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The role of adrenergic activation on murine luteal cell viability and progesterone production

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ABSTRACT

Sympathetic innervations exist in mammalian CL. The action of catecholaminergic system on luteal cells has been the focus of a variety of studies. Norepinephrine (NE) increased progesterone secretion of cattle luteal cells by activating β-adrenoceptors. In this study, murine luteal cells were treated with NE and isoprenaline (ISO). We found that NE increased the viability of murine luteal cells and ISO decreased the viability of luteal cells. Both NE and ISO promoted the progesterone production. Nonselective β-adrenergic antagonist, propranolol reversed the effect of ISO on cell viability but did not reverse the effect of NE on cell viability. Propranolol blocked the influence of NE and ISO on progesterone production. These results reveal that the increase of luteal cell viability induced by NE is not dependent on β-adrenergic activation. α-Adrenergic activation possibly contributes to it. Both NE and ISO increased progesterone production through activating β-adrenergic receptor. Further study showed that CyclinD2 is involved in the increase of luteal cell induced by NE. 3β-Hydroxysteroid dehydrogenase, LHR, steroidogenic acute regulatory protein (StAR), and PGF2α contribute to the progesterone production induced by NE and ISO.

1. Introduction

The CL is a transient but dynamic endocrine gland in mammal ovaries. The CL develops from the dominant ovarian follicle after ovulation and is composed of residual follicular cells (small and large luteal cells) and other cells (endothelial, immune, fibroblasts). The normal CL plays central role in controlling the estrous cycle, establishment and maintenance of pregnancy through regulating progesterone secretion [1]. The progesterone synthesis in luteal cells is regulated endocrinologically by LH from the pituitary in the menstrual cycle and human chorionic gonadotropin (hCG) from villous trophoblasts in the early stage of pregnancy [2]. In addition, studies show that other endocrine molecules and paracrine signal molecules play important role in this process, such as ProstaglandinE2, IGF system, angiogenic factors, and factors from immune cells [3,4]. Our previous study also showed that Notch signaling regulated progesterone production in mural luteal cells [5]. The luteal regression charactered by functional luteolysis (reduction in progesterone production) and structural luteolysis (apoptosis of luteal cells), is also necessary for the cyclicity of the reproductive process. Prostaglandin F2α (PGF2α) plays central role in this process [6,7]. Extensive sympathetic innervations exist in mammalian ovary. Neurotransmitters, such as catecholamines, can be released into ovaries and work as intraovarian regulators. About 75% of ovarian catecholamine is from sympathetic nerves.

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Ovaries can also synthesize small amount of intrinsic catecholamine [8]. The adrenergic innervation was found in all parts of the ovary. The 3β-hydroxysteroid dehydrogenaze (3β-HSD) activity was decreased after denervation of the ovary [9]. It was reported that progressive ingrowth of adrenergic nerves had been observed during the luteal phase [10]. Higher concentrations of NE existed in newly formed CL than that in developed CL, regressed CL or CL from pregnant females [11]. Among the catecholamines, NE is the most abundant neurotransmitter released by sympathetic nerves in the mammalian ovary [12]. Studies showed that NE increased progesterone and oxytocin (OT) secretion of cattle luteal cells by activating β-adrenoceptors, and increased CYP11A (P450scc) and 3β-HSD activity [13–15]. However, the function of adrenergic activation in murine CL is still unclear. In this study, we investigated the influence of adrenergic activation on mural luteal cell viability and steroids hormone production and analyzed the possible mechanism.

2. Materials and methods

2.1. Animals and treatment

Immature Kunming mice (26-day-old) were purchased from Animal Facility of Nanchang University and housed in a temperature and light controlled facility with free access to water and food. The experimental protocols were approved by the ethical committee of Nanchang University. After being housed for 1 day, the mice were injected intraperitoneally with36 IU of pregnant mare's serum gonadotropin (NingBo Biological Technology, Zhejiang, China) to induce follicular maturation. Seventy two hours later, they were administered of 36 IU hCG (NingBo Biological Technology, Zhejiang, China) to induce ovulation and luteinization. The ovaries were collected 7 days after hCG treatment.

