Dynamics of expression and localization of the cannabinoid system in granulosa cells during oocyte nuclear maturation

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Objective: To describe the expression of cannabinoid receptors CB1 and CB2 and cannabinoid-degrading enzymes fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGLL) in human granulosa cells and to investigate their differential distribution with respect to CB1 at various stages during the nuclear maturation of the oocyte.
Design: Analysis of granulosa cells from germinal vesicle (GV), metaphase I (MI), and MII oocytes by quantitative reverse transcriptase–polymerase chain reaction, Western blot, and indirect immunofluorescence assays.
Setting: Academic research laboratory.
Patient(s): Patients from the Human Reproduction Unit of Cruces University Hospital undergoing intracytoplasmic sperm injection.
Intervention(s): We analyzed the granulosa cells of 300 oocytes from 53 patients. The oocyte maturation stages were 75 at GV stage, 51 at MI, and 174 at MII.
Main Outcome Measure(s): The mRNA and protein expression of CB1, CB2, FAAH, and MGLL and localization in granulosa cells at each oocyte maturation stage.
Result(s): CB1, FAAH, and MGLL are present in human granulosa cells during oocyte maturation, but the presence of CB2 receptor is not entirely clear in those cells. CB1 and FAAH were detected in the periphery of the granulosa cells from the GV to the MII oocytes, and they colocalized in some portions of the cell membrane. On the other hand, MGLL immunostaining was more homogeneous across the cell and overlapped with CB1 only weakly.
Conclusion(s): The presence of the cannabinoid system in granulosa cells suggests a possible role of this system in the nuclear maturation of the oocyte. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Cannabinoid, granulosa cells, oocyte maturation

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Oocyte maturation is a highly coordinated process that includes the progression of the oocyte from the first meiotic blockage at the germinal vesicle (GV) stage to the second meiotic blockage at the metaphase II (MII) stage. This progression occurs inside the follicle owing to a careful communication between oocytes and granulosa cells. These granulosa cells are somatic cells that surround the oocyte, but, when the follicle antrum is formed, they separate into mural granulosa cells forming the follicle inner wall and into cumulus granulosa cells, which are closely communicated with each other and with the oocyte through gap junctions (1).

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Oocyte maturation can also be carried out in vitro as long as the oocyte is cultured without removing the surrounding granulosa cells. This cumulus-oocyte complex (COC) is the structure that is ovulated in vivo. For oocyte maturation bidirectional communication is necessary, via gap junctions and via paracrine signaling, between the oocyte and granulosa cells (2). In this sense, many substances from the COC have been implicated in the regulation of the oocyte maturation; some of these act once they are translocated by the gap junction, and others act on receptors present on the cumulus cells and/or the oocyte (3).

Among all these substances, the cannabinoid compounds have been highlighted in some studies. Endocannabinoids are fatty acid derivatives that exert their effects by binding to membrane Gi/o protein-coupled receptors CB1 and CB2. Anandamide (AEA) and 2-arachiconoylglycerol (2-AG) are the better characterized endocannabinoids. AEA and 2-AG are degraded by fatty acid amide hydrolase (FAAH), which is found on the internal membranes of cells, although 2-AG is degraded by monoglyceride lipase (MGLL) as well. Several studies have been published concerning the role of cannabinoids in the female reproductive system (4–6), and the cannabinoid receptors and degradation enzymes have been found in various parts of the mammalian femalereproductive system as uterus, oviduct (7–10), preimplantation embryos, and placenta (11–13).

In regard to the oocyte maturation, it is known that AEA is present in the human follicular fluid (14) and that its concentration in follicles with mature oocytes is higher than in follicles with immature oocytes (15). The localization of the cannabinoid receptors and enzymes in rat (16) and human ovary led to the hypothesis that AEA plays a role in folliculogenesis, preovulatory follicle maturation, oocyte maturity, and/or ovulation (15). The hypothesis about oocyte maturation was reinforced with the localization of cannabinoid receptors (17) and cannabinoid-degrading enzymes (authors' unpublished data) during the nuclear maturation of human oocytes, where each protein is relocated during that maturation.

