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FASTING LOWERS GASTRIN-RELEASING PEPTIDE AND FSH mRNA IN THE OVINE ANTERIOR PITUITARY GLAND

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ABSTRACT: Estrogen receptor beta (ER-β), LH, and FSH are important mediators of reproduction. Follicle recruitment and development is stimulated by FSH. During anorexia, serum concentrations of FSH and LH decrease. Gastrin-releasing peptide (GRP), neuromedin B (NMB), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) and thyroid-stimulating hormone (TSH) are important metabolic regulators expressed in the anterior pituitary gland (AP). Gastrin-releasing peptide stimulates release of ACTH, is associated with melanocortin in regulating food intake, and is a regulatory peptide in the female reproductive tract. In cattle, pituitary GRP expression was markedly up-regulated after resumption of estrus following parturition, indicating a connection between gene expression of GRP and reproductive function. The objective of this study was to determine effects of fasting during the luteal phase of the estrous cycle on gene expression in the anterior pituitary gland during the subsequent periovulatory period. Estrus was synchronized in mature (≥ 3 yr old) western white-faced ewes with prostaglandin $F_2\alpha$ ($PGF_2\alpha$). Randomly selected ewes were fed grass hay ad libitum (control = 10) or were withheld from feed on days 7 – 11 of their estrous cycle (d 1 = estrus; fasted = 10). On d 12, fasted ewes were returned to feed and all ewes were treated with $PGF_2\alpha$ (0 hrs). Pituitaries were collected 72 h after $PGF_2\alpha$. Ovaries were observed for presence of pre-ovulatory follicle or newly formed CL. Pituitaries were analyzed (n = 5 each group) from ewes that had ovulated. Fasting decreased ($P < 0.05$) gene expression of GRP and FSH. Differences in gene expression were not noted ($P \geq 0.26$) in mRNA levels of PGC-1α, TSH, NMB, ER-β, or LH. Mediation of metabolic effects on reproductive function may be regulated by GRP affecting expression of FSH.

Key Words: Fasting, Pituitary, GRP, FSH.

Introduction

Research continues to determine how food intake and metabolism effect reproduction. Factors known to mediate food intake are gastrin-releasing peptide (GRP), neuromedin B (NMB), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), and thyroid-stimulating hormone (TSH). Bombesin-like peptides, GRP and NMB, have anorexigenic effects (Frank, 2001; Polya, 2003). Production of TSH from the anterior pituitary gland is directly acted upon by GRP. Along with metabolic effects, GRP stimulates the release of LH from AP cells suggesting a role in mediating reproductive function (Evans, 1999). Immunoreactive GRP is found in mature lactotrophs, corticotrophs, and immature somatotrophs, thyrotrophs, and gonadotrophs in the rat. (Houben, 1991).

A major regulator of mitochondria, PGC-1α, affects metabolic homeostasis and, possibly, cellular energy expenditure (Cantó, 2009). It also plays a role in reproduction by interacting with estrogen receptor alpha to produce progesterone in rat ovarian granulosa cells (Chen, 2008). Other genes important in reproductive function are those encoding for FSH, LH, and estrogen receptor beta (ER-β). In addition to reproductive effects, ER-β mediates glucose metabolism (Foryst-Ludwig, 2008). Luteinizing hormone and FSH are responsive to changing metabolism and body condition. Serum concentrations of FSH and LH are decreased in anorexic individuals (Tomova, 2007).

The objective of this study was to determine if fasting during the luteal phase in mature female ewes affected anterior pituitary gland levels of mRNA for ER-β, LH, FSH, GRP, NMB, PGC-1α, and TSH during the subsequent periovulatory period.

Materials and Methods

Animals,Treatment. Mature (≥ 3 yr old) western white-faced ewes were synchronized with two 10 mg doses of $PGF_2\alpha$ (Lutalyse, Pharmacia &Upjohn Co., Kalamazoo, MI) on d 1 and d 10 (d 1 = first day of estrous). Following the injection of $PGF_2\alpha$ on d 10, estrous behavior was monitored with two vasectomized rams for 4 d. Ewes with synchronized estrous cycles (n = 20) were randomly allotted to control (n = 10) or fasted (n = 10) groups. Ewes were housed separately by treatment in adjacent pens. Control ewes were fed grass hay ad libitum. Fasted ewes were withheld from feed on d 7 to 11 of their estrous cycle. On d 12, fasted ewes were returned to feed and all ewes were given a 10 mg injection of $PGF_2\alpha$. Pituitaries and ovaries were collected 72 h after $PGF_2\alpha$ administration. Pituitaries from ewes that ovulated (n = 5 each group) were snap frozen for analysis.