2.2. Chemicals

Norepinephrine (NE), isoproterenol (ISO), propranolol (Pro), and β-actin (A5316) were ordered from Sigma (St. Louis, MO, USA). 3β-Hydroxysteroid dehydrogenase (sc-30820) was ordered from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Steroidogenic acute regulatory protein (StAR) (D10H12) was purchased from Cell Signaling Technology (Beverly, Massachusetts). Trizol reagent was ordered from Invitrogen (Carlsbad, CA, USA). GoScript Reverse Transcription System (A5001) and Dead End apoptosis detection kit were purchased from Promega (Madison, WI, USA). Brilliant SYBR Green QPCR Master Mix (FP-202) was ordered from TIANGEN (Beijing, China). Collagenase (type I), DNase I (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA).

2.3. Luteal cell culture

The isolation of cells from luteinized ovaries was performed as described procedure [16]. Take out the luteinized ovaries and remove the fat and capsule tissue. After mechanical dissection, ovaries were digested in medium containing 1 mg/mL collagenase, 0.025% trypsin, and 0.02 mg/mL Dnase I for 10 minutes at 37 °C. The digested suspension was filtered through 75-μm strainers to remove debris and was centrifuged at 500 × *g* for 5 minutes. After two washes, the cells were seeded with DMEM/F12 culture medium, supplemented with 5% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin sulfate and cultured overnight for adhesion. After this period, the cells were cultured in fresh medium with indicated reagents.

2.4. MTT test

Luteal cells (5×10^3) were seeded in 96-well plates and incubated in the fresh medium with 1-μM NE, 10-μM NE, 100-μM NE, 1-μM ISO, 10-μM ISO, and 100-μM ISO. Cell viability was monitored by MTT assay after 48hours. MTT reagent of 20 μL was added in each well of 96-wells plate and incubated for 4 hours, the supernatant was removed, and the cells were treated with 150-μL/well DMSO for 10 min. Absorbance at 570 nm was recorded using an enzyme-linked immunosorbent assay plate reader. For the experiment of blocking β-adrenergic activation, propranolol was added to the medium 30 minutes before NE and ISO.

2.5. Terminal dexynucleotidyl transferase(TdT)-mediated dUTP nick end labeling assay

Terminal dexynucleotidyl transferase(TdT)-mediated dUTP nick end labeling assay was performed using Dead End Apoptosis Detection Kit according to the manufactures' instruction. Briefly, cells were fixed with 4% PFA at 4 °C for 25 minutes and incubated with a reaction mix containing Nucleotide mix (including fluorescein-12-dUTP) and TDT–terminal deoxynucleotidyl transferase for 1hour at 37 °C. Then, the cells were washed with 2XSSC and PBS. The nuclei were stained with 4′,6-diamidino-2-phenylindole. Apoptotic cells with Green fluorescence were examined by OLYMPUS fluorescence microscope.

2.6. Hormone measurements

Luteal cells were cultured in serum-free medium and treated with indicated reagents. After 48 hours, we harvested the supernatant of media and stored at −80 °C for hormone measurement. The estradiol and progesterone were measured with a commercial radioimmunoassay kit at a commercial laboratory (Beijing Sino-uk institute of Biological Technology). The intra-assay and interassay coefficients of variation is less than10%. The cross reactivities with other peptides and steroid hormones in these kits did not exceed 4%. The sensitivity of the progesterone and estradiol are 0.25 ng/mL and 2 pg/mL, respectively. For the experiment of blocking β-adrenergic activation, propranolol was added to the medium 30 minutes before NE and ISO.

2.7. RNA extraction and real-time polymerase chain reaction (PCR)

Luteal cells were lysed with Trizol Reagent. Total RNA was extracted according to the manufacturer's instruction.