Taking into account the necessary bidirectional communication between the oocyte and the granulosa cells, our study aimed to characterize in depth, using a variety of experimental methods, the differential expression of the two cannabinoid receptors and the two degrading enzymes in human granulosa cells that surround oocytes at each stage of meiotic resumption.

MATERIAL AND METHODS

Granulosa Cells Collection

Granulosa cells were obtained from 53 patients (ages 25–40 years; mean, 36.7 ± 2.6) undergoing intracytoplasmic sperm injection (ICSI) at the Human Reproduction Unit of the Cruces University Hospital. Ethical approval was obtained from the Clinical Research Ethical Committee of the Basque Health System (Osakidetza, CEIC reference no.r E07/54, 3/2008). Informed consent was obtained from all patients.

We analyzed the granulosa cells of 300 oocytes from 53 patients. Of the 300 oocytes studied, 75 were at the GV stage, 51 at MI, and 174 at MII. The main ICSI indications were male factor (79.9%) and failure of IUI. The main female-associated conditions were endometriosis (9.8%) and tubal factor (11.2%). Patients had no history of cannabinoid drug consumption.

The ovarian stimulation protocol has been published elsewhere (18, 19). Briefly, it consists of down-regulation with GnRH agonist and triptorelin acetate on a long protocol or with GnRH antagonist and the cetrorelix protocol, ovarian stimulation with recombinant FSH and highly purified urinary menopausal gonadotropins or recombinant LH, and ovulation being triggered with 250 μg recombinant hCG. Q2

Oocyte retrieval was performed 35–37 hours after hCG administration. Follicles were aspirated with a negative pressure of 115–120 mmHg with a single lumen 18-gauge oocyte pick-up needle (K-OPS-6035-RWH-B-ET; Cook) under transvaginal ultrasound guidance. Follicular fluids were observed at low magnification (×40–100) under the stereomicroscope at 37°C. When a COC was found, the stage of maturity was estimated by noting the volume, density, and condition of the surrounding coronal and cumulus cells, according to published criteria (20), and classified into one of four categories: [1] mature, [2] slightly immature, [3] completely immature, or [4] hypermature. The oocytes were then incubated for 2 hours in IVF Medium (IVF Medium, Medicult, Origio) at 37°C and 6% CO_2 in air. Immediately before micromanipulation for the ICSI procedure, oocytes were denuded from the cumulus oophorus one by one in 30-μL droplets by a brief exposure to 40 IU/L hyaluronidase solution (Hyadase; Medicult, Origio), followed by mechanical removal of the corona radiate cells with the use of plastic pipettes of defined diameters (denuding pipette; Cook). The droplets containing the cumulus and granulosa cells after oocyte denuding were recovered in Eppendorf tubes, taking into account the nuclear maturation stage of the oocyte for separation into granulosa cells from GV, MI, or MII, and were centrifugated at 800 g. Finally, the medium containing the hyaluronidase solution was removed and the cells were placed at 4°C for early use or in the freezer at −80°C for later study.

Reverse Transcription

RNA from granulosa cells (obtained from 10 oocytes of each stage; n = 3), cerebral cortex (positive control for CB1, FAAH, and MGLL) and Jurkat cells (positive control for CB2), were isolated with the Dynabeads mRNA Purification Kit (Ambion). The procedure for obtaining the cDNA was performed with ImProm-II Reverse Transcription System (Promega). Briefly, about 150 ng of RNA and random primers were heated at 65°C for 10 minutes and chilled on ice for 5 minutes. Then, once the reverse transcription mix was added, it was annealed at 25°C for 5 minutes. A first-strand synthesis reaction was carried out at 55°C for 60 minutes, and the reverse transcriptase was inactivated at 70°C for 15 minutes.

Real-time Quantitative Polymerase Chain Reaction (PCR) Analysis

Quantitative PCR was performed in three replicates with the StepOne thermocycler using a TaqMan assay (Applied