 1 gene (DGAT1 K232A: amino acid lysine or alanine at position 232), which is known to affect body fat (Thaller et al., 2003) and milk fat content (Kühn et al., 2007). All cows were bought from one commercial farm at the end of their second to fourth lactation and had >10,000 kg milk/305 d in an earlier lactation. Animals were kept in tie stalls and had free access to water and mineral salt blocks; milking was terminated 7 weeks prior to the expected calving date. In week 5 prior to calving and again in week 2 postpartum (pp), cows were kept in respiratory chambers (15 °C) and the RQ, oxygen consumption, fat oxidation, and carbohydrate oxidation were determined every 6 min for 24 h during ad libitum feeding as described previously (Derno et al., 2013). Animals were fed a total mixed ration (TMR) twice daily (07.00 a.m. and 03.00 p.m.) consisting after calving of grass silage, corn silage, barley straw, hay, concentrate, extracted rapeseed meal, molassed sugar beet pulp, mineral feed and propylene glycol (net energy lactation = 7.1 MJ/kg of dry matter [DM], utilizable protein = 163 g/kg of DM) according to the recommendations of the German Society of Nutritional Physiology (2001) Gesellschaft, 2001. Feed intake was recorded daily and dry matter content of the TMR was measured weekly to determine dry matter intake (DMI). Cows were milked twice daily (03.30 a.m. and 03.00 p.m.) and milk samples were taken as a pool sample from one morning and one afternoon milking every week. Body weight and back fat thickness, the latter measured by ultrasound (Aloka SSK-500, PPG Hellige GmbH, Freiburg, Germany), were determined weekly. Liver biopsies were taken before morning feeding at *d*3, 18, and 30 postpartum (pp). To this end, the skin around the 12th intercostal space was anaesthetized with 10 mL Isocain 2% (Serumwerke Bernburg, Germany) and a sample was taken with a tailor-made biopsy needle (Ø 6 mm). Tissue gained was immediately snap-frozen and stored at −80 °C until analyzed for total liver fat content. Blood samples from the jugular vein were taken in EDTA containing tubes once weekly, centrifuged (20 min, 1565*g*, 4 °C) and the obtained plasma stored at −80 °C. Thirty min before slaughter on d 40 pp, access to feed was cut off, a blood sample was taken and the animals were slaughtered in the institutional slaughterhouse at 7.30 a.m. by exsanguination following captive bolt stunning.

2.2. Brain sampling

The head was instantaneously displaced and the skullcap was opened at the dorsal side. The dura mater was removed and the optic nerves, the pituitary gland, and the brain stem were disconnected. The time elapsed from head displacement to brain withdrawal was approximately 15 min. The brain was immediately chilled on ice. A frontal section was made bounding ventrally at the optic chiasm and dorsally at the mammillary bodies. The hypothalamic region was isolated by two diagonal sections ranging from the fornix directly to the most ventro-lateral side of the optic tract. The tissue was liberated of external blood vessel network, cut along the median axis, and the right hemisphere was embedded in Tissue-Tek® (Sakura Finetek Europe B.L.; No. 4583, Germany), frozen on liquid nitrogen and stored at −80 °C. The ARC from the left hemisphere was separated and stored in RNAlater® (Qiagen GmbH, No. 76106, Germany) at −20 °C until analysis.

2.3. Analysis of plasma metabolites and liver fat

Plasma leptin concentrations were measured using an ELISA as previously described by Sauerwein et al. (2004). Plasma NEFA, urea, beta-hydroxybutyric acid (BHBA), triglycerides, and glucose concentrations were determined by an automatic analyzer (Abx Pentra 400; Horiba, France, Clinic for Cattle, University for Veterinary Medicine Hannover, Germany) using NEFA kit #600-215S (Wako Chemicals, Germany), urea kit #LT-UR 0050 (Labor + Technik GmbH, Berlin, Germany), BHBA kit #RB 1008 (Randox Laboratories GmbH, Germany), triglyceride kit #A11A01640 (Abx-Horiba), and glucose kit #553-230 (MTI diagnostics GmbH, Germany). Based on the NEFA concentration on the day of slaughter on day 40 pp, which allows direct comparison to variables of hypothalamic tissue, cows were assigned to the *L* (lowest NEFA < 400 μmol/l; $n = 9$) or *H* (highest NEFA > 400 μmol/l; $n = 9$, $P < 0.001$) group. A plasma fatty acid profile was determined as described previously (Laeger et al., 2013) with slight modifications. Briefly, 5 μL 1 mM butylated hydroxytoluene, 25 μL of 2.3 mM heptadecanoic acid (dissolved in ethanol and used as internal standard), 0.5 g NaCl, and 12.5 μL of 2 N HCl were added to 250 μL of EDTA plasma on ice. After two subsequent extraction steps with 125 and 100 μL ethyl acetate, respectively, and subsequent centrifugation (30 and 60 s, respectively, 9600*g* at room temperature), combined organic layers were dried with 0.5 g anhydrous sodium sulphate. The supernatant was treated with 25 μL trimethylsulfonium hydroxide and stored at −20 °C until analyzed on a HP-ULTRA 2 column (50 m, 0.32 ID; 0.52 μm) by GC–MS (GC-2010 and GCMS-QP2010, Shimadzu; Japan). The concentration of plasma volatile fatty acids (VFA) was determined by gas chromatography-flame ionization detector (GC-FID; Series 17A; Shimadzu Corp., Kyoto, Japan) on a RTX-1701 column (30 m, 0.32 i.d., 0.5 μm) described previously (Kristensen, 2000). The portions of single fatty acids are calculated as percentage of total fatty acid concentration (Schäff et al., 2012). Plasma concentrations of all