Modulation of microvascular permeability in the preovulatory rat ovary by an ovulatory gonadotropin stimulus

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Objective: To characterize the size selectivity of the rat ovarian vasculature and its changes after gonadotropin induction of ovulation.
Design: Experimental study.
Setting: Obstetrics and Gynecology Department.
Animal(s): Immature, female Sprague-Dawley rats.
Intervention(s): Rats were pretreated with equine chorionic gonadotropin, and ovaries were retrieved either 48 hours later or at any of several time points during ovulation induced by hCG. Fluorescein isothiocyanate–labeled Ficoll was injected 10 minutes before ovarian sampling, and the distribution of Ficoll was measured in plasma and ovarian extracts.
Main Outcome Measure(s): The Ficoll data were analyzed according to a two-pore model to acquire information on small (r_S) and large (r_L) pore radii as well as the number of large pores reflected by the large pore fraction of the hydraulic conductance (LpS%) at each periovulatory time interval.
Result(s): Before hCG, r_S and r_L were 54.7 ± 1.2 Å (mean ± SEM) and 149.3 ± 5.3 Å, respectively. At this preovulatory stage, LpS% was 7.1% ± 3.2%. Stimulation with hCG caused close to a three-fold increase in LpS% at 2 and 4 hours (20.9% ± 1.8% and 20.7% ± 2.5%, respectively) and approximately 15% enlargements of r_S and r_L. Thus, the change in LpS% represents a dramatic increase in the number of large pores and not an increased size of preexisting large pores, since the small and large pore radii changed in parallel.
Conclusion(s): These results indicate that capillary permeability of the ovarian blood-follicle barrier is modulated by gonadotropin, mainly through increased numbers of large pores, similar to a classical inflammatory response. (Fertil Steril® 2013;99:903–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: Ovary, ovulation, permeability, permselectivity, rat

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The preovulatory follicle, which ruptures at ovulation, is composed of a centrally located fluid-filled antrum that is surrounded by an avascular granulosa layer and the peripheral theca layer with its capillary network close to the basal lamina. Vascularization and its functional changes in the ovary may be of physiological importance in ovulation, as exemplified by the more prominent vascularization in the dominant follicle of the rhesus macaque ovary compared with that of nondominant follicles (1).

Shortly after the LH surge, a rapid swelling and hyperemia of the rat preovulatory follicle is observed (2), and microscopy studies of rabbit ovaries have revealed edema formation (3, 4). It has been proposed that these follicular changes are caused by increased ovarian vascular permeability and blood flow (5, 6), as well as by structural degradation of the follicular wall (7). Observations of leakage of injected carbon particles in the theca interna of the ovulating rabbit follicle (8) and increased ovarian uptake of

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radiolabeled albumin in the ovulating rat ovary (9) indicate augmented permeability during this period.

The proposed LH-induced increase in ovarian transport of macromolecules would most likely involve changes in the ovary of permselectivity, a term that refers to the restriction of macromolecular passage through the capillary walls due to molecular size, charge, or physical configuration. This would relate to the existence of a dynamic blood-follicle barrier, which is proposed to be a molecular sieve that restricts the passage of solutes from ovarian blood vessels to the extracellular space (10–12). However, increased transport of solutes across the blood-follicle barrier could also be due to increased capillary hydrostatic pressure, increased blood flow, and/or recruitment of more capillaries increasing the surface area available for exchange without altering the permselectivity per se (13).

In the mouse ovary, this blood-follicle barrier seems to be both charge and size selective (14), since negatively charged inter-α-inhibitor (IαI) entered the follicle only after preovulatory stimulation by gonadotropin and because passage of intermediate-size molecules was gonadotropin-dependent (14). These findings of permselectivity in the mouse ovary are in accordance with observations of permselectivity in various other organs (13). In theory, the size selectivity and the charge selectivity of the blood-follicle barrier may be ascribed to different components of the vascular wall with independent regulation (15).

The hypothesis of the study was that the size-selective properties of ovarian microvasculature change during ovulation.

MATERIALS AND METHODS

Animals

Immature female Sprague-Dawley rats (B&K Universal) were kept under standardized conditions of temperature and light (light on between 05:00 and 19:00) with pelleted food and water ad libitum. All experiments were approved by the Regional Animal Ethics Committee of Gothenburg.

