by the mid-cycle surge of luteinizing hormone (LH). The CL is a transient ovarian endocrine structure that maintains pregnancy in primate during the first trimester and in rodents during the entire pregnancy by producing steroid hormone progesterone (P4). CL growth and differentiation are tightly regulated by both survival and cell death signals, including endocrine (LH), intra-ovarian regulators, and cell-cell interactions. Neuregulin-1 (NRG1) is a member of the epidermal growth factor-like factor family that mediates it's effect through the erythroblastoma (ErbB) family. However, the detailed mechanisms associated with the interplay of NRG1 and its receptors in CL function is not known. Therefore, we examined the role and action of NRG1 and its receptors in the gonadotropin signaling pathway that impacts CL functions. Immunocolocalization of NRG1 and ErbB2/3 in pregnant rat CL on day 14 and 21 suggest that both NRG1 and ErbB2/3 are differentially expressed in CL. Moreover, both NRG1 and ErbB2/3 are highly expressed in rat CL on day 14 compared to day 21. Furthermore, *in vitro* studies revealed that rat luteal cells (LCs) treated with exogenous tumor necrosis factor-α (TNFα, an inflammatory cytokine) promoted apoptosis in LCs in a dose and time-dependent manner. However, the effects of TNFα was attenuated in presence of exogenous NRG1. Under these experimental conditions, immunoblot analysis indicated that exogenous TNFα treatment in the presence of NRG1 inhibits apoptosis through increased levels of the anti-apoptotic proteins Bcl2 and Bclxl, and activation of ErbB2-ErbB3-PI3K-Akt signaling pathway. Collectively, these studies provide new insights on the NRG1-mediated anti-apoptotic mechanism in LCs through ErbB3-ErbB2-PI3K-Akt→Bcl/Bcl-xL pathway and may have important clinical implications.

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Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Hepatic Dysregulation of Bile Acid Homeostasis in Hyperandrogenemic Female Mouse Model of Polycystic Ovary Syndrome

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Introduction and Purpose: Polycystic Ovary Syndrome (PCOS) is recognized as the most common endocrine disorder in women of reproductive age. Notably, PCOS women with hyperandrogenism have a pronounced increased risk for cardio-metabolic comorbidities compared with healthy individuals. Bile acids are endocrine signaling molecules that modulate hepatic lipid, glucose, and energy metabolism by aiding in absorption of lipids. Alteration of bile acid homeostasis affects overall metabolic homeostasis and contributes to pathogenesis of an array of metabolic diseases, although the molecular mechanisms of this have not been studied in PCOS. **Methods:** Four-week old C57BL/6N female mice were implanted subcutaneously with dihydrotestosterone (DHT, 8.0 mg) or vehicle silastic tubes (n=8/grp). Weekly body weight, food intake, and body composition was assessed. Fasting serum was obtained and the oral glucose tolerance test (OGTT) was performed in the last week of treatment. Animals were euthanized on treatment day 90 and livers were harvested. Expression levels of mRNA were assessed using RT-qPCR. **Results:** DHT treated females had significantly higher liver mass (1,387 ± 51 vs 1,197 ± 29 g, $p<0.05$), increased lean mass (21.25 ± 0.27 vs 19.58 ± 0.23 g, $p<0.05$) and increased fat mass (4.83 ± 0.47 vs 3.59 ± 0.36 g, $p<0.05$) compared to the vehicle counterparts. These hyperandrogenemic females additionally showed altered glucose homeostasis, having increased fasting glucose (201.10 ± 11.11 vs 152.80 ± 9.23 mg/dL, $p<0.05$) and an increased area under the curve (209.2± 11.0 vs 160.8± 3.5 mg.min/dL, $p<0.05$) following OGTT. Hepatic expression of both classic (Cyp8b1, 1.4 ± 0.1-fold, $p<0.05$) and alternative (Cyp7b1, 2.0 ± 0.3-fold, $p<0.05$) bile acid synthesis cytochrome P450 enzyme genes were significantly upregulated in DHT treated animals. Additionally, expression of sulfotransferase Sult2a2 was completely abolished in DHT treated animals compared with vehicle animals, indicating the possibility of androgen regulation of the sulfonation of bile acids marked for elimination. Liver expression of both the bile acid receptor G-protein coupled bile acid receptor 1 and the androgen receptor were both significantly downregulated (Gpbar1: 0.68 ± 0.08-fold, AR: 0.46 ± 0.04-fold, $p<0.05$) in DHT treated animals. **Conclusions:** Bile acid synthesis, transport, and elimination are tightly controlled processes in the liver to maintain a constant bile acid pool and limit reabsorption. Together, our results highlight the potential role of androgens in DHT-treated female mice in the dysregulation of bile acid homeostasis and its potential contribution to influence metabolic dysfunction. (Supported by NIH grants NIGMS P20GM-121334 to LLYC and DGR, and NIH NIDDK R21DK-113500 to DGR and the Mississippi Center of Excellence in Perinatal Research.)