RNA Isolation and cDNA Synthesis. Approximately 100 mg of anterior pituitary gland tissue from each animal was homogenized in 1 mL of TRI reagent (Sigma Aldrich Chemical; St. Louis, MO). Concentrations of RNA were determined using a NanoDrop spectrophotometer. Then RNA was purified using an RNEASY kit (Qiagen Inc; Santa Clara, CA). A 20 µL reaction using 4 µL reverse transcription buffer (5X), 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA) and 2.0 µg RNA was used to

synthesise cDNA. The thermocycler program ran for 5 min at 25 ºC, 30 min at 42 ºC, 5 min at 85 ºC and held at 4 ºC. Synthesized cDNA was diluted 6-fold with nuclease-free water and stored at -20 ºC.

Semi-Quantitative Real Time RT-PCR. Diluted cDNA was mixed with SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), nuclease-free water, and a forward and reverse primer. Primer3 software was used to design primers for ovine ER-β, LH, FSH, GRP, PGC-1α, TSH, and bovine NMB. Semi-quantitative RT-PCR was performed using 40 cycles of 95 ºC for 30 sec and 1 cycle of 62 ºC for 30 sec. Following amplification, cDNA were melted to ensure quality of amplification by incubating RT-PCR products for 10 sec at each step with an increase in temperature by 0.5 ºC from 55 ºC to 95 ºC in each cycle. All gene expression levels were quantified relative to GAPDH.

Statistical Analysis. All mRNA data were analyzed by SAS (Version 9.0). A one-tailed t-test was used to determine mean differences in the average fold change of mRNA expression within the anterior pituitary glands of fasted ewes compared to control ewes.

Results

Gene expression of GRP ($P = 0.03$) and FSH ($P = 0.04$) were down-regulated in the anterior pituitary gland of fasted ewes compared to control ewes (Fig. 1). Expression of mRNA for ER-β, LH, PGC-1α, TSH and NMB did not differ ($P \geq 0.26$) between treatments (Table 1).

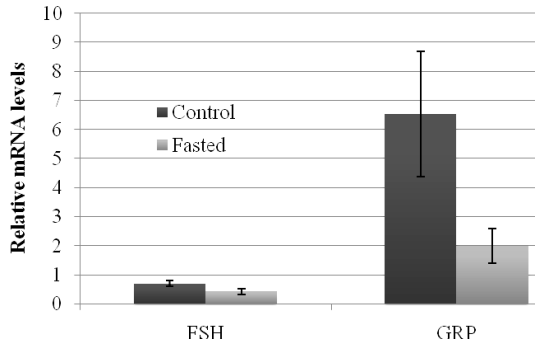

Figure 1. Expression of FSH and GRP mRNA in the anterior pituitary gland of control and fasted ewes. ($P < 0.05$)

Table 1. Relative concentrations of mRNA in the anterior pituitary gland of fasted compared to control ewes.

Gene	Fold Change	*P* value
ER-β	1.13	0.34
LH	0.84	0.26
PGC-1α	1.11	0.36
TSH	1.13	0.42
NMB	0.93	0.41

Discussion

Differences in ER-β levels were expected because ER-β is a mediator of insulin/glucose metabolism and previous studies demonstrated decreased insulin during fasting (Kiyma, 2004). The glucose/insulin system is dynamic and changes rapidly, suggesting ER-β mRNA levels may have readjusted following fasting.

The lack of food in the stomach from fasting could cause an increase in orexigenic hormones and a decrease in anorexigenic hormones. Ruminant animals, such as sheep, have a slower rate of digestive passage than non-ruminants which could suggest that effects of fasting on anorexigenic hormones may be less acute than in non-ruminants. Such a delay in the need for anorexigenic hormones may relate to decreased GRP gene expression in the AP.

A decrease in TSH and LH would be expected since low doses of synthetic porcine GRP injected into male rats stimulated LH release and suppressed TSH secretion (Güllner, 1983). The lack of differences in the current study could be associated with the cyclicity of females or may reflect differences among species. Alternatively GRP may regulate release of TSH and LH but not regulate its synthesis.

Levels of FSH decrease with long term anorexia. This decreased level of FSH, along with LH can cause women to experience amenorrhea. While LH increases rapidly FSH levels gradually return to normal following food intake in anorexic individuals. The pathway involved in regulating this change in reproductive function is relatively unknown.

Implications

This study suggests GRP may play a role in mediating nutritional induced changes in reproductive function through FSH. Since both FSH and GRP are produced by AP gonadtrophs, the current results provide support for an autocrine / paracrine mechanism of regulation.

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