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Lactogenic hormones stimulate expression of lipogenic genes but not glucose transporters in bovine mammary gland

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Abstract

During the onset of lactation, there is a dramatic increase in the expression of glucose transporters (GLUT) and a group of enzymes involved in milk fat synthesis in the bovine mammary gland. The objective of this study was to investigate whether the lactogenic hormones mediate both of these increases. Bovine mammary explants were cultured for 48, 72, or 96 h with the following hormone treatments: no hormone (control), IGF-I, insulin (Ins), Ins + hydrocortisone + ovine prolactin (InsHPrl), or Ins + hydrocortisone + prolactin + 17β-estradiol (InsHPrlE). The relative expression of β-casein, α-lactalbumin, sterol regulatory element binding factor 1 (SREBF1), fatty acid synthase (FASN), acetyl-CoA carboxylase α (ACACA), stearyol-CoA desaturase (SCD), GLUT1, GLUT8, and GLUT12 were measured by real-time PCR. Exposure to the lactogenic hormone combinations InsHPrl and InsHPrlE for 96 h stimulated expression of β-casein and α-lactalbumin mRNA by several hundred-fold and also increased the expression of *SREBF1*, *FASN*, *ACACA*, and *SCD* genes in mammary explants ($P < 0.01$). However, those hormone combinations had no effect on GLUT1 or GLUT8 expression and inhibited GLUT12 expression by 50% after 72 h of treatment ($P < 0.05$). In separate experiments, the expression of GLUTs in the mouse mammary epithelial cell line HC11 or in bovine primary mammary epithelial cells was not increased by lactogenic hormone treatments. Moreover, treatment of dairy cows with bovine prolactin had no effect on GLUT expression in the mammary gland. In conclusion, lactogenic hormones clearly stimulate expression of milk protein and lipogenic genes, but they do not appear to mediate the marked up-regulation of GLUT expression in the mammary gland during the onset of lactation.

Keywords: Gene expression; Glucose transporters; Mammary gland; Lactogenic hormones

1. Introduction

Glucose is an important nutrient in general, but it is absolutely essential for milk production in the mammary gland of lactating animals because it is an energy source as well as a key substrate for synthesis of milk protein and lipids, and, especially, of lactose. The mammary gland does not synthesize glucose; thus, it needs to take up glucose from blood. Glucose uptake by mammary epithelial cells may play a rate-limiting role in milk production [1].

Glucose transport across the plasma membrane of mammary epithelial cells is mainly mediated by facilitative glucose transporters (GLUTs). The family of GLUTs consists of 13 isoforms, which are designated

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as GLUT1 to GLUT12 and H^+/myo-inositol cotransporter [2]. These transporters are structurally conserved and have different tissue distributions, transport kinetics, and regulatory properties. The lactating mammary gland mainly expresses GLUT1, GLUT8, and GLUT12 isoforms [3–5].

The demand for glucose by the mammary gland increases dramatically at the onset of lactation. The glucose transport activity increases 40-fold from the virgin state to the midlactating state [6,7]. Consequently, there is a coordinated increase in the expression of GLUTs in the mammary gland during the onset of lactation. From late pregnancy to early lactation, the mRNA abundance of GLUT1, GLUT8, and GLUT12 in bovine mammary gland increases markedly, by several-fold to several hundred-fold [1].

Expression of genes that code for enzymes and proteins required for de novo lipogenesis is also strikingly upregulated in the mammary gland during lactogenesis. For example, expression of sterol regulatory element binding factor 1 (SREBF1), the master regulator of lipid synthesis [8], increases approximately 2-fold in both mouse and cow mammary gland from late pregnancy to early lactation [9,10]. Expression of its known targets, fatty acid synthase (FASN) and acetyl-CoA carboxylase α (ACACA), also increases during the same period [9,10]. In addition, stearoyl-CoA desaturase (SCD) is upregulated more than 40-fold from late pregnancy to lactation [9].

Mammary development and milk synthesis are regulated both by systemic hormones and by local factors. It has been established that prolactin (Prl) and glucocorticoids (GCs) are the main mediators of secretory cell differentiation and lactogenesis [11,12]. In cows, concentrations of Prl, GCs, and GH in blood increase during late pregnancy and peak near parturition [11]. Concentrations of estrogens (Es) also increase gradually during late pregnancy, then surge several days before parturition. It has been shown that Prl-induced secretion of α-lactalbumin is markedly enhanced by adding 17β-estradiol to mammary explants [11]. Locally produced IGF-I is believed to mediate the effects of GH on the mammary gland, and the numbers of IGF-I receptors on mammary epithelial cells increase markedly during late gestation [11]. In addition, disruption of Prl signaling in the mammary gland decreased GLUT1 expression [13,14]. On the basis of these facts, we hypothesized that some combination of Prl, GCs, E, and/or IGF-I is responsible for the upregulation of GLUTs and lipogenic genes in the bovine mammary gland during onset of lactation. Our objective was to investigate the effects of those hormones on the expression of GLUTs and lipogenic genes in the mammary gland.

2. Materials and methods

2.1. Mammary tissue biopsy and explant culture

All animal use was approved by the University of Vermont Institutional Animal Care and Use Committee. Mammary tissues were obtained via biopsy from the rear quarters of 2 Holstein heifers and 2 multiparous Holstein dry cows approximately 37 d prepartum (37 ± 5 d). The biopsy procedures were performed as described previously [15].

Immediately after biopsy, a small piece (5 mg) of tissue was fixed in 4% paraformaldehyde for 4 h at 4°C. After fixation, the tissue was rinsed with 3 changes of cold PBS (2.67 mM potassium chloride, 1.47 mM potassium phosphate monobasic, 137.93 mM sodium chloride, and 8.06 mM sodium phosphate dibasic), immersed in 0.5 M sucrose in PBS overnight at 4°C, preserved in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA), and then frozen in liquid nitrogen chilled isopentane. Frozen tissues were stored at −80°C until use. The remaining tissue (~1 g) was cut into small pieces (~50 mg per piece). Two pieces of fresh tissue were immediately frozen in liquid nitrogen for RNA isolation. The remaining tissue was placed in 50-mL tubes containing basic medium [Medium 199 with Earle's salts and L-glutamine (Sigma, St. Louis, MO, USA), containing 26.2 mM sodium bicarbonate, 10 mM sodium acetate, 15 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B; room temperature] and transported to the laboratory within 30 min. In a sterile, laminar flow hood, tissue was washed 3 times with PBS containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B and then diced into explants (~1 mm^3). Explants were washed 3 times with basic medium which was removed by use of a strainer after each wash. Explants were placed on siliconized lens paper (Whatman, Piscataway, NJ, USA) floating on culture media supplemented with various hormone treatments. Lens paper was siliconized as described previously [16]. There were 5 hormone treatments: NH (basic medium without hormone), IGF-I (basic medium + 200 ng/mL IGF-I), Ins (basic medium + 5 μg/mL insulin), InsHPrl (basic medium + 5 μg/mL Ins + 5 μg/mL ovine Prl + 1 μg/mL hydrocortisone), and InsHPrlE (basic medium + 5 μg/mL Ins + 5 μg/mL