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Involvement of BMP-15 in Glucocorticoid Actions on Ovarian Steroidogenesis by Rat Granulosa Cells

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Glucocorticoid receptor (GR) are known to be expressed in the ovary and glucocorticoids are shown to exert direct effects on granulosa cell functions. In the clinical setting, menstrual abnormality, amenorrhea and hypermenorrhea can be shown in patients with glucocorticoid excess. On the other hand, glucocorticoids can also be used for the treatment of PCOS with hyperandrogenism. However, the effects of glucocorticoids on the reproductive system have not been fully elucidated. In the present study, we investigated the

influence of glucocorticoids on follicular steroidogenesis using primary culture of rat granulosa cells, by focusing on the ovarian bone morphogenetic proteins (BMPs) acting as a luteinizing inhibitor. Granulosa cells isolated from female immature rats were treated with follicle-stimulating hormone (FSH) in the presence of dexamethasone (Dex) in serum-free conditions. After treatment with Dex for 48 h, the changes of estradiol (E2) and progesterone (P4) production and cAMP synthesis induced by FSH treatments were measured by ELISA. Total RNAs of granulosa cells treated with FSH, Dex and BMPs were extracted and mRNA levels of steroidogenetic factors and enzymes, BMP receptors and Id-1 were quantified by real-time RT-PCR. Phosphorylation of Smad1/5/9 induced by BMPs was evaluated by Western blotting using cell lysates in the presence or absence of Dex. As a result, it was revealed that Dex treatment decreased FSH-induced E2 production by granulosa cells. In accordance with the steroid results, Dex suppressed FSH-induced P450arom mRNA expression as well as FSH-induced cAMP synthesis by granulosa cells. By contrast, Dex treatment augmented FSH-induced P4 production by granulosa cells in a concentration-dependent manner. Dex treatment was found to enhance basal and FSH-induced mRNA levels of P4-synthetic enzymes including P450scc and 3βHSD. Of note, Dex treatment activated the BMP target gene Id-1 transcription and Smad1/5/9 phosphorylation, in particular, induced by BMP-15 among various BMP ligands including BMP-2, -4, -6, -7, -9 and -15. It was also revealed that Dex treatment increased mRNA levels of ALK-6, a type-I receptor for BMP-15, and that BMP-15 treatment in turn upregulated GR mRNA levels expressed by granulosa cells. Given that BMP-15 acts as an inhibitor for P4 production by suppressing FSH-receptor actions, it was suggested that glucocorticoid is functionally linked to the enhancement of endogenous BMP-15, leading to the negative feedback toward the P4 overproduction induced by FSH and Dex in granulosa cells. Collectively, it was revealed that glucocorticoids elicit differential effects on the ovarian steroidogenesis of E2 and P4, in which GR and BMP-15 actions are mutually enhanced in granulosa cells.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Involvement of NR5A1 and NR5A2 in the Regulation of Steroidogenesis by Clock Gene and BMPs by Human Granulosa Cells

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We previously reported that the expression levels of Clock gene are linked to the expression levels of steroidogenetic enzymes in human granulosa cells (EJ 2019). However, the downstream molecules of the Clock gene actions in the regulation of ovarian steroidogenesis have yet to be elucidated. In the present study, we investigated the roles of the transcription factors, NR5A1 (also known as SF-1) and NR5A2 (LRH-1), which play key roles in the reproductive function as well as steroidogenesis by focusing on the functional link between Clock gene and bone morphogenetic protein (BMP) signaling using human granulosa KGN cells. First of all, we examined the effects of BMPs/growth differentiation factor (GDF) on forskolin (FSK)-induced steroidogenesis. As a result, FSK-induced mRNA levels of StAR and P450scc, but not P450arom, were potently suppressed by treatments with BMP-6, -9, -15 and GDF-9. The expression levels of NR5A1 and NR5A2 mRNA were also upregulated by FSK treatment, while the BMP-target gene Id-1 mRNA levels were stimulated by the treatment with BMPs. Of interest, treatments with BMPs/GDF increased FSK-induced NR5A1 mRNA levels but suppressed FSK-induced NR5A2 mRNA levels by granulosa cells. The expression levels of NR5A1 mRNA were positively correlated with the changes of P450arom and 3βHSD mRNA, whereas the expression levels of NR5A2 mRNA were correlated with that of StAR and P450scc mRNA. In addition, the expression levels of NR5A1 and NR5A2 mRNAs were positively correlated with the levels of Clock mRNA. In particular, Clock mRNA levels showed highly positive correlation with the levels of NR5A2 mRNA compared with NR5A1 mRNA. Of note, Id-1 mRNA levels were positively correlated with the levels of NR5A1 mRNA, but negatively correlated with that of NR5A2 mRNA. Furthermore, the inhibition of Clock gene expression by siRNA attenuated the expression levels of NR5A1 and NR5A2 mRNA, resulting in decreased mRNA levels of StAR and P450arom in the presence of FSK. Thus, the present results suggested a novel mechanism by which Clock expression is functionally linked to the expression of NR5A1 and NR5A2, the latter of which is further regulated by BMP signaling by granulosa cells. The interaction among Clock, NR5A1/NR5A2 and BMPs may be involved in the fine tuning of steroidogenesis by ovarian follicles.

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OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Lack of Association Between ACE2 Expression and Serum Testosterone Concentrations in Peripheral Mononuclear Cells in Males

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Background: Male sex is a risk factor for developing severe COVID-19 illness, hospitalization, and mortality. It is possible that the male sex hormone, testosterone, contributes to the morbidity from COVID-19. SARS-CoV2 viruses use cell membrane protein Angiotensin-Converting Enzyme 2 (ACE2) receptor and undergo S protein priming by the Type II Transmembrane Serine Protease (TMPRSS2) to enter the cells. Hence, the expression level of ACE2 and TMPRSS2 may affect disease susceptibility and possible severity. TMPRSS2 is regulated by the androgen receptor. We, therefore, examined if an association exists between serum testosterone concentrations and ACE2 or TMPRSS2 expression level in men.
Methods: We analyzed fasting serum samples and peripheral blood mononuclear cells (MNC) from 42 men. Total and free testosterone and estradiol were measured by