Physiol. Res. 66: 1057-1059, 2017

RAPID COMMUNICATION

Ovarian Steroid Hormone Secretion Activity Examined After Supplementation of Green Tea Extract

S. ROYCHOUDHURY[1,2], M. HALENAR[2], V. TUPA[2], K. MICHALCOVA[2], S. NATH[1], M. KACANIOVA[3], A. KOLESAROVA[2]

[1]Department of Life Science and Bioinformatics, Assam University, Silchar, India, [2]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic, [3]Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

Received June 2, 2017
Accepted September 12, 2017

Summary

This study aimed at examining the secretion activity of steroid hormones progesterone and 17β-estradiol by porcine ovarian granulosa cells after addition of green tea extract. Granulosa cells were incubated with green tea extract (at doses of 0.01, 0.1, 1, 10 and 100 μg.ml^{-1}). Another set of cells were incubated with green tea extract at the above doses along with additional supplementation of follicle stimulating hormone (FSH) at 10 μg.ml^{-1}. Release of hormones by granulosa cells was assessed by EIA after 24 h exposure. Secretion of steroid hormones was not affected either by green tea extract alone or after FSH supplementation with green tea extract. Results indicate that ovarian steroidogenesis is not affected by green tea under conditions used in the experiment.

Key words

Green tea • Ovarian granulosa cells • Anti-microbial activity • FSH • Steroid hormones

Corresponding author

A. Kolesarova, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra 949 76, Slovak Republic. E-mail: Adriana.Kolesarova@uniag.sk

Green tea (*Camellia sinensis* L., Theaceae) is a widely consumed beverage all over the world. Health-promoting activities of green tea extract have been attributed to flavonoid-like polyphenols known as catechins (Khan and Mukhtar 2007). The unique property of green tea catechins has the potential to improve reproductive health, including the quality of male and female gametes (Roychoudhury *et al.* 2017). Ovarian granulosa cells play a central role in steroidogenesis, which is critical for female reproduction (Harvey *et al.* 2009). Steroid hormone secretion by ovarian granulosa cells ensures a receptive environment for the implantation and development of the early embryo (Albertini *et al.* 2003). The aim of this *in vitro* study was to examine the steroid hormone (progesterone, 17β-estradiol) secretion by porcine ovarian granulosa cells after addition of green tea extract and green tea extract + follicle stimulating hormone (FSH). Briefly, 50 g plant materials (packaged leaves of Chun Mee green tea of Chinese origin) were grounded mechanically into fine powder and were extracted with 350 ml distilled water by boiling under reflux for 30 min. The extract was filtered and evaporated to dryness to yield the dry extract (yield ~ 50 %) (Chatterjee *et al.* 2012). Just before the addition to the cells, extract was dissolved first in DMSO (concentration 10 mg.ml^{-1}) and then in culture medium. The maximal concentration of DMSO in culture (and added to control) was 0.1 %. Ovarian granulosa cells were collected from the follicles (3-5 mm) of prepubertal gilts according to EU and Slovak guidelines for animal care, manipulation and use, were washed in sterile DMEM/F12 1:1 medium (BioWhittaker, Verviers, Belgium), and resuspended in the same medium supplemented with 10 % fetal bovine

Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

serum (FBS, BioWhittaker, Verviers, Belgium) with 1 % antibiotic-antimycotic solution (Sigma, St. Louis, MO, USA) at a final concentration of 10^6 cells.ml^{-1} medium. Granulosa cell suspension (1 ml/well) was dispensed in 24-well culture plates (Nunc, Roskilde, Denmark) and incubated at 37 °C and 5 % CO_2 in humidified air until 60-75 % confluent monolayer was formed (3-5 days), at that point medium was renewed. Further culture was performed in 1 ml culture medium in 24-well culture plates (Kolesarova *et al.* 2012, Packova *et al.* 2015, Roychoudhury *et al.* 2015). After medium replacement experimental cells were cultured for 24 h without (control) or with green tea extract (at doses of 0.01, 0.1, 1, 10 and 100 μg.ml^{-1}), and green tea extract (at doses of 0.01, 0.1, 1, 10 and 100 μg.ml^{-1}) + FSH (Sigma Aldrich, Steinheim, Germany) at 10 μg.ml^{-1}. Concentrations of progesterone and 17β-estradiol were determined in duplicate in the incubation medium by EIA (Packova *et al.* 2015, Roychoudhury *et al.* 2014). All EIAs were validated for use in samples of culture medium. For progesterone, intra- and interassay coeficients of variation did not exceed 4 % and 9.3 %, respectively. For 17β-estradiol, intra- and interassay coeficients of variation did not exceed 9 % and 10 %, respectively. Each treatment group was represented by 4 wells. Assays for hormone concentration in the incubation medium were performed in duplicates. Each experiment was performed thrice. Significant differences between the experiments were evaluated using one-way ANOVA followed by paired Wilcoxon-Mann-Whitney test (Systat Software, GmbH, Erkhart, Germany). Differences from control at $P<0.05$ were considered significant. In culture, granulosa cells formed a monolayer and secreted progesterone and 17β-estradiol. Release of these steroid hormones was not affected by green tea extract at the doses used. FSH supplementation to green tea extract showed an increasing trend of progesterone and 17β-estradiol release in comparison to green tea alone, however, the differences were not statistically significant (Figs 1 and 2). In an earlier study, treatment with 10 ng.ml^{-1} ovine FSH did not affect estradiol release although progesterone secretion was stimulated by bovine granulosa cells (Wrathall and Knight 1993). Experimental granulosa cells were suitable for testing and analysis of green tea extract as they formed cell monolayer and released of hormones into the culture medium. Epigallocatechin-3-gallate (EGCG), a major green tea catechin constituting more than 50 % of total catechins (catechins make up approx. 40 % of dried tea extract) was able to increase progesterone release at a dose of 10 μg.ml^{-1}, but not at higher doses. On the contrary, another study noted inhibition of progesterone as well as estradiol secretion by granulosa cells at 5 and 50 μg.ml^{-1} EGCG (Basini *et al.* 2005). These differences could be explained by the differences source of ovarian cells, for example, ovaries from mature pigs versus prepubertal pigs, as used in our study. Variation in the composition of green tea may be another factor behind the different study results obtained from separate studies.

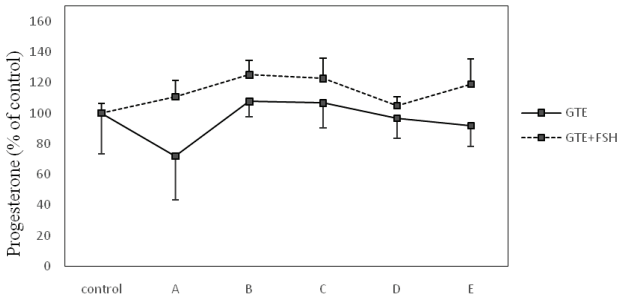

Fig. 1. Progesterone release by cultured porcine ovarian granulosa cells after addition of: (**A**) green tea extract (GTE), and (**B**) green tea extract + FSH. EIA after 24 h of culture. Each bar represents the mean (± SD) progesterone % of control.

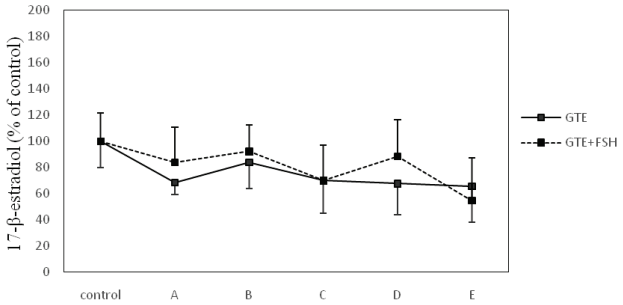

Fig. 2. 17β-estradiol release by cultured porcine ovarian granulosa cells after addition of: (**A**) green tea extract (GTE), and (**B**) green tea extract + FSH. EIA after 24 h of culture. Each bar represents the mean (± SD) progesterone % of control.

The composition of green tea depends on factors such as geographical location (climate, soil), agricultural practices (fertilizers, deadheading) and the properties of the plant itself (variety, age of the leaf, and position of the leaf on the harvested shoot) (Cabrera *et al.* 2006). Ten-day infusion green tea extract showed reproductive improvement in estradiol valerate-induced polycystic ovarian syndrome in rats, including decrease in the levels of serum luteinizing hormone (LH) and testosterone, but FSH levels remained unchanged (Ghafurniyan *et al.* 2015). In adult albino male rats, serum levels of

gonadotropic hormones FSH and LH as well as steroid hormone testosterone were reduced by green tea extract after 26 days of treatment *in vivo* (Das and Karmakar 2015). Earlier *in vivo* study on albino male rats for 26 days reported inhibition of testicular delta(5)3beta-and 17beta-hydroxysteroid dehydrogenase, including serum testosterone and LH levels but FSH levels did not change (Chandra *et al.* 2011). In the present study, the inability of FSH to stimulate the release of either progesterone or estradiol by porcine ovarian granulosa cells may reflect a paucity of functional FSH receptors (Wrathall and Knight 1993).