Experimental Protocol

At 26 days of age, the rats were pretreated with 10 IU of equine chorionic gonadotropin (eCG: Sigma Chemical Company) SC to promote follicular growth and maturation to a first generation of preovulatory follicles 2 days later. Ovarian samples were taken 48 hours after eCG (0 hours sample; n = 7) or at time points 30 minutes (n = 4), 1 hour (n = 4), 2 hours (n = 7), 4 hours (n = 7), and 8 hours (n = 4) after IP administration of 15 IU of hCG (Serono). The rats were anesthetized with ketamine (40 mg/kg; Parke Davis) and xylazine (6.5 mg/kg; Bayer) given IP 20 minutes before ovarian sampling. The femoral veins were exposed by a small incision, and fluorescein isothiocyanate (FITC)-Ficoll (0.8 mg/rat; Pharmacia-Upjohn), dissolved in 200 μL of 0.154 M NaCl, was injected IV. A laparotomy was performed 10 minutes later, and both ovaries were excised and washed in 0.154 M NaCl. Periovarian adipose tissue and oviducts were carefully trimmed away from the ovary. Rats were then heparinized (250 IU) through the femoral vein, and blood samples (1 mL) were collected from the aorta.

Since FITC-Ficoll is a light-sensitive substance, all the procedures below were conducted under restricted light conditions. Each ovarian sample was homogenized with Pellet Pestle (Kontes) in 100 μL of 0.154 M NaCl and centrifuged (10,800 rpm; 5 minutes), and the supernatants were kept at −20°C. Blood content in the ovarian homogenate was assessed by measuring hemoglobin concentrations in the sample preparation and was less than 3% of ovarian wet weight in all experiments. Blood samples were centrifuged (10,800 rpm, 5 minutes), and the plasma fraction was stored at −20°C until later analysis.

FITC-Ficoll Standard Curve

Ficoll is an almost spherical molecule, which has a frictional ratio close to 1.0 and a net surface charge of 0. Five samples of Ficoll with known molecular radii were labeled with FITC and used as standards as described elsewhere (15, 16). The labeled Ficoll molecules were subjected to gel filtration on a Superose 12PC3.2/30 column (SMART HPLC, Pharmacia Biotech AB), and the fluorescence of FITC-Ficoll was measured using a DIONEX RF 2000 Fluorescence Detector (Dionex Softrom GmbH). The eluted volume to the fluorescence curves was recorded by Cromeleon software (Dionex Softrom GmbH). The known Stokes-Einstein radii of the Ficoll standard molecules were correlated to the corresponding elution fraction to construct standard curves. The concentration of Ficolls could hereby be determined for a wide range (12–74 Å) of molecular sizes.

Analysis of Ficoll Concentrations

Ovarian extracts and plasma samples were diluted 1:16 with phosphate buffer (0.05 M, with 0.154 M NaCl at pH 7.0) and filtrated to remove particles and contaminants. These filtrates were analyzed on a Superose 12PC3.2/30 column, and curves of fluorescence to eluted volume were obtained. From each sample, a volume of 5–10 μL was analyzed at an emission wavelength of 520 nm and an excitation wavelength of 492 nm. During analysis, flow rate (1 mL/minute), sampling frequency (1/second), pressure (4 MPa), and temperature (8°C) were maintained constant. The results were then converted to the Stokes-Einstein radius to the absorbance function of FITC-Ficoll based on the standard curves obtained as above. The ovarian fluid concentrations (C_{OF}) were corrected for the small amounts of residual blood determined by separate analysis of hemoglobin content. Finally, C_{OF} over plasma Ficoll concentration (C_P) ratios were estimated for approximately 200 data pairs of Stokes-Einstein radii between 12 Å and 74 Å. By these procedures, the error in the C_{OF}/C_P-ratios for Ficoll was estimated to be less than 1% for most molecular sizes.

Calculations

The results were analyzed according to the two-pore model, as discussed in detail elsewhere (13). Small pore radius (r_S), large pore radius (r_L), and the large pore fraction of the total hydraulic conductance (LpS%) were calculated by nonlinear