Estradiol-Dependent Effect of Nitric Oxide on Meiotic Maturation of Mouse Oocytes

T. Yu. Voznesenskaya and T. V. Blashkiv

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 10, pp. 368-370, October, 2005
Original article submitted March 4, 2005

Estradiol dipropionate decreased the number of oocytes in mouse ovary and suppressed meiotic maturation of these cells. Administration of NO donors sodium nitroprusside and S-nitroso-N-acetylpenicillamine increased the ability of ovarian oocytes to complete meiotic maturation and counteracted the effect of estradiol dipropionate. Our results suggest that estradiol is involved in the regulation of production and release of ovarian NO.

Key Words: *NO; estradiol; mouse oocytes*

Hormones are involved in the regulation of NO synthase activity in cells. For example, estrogens increase enzyme activity in the uterus of nonpregnant sheep. During pregnancy this mechanism mediates the increase in blood supply to the uterus. Angiotensin II partially blocks contraction of papillary muscles in the heart, which is realized via modulation of NO synthesis [3].

Published data show that estradiol can act via nongenomic stimulation of the membrane-intracellular transmitter system and can produce a genomic effect that is typical of steroids and depends on protein synthase activity [6]. A positive correlation was revealed between nitrate content in the follicular fluid, number of oocytes resuming meiotic maturation in IVF women, and serum estradiol concentration on the day of treatment with chorionic gonadotropin [8]. Biochemical and immunochemical studies revealed endothelial NO synthase (eNOS) in granular and thecal cells of the follicle and oocyte [5]. Previous experiments showed that the intensity of NO production is maximum in granular cells of small follicles [4]. The existence of little difference in the concentration of NO in large and small follicles suggests that NO induces different changes in these follicles [7]. Here we studied the effect of NO donors on mouse oocytes and evaluated the influence of exogenous estradiol on meiotic maturation of oocytes from large and small follicles.

Department of Immunology and Cytotoxic Sera, A. A. Bogomolets Institute of Physiology, Ukrainian Academy of Sciences, Kiev. ***Address for correspondence:*** imm@biph.kiev.ua. T. Yu. Voznesenskaya

MATERIALS AND METHODS

Experiments were performed on adult female CBA mice (20-25 g) during proestrus. The ovaries were removed 20 h after administration of NO donors under ether anesthesia. We performed 2 series of experiments. In series I, NO donors sodium nitroprusside (Sigma) and S-nitroso-N-acetylpenicillamine (Sigma) were injected intraperitoneally in a single dose of 0.5 mg/kg. This dose was selected taking into account published data [4] and results of experiments with CBA females receiving the test preparations in doses of 5.00, 0.50, and 0.05 mg/kg. Control animals intraperitoneally received an equivalent volume of physiological saline. Oocytes were isolated from the ovaries. In series II, sodium nitroprusside and S-nitroso-N-acetylpenicillamine were administered 24 h after estrogen treatment. Estrogens (oil solution of estradiol dipropionate) were injected intraperitoneally in a single dose of 0.025 mg/kg. This contraceptive dose was selected taking into account published data [2] and results of experiments with CBA females receiving the test preparations in doses of 0.00020, 0.00125, and 0.02500 mg/kg. Control animals intraperitoneally received an equivalent volume of sterile oil and physiological saline instead of estradiol dipropionate and NO donors, respectively. Small (158-164 μ) and large

0007-4888/05/1404-0378

follicles (359-367 μ) were nonenzymatically isolated from the ovaries and classified by volume [1]. Oocytes were obtained from follicles of each group. In all series the oocytes were washed, counted, and subjected to morphological study. We examined the germinal vesicle (GV), perivitelline space, and cytoplasm (density, degree of granulation, and signs of fragmentation and degeneration). The oocytes were divided into 2 groups. The cells of satisfactory quality had GV and regularly granulated cytoplasm. Atypical cells of low quality had GV, widened perivitelline space, and irregularly granulated cytoplasm. These cells were characterized by signs of fragmentation or degeneration. Satisfactory-quality oocytes were cultured in chambers with 0.40 ml DME medium that contained 15 mmol/liter HEPES and 1.71 mmol/liter calcium at 37°C using a sterile box. The count of morphologically atypical oocytes was estimated as the ratio between the number of low-quality oocytes and total number of oocytes (in percents). We counted oocytes with dissolved GV that were able to resume meiosis (RM) after 2-h culturing. We also estimated the number of oocytes forming the first polar body (PB) after 20-h culturing. The ratio of these cells was expressed in percents of the initial number of oocytes with GV. The results were analyzed by Student's *t* test.