In conclusion, our results indicate that used doses of green tea extract did not affect steroid secretion by ovarian granulosa cells but FSH supplementation to green tea extract showed an increasing trend of progesterone and 17β-estradiol release. Although these data contribute to new insights regarding the action of green tea extract, it is necessary to examine the individual steps of steroidogenesis in ovarian cells.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects no. VEGA, 1/0039/16, KEGA 011SPU-4/2016, Slovak Research and Development Agency, APVV-0304-12, APVV-15-0543 and European Community under project no. 26220220180: Building Research Centre "AgroBioTech".

References

ALBERTINI DF, BARRETT SL: Oocyte somatic cell communication. *Reprod Suppl* **61**: 49-54, 2003.

BASINI G, BIANCO F, GRASSELLI F: Epigallocatechin-3-gallate from green tea negatively affects swine granulosa cell function: *Domest Anim Endocrinol* **28**: 243-256, 2005.

CABRERA C, ARTACHO R, GIMENEZ R: Beneficial effects of green tea - a review: *J Am Coll Nutr* **25**: 79-99, 2006.

CHANDRA AK, CHOUDHURY SR, DE N, SARKAR M: Effect of green tea (Camellia sinensis L.) extract on morphological and functional changes in adult male gonads of albino rats. *Indian J Exp Biol* **49**: 689-687, 2011.

CHATTERJEE P, CHANDRA S, DEY P, BHATTACHARYA S: Evaluation of anti-inflammatory effects of green tea and black tea: a comparative *in vitro* study. *J Adv Pharm Technol Res* **3**: 136-138, 2012.

DAS SK, KARMAKAR SN: Effect of green tea (Camellia sinensis L.) leaf extract on reproductive system of adult male albino rats: *Int J Physiol Pathophysiol Pharmacol* **7**: 178-184, 2015.

GHAFURNIYAN H, AZARNIA M, NABIUNI M, KARIMZADEH L: The effect of green tea extract on reproductive improvement in estradiol valerate-induced polycystic ovary polycystic ovarian syndrome in rat. *Iran J Pharm Res* **14**: 1215-1233, 2015.

HARVEY CN, ESMAIL M, WANG Q, BROOKS AI, ZACHOW R, UZUMCU M: Effect of the methoxychlor metabolite HPTE on the rat ovarian granulosa cell transcriptome *in vitro*. *Toxicol Sci* **110**: 95-106, 2009.

KHAN N, MUKHTAR H: Tea polyphenols for health promotion. *Life Sci* **81**: 519-533, 2007.

KOLESAROVA A, CAPCAROVA M, MARUNIAKOVA N, LUKAC N, CIERESZKO RE, SIROTKIN AV: Resveratrol inhibits reproductive toxicity induced by deoxynivalenol. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **47**: 1329-1334, 2012.

PACKOVA D, CARBONELL-BARRACHINA AA, KOLESAROVA A: Ellagitannins – compounds from pomegranate as possible effector in steroidogenesis of rabbit ovaries. *Physiol Res* **64**: 583-585, 2015.

ROYCHOUDHURY S, DETVANOVA L, SIROTKIN AV, TOMAN R, KOLESAROVA A: *In vitro* changes in secretion activity of rat ovarian fragments induced by molybdenum. *Physiol Res* **63**: 807-809, 2014.

ROYCHOUDHURY S, MASSANYI P, SLIVKOVA J, FORMICKI G, LUKAC N, SLAMECKA J, SLAMA P, KOLESAROVA A: Effect of mercury on porcine ovarian granulosa cells *in vitro*. *J Environ Sci Health A Toxicology Hazard Subst Environ Eng* **50**: 839-845, 2015.

ROYCHOUDHURY S, AGARWAL A, VIRK G, CHO CL: Potential role of green tea catechins in the management of oxidative stress-associated infertility. *Reprod Biomed Online* **34**: 487-498, 2017.

WRATHALL JH, KNIGHT PG: Production of immunoactive inhibin by bovine granulosa cells in serum-free culture: effects of exogenous steroids and FSH. *Domest Anim Endocrinol* **10**: 289-304, 1993.