RESULTS

Administration of sodium nitroprusside and S-nitroso-N-acetylpenicillamine increased the number of ovarian oocytes to 23.0±0.7 ($p<0.05$) and 26.1±0.7 cells ($p<0.01$), respectively, compared to the control (18.2±1.2 cells); the number of morphologically atypical ovarian oocytes (with widened perivitelline space and irregularly granulated cytoplasm and with signs cytoplasm fragmentation of increased to 11.7 and 15.7%, respectively (*vs*. 5.8% in the control). Sodium nitroprusside and nitric oxide donor S-nitroso-N-acetylpenicillamine increased the number of oocytes resuming the first meiotic division *in vitro* to 55.4±1.3 ($p<0.05$) and 59.5±1.5% ($p<0.01$), respectively (*vs.* 47.5±1.4% in the control); the number of PB oocytes increased to 67.2±1.3 ($p<0.05$) and 75.0±1.4% ($p<0.01$), respectively, compared to the control (60.0±2.5%). These data indicate that NO donors regulate meiotic maturation of oocytes. Published data show that NO acts as a local modulator of granular cell function, but has no effect on cell proliferation [4].

Estradiol dipropionate decreased the number of oocytes, which can be isolated from one ovary to 12.0±0.8 cells ($p<0.05$, compared to 18.2±1.2 and 14.5±0.6 in the NaCl and control groups, respectively). Moreover, estradiol dipropionate suppressed the ability of oocytes from small and large follicles to RM. The number of RM oocytes from small follicles of animals receiving estradiol dipropionate and NaCl was 10.0±1.4 ($p<0.01$) and 12.5±1.1% ($p<0.01$), respectively (*vs*. 22.2±0.2% in the control). After treatment with estradiol dipropionate the number of RM oocytes from large follicles was 32.4±0.3%. Estradiol dipropionate decreased the number of oocytes from small (35.0±1.7 *vs*. 85.7±0.8% in the control, $p<0.05$) and large follicles (25.0±1.4 *vs*. 92.5±0.4% in the control, $p<0.01$) that *in vitro* formed the first PB. Our findings suggest that estradiol dipropionate decreases the number of ovarian oocytes and suppresses meiotic maturation of these cells. It can be hypothesized that after administration of estradiol dipropionate mouse oocytes do not maturate in the follicles and undergo ovulation at an earlier stage of development. These data seem to explain the mechanism for contraceptive action of estradiol dipropionate.

Administration of sodium nitroprusside and S-nitroso-N-acetylpenicillamine after stimulation with estradiol dipropionate increased the number of ovarian oocytes to 15.3±1.1 and 16.0±0.7 cells, respectively (*vs.* 12.0±0.8 cells in mice receiving estradiol dipropionate, $p<0.01$). The test substances *in vitro* increased the ability of oocytes from small follicles to RM to 17.5±0.8 ($p<0.01$) and 22.7±1.3% ($p<0.01$), respectively, *vs*. 10.0±1.4% in the estradiol dipropionate group. Sodium nitroprusside and S-nitroso-N-acetylpenicillamine also increased the ability of oocytes from large follicles to RM to 18.3±1.3 ($p<0.05$) and 23.3±0.9% ($p<0.01$), respectively, *vs*. 12.5±1.1% in the estradiol dipropionate group. Sodium nitroprusside and S-nitroso-N-acetylpenicillamine increased the number of PB oocytes from small (56.7±1.0 ($p<0.01$) and 65.2±1.1% ($p<0.01$), respectively, *vs*. 35.0±1.7% in the estradiol dipropionate group) and large follicles (38.2±1.1 ($p<0.01$) and 45.2±1.7% ($p<0.01$), respectively, *vs*. 25.0±1.4% in the estradiol dipropionate group). Therefore, sodium nitroprusside and S-nitroso-N-acetylpenicillamine abolish the effect of estradiol dipropionate. Hence, NO is involved in the influence of estradiol dipropionate on mouse oocytes.

Our results indicate that NO donors increase the number of ovarian oocytes and stimulate meiotic maturation of these cells. We conclude that estradiol plays a role in the regulation of production and release of ovarian NO. The estradiol-dependent effect of NO should be taken into account in the development of new contraceptive drugs.

REFERENCES

1. T. V. Blashkiv, I. N. Alekseeva, R. I. Yanchii, *et al*., *Neirofiziologiya*, **32**, No. 3, 187-190 (2000).
2. T. I. Ivanenko, V. P. Fedotov, and V. M. Rzheznikov, *Eksp. Klin. Farmakol*., **59**, No. 4, 34-37 (1996).

3. A. Kh. Urazaev and A. L. Zefirov, *Usp. Fiziol. Nauk*, **30**, No. 1, 54-72 (1999).
4. G. Basini, M. Barstta, N. Ponderato, *et al*., *Reprod. Fertil. Dev*., **10**, No. 6, 471-478 (1998).
5. A. Jablonka and L. Olson, *Endocrinology*, **139**, 2944-2954 (1998).
6. M. Joswig, V. Hach-Wunderle, R. Ziegler, *et al*., *Exp. Clin. Endocrinol. Diabetes*, **197**, No. 8, 477-487 (1999).
7. N. Sugino, S. Takiguchi, M. Ono, *et al*., *Hum. Reprod*., **11**, No. 11, 2484-2487 (1996).
8. M. Tao, H. Kodama, S. Kagabu, *et al*., *Ibid*., **12**, No. 10, 2220-2225 (1997).