Tissues, Pathology, and Diagnostic Microscopy

LS.2.P094
Effects of genistein on gonadotropic cells in middle-aged female rats

I. Medigović[1]

[1]Institute for Biological Research "Siniša Stanković", Department of Citology, Belgrade, Serbia

ivana84@ibiss.bg.ac.rs
Keywords: genistein, estradiol, confocal microscopy, gonadotropic cells, middle-aged female rats

Genistein (G) is a major isoflavone aglycone found in soy based products [1]. It is structurally similar to estradiol and binds to both types of estrogen receptors, with higher affinity for ERβ [2]. Besides mild estrogenic/antiestrogenic activity, genistein also has antiproliferative, antiangiogenic and the antioxidative activities [3]. The present study was conducted to examine the effects of genistein exposure on morphology and immunofluorescent properties of pituitary follicle stimulating (FSH) and luteinizing (LH) cells in middle-aged female rats. Treatment with estradioldipropionate (E) was used as a positive control.

Middle-aged, 12 months old, female rats subcutaneously received 0.625 mg/kg of E, dissolved in olive oil, or 35 mg/kg of G dissolved in a mixture of olive oil and ethanol (9:1), daily, for 4 weeks. Each of the treated groups had a corresponding control group. Thus, females of the control G group (CG) received sterile olive oil and ethanol, while those of the E control group (CE) were given sterile olive oil. Intact control group (C) was also established. All animals were sacrificed 24 h after the last injection. The pituitary glands were excised, fixed in 4% paraformaldehyde for 24h and processed for embedding in paraffin. Tissue sections were deparaffinized in xylene and rehydrated through a decreasing series of ethanol. FSH and LH cells were localized by the immufluorescence using polyclonal anti-rat βFSH (1:300 v/v) and polyclonal anti-rat βLH antibodies (1:500 v/v) as primary antibodies. Donkey anti-goat Alexa Fluor 488 (Invitrogen, 1:200) served as secondary antibody. Images were obtained using a confocal laser scanning microscope Leica TCS SP5 II Basic (Leica Microsystems CMS GmbH; Germany). An Ar-ion 488 nm laser was used for excitation of green fluorescence. Imaging was done with a 40x or 63x objective. Analysis of confocal microscopy images was performed using the Quantify option in LAS AF Lite software (Leica Microsystems CMS GmbH; Germany). Relative intensity of fluorescence (RIF) was calculated according to Waters and Swedlow [4]. Intensity of fluorescence was measured on 100-150 FSH or LH cells per animal, in which the nucleus was apparent.

In this study, we demonstrated a method to quantitate the antigens visualized with indirect immunofluoresence and viewed with confocal microscopy. Since use of confocal microscopy restricted the thickness of optical section from which measurements of emitted fluorescence were obtained, direct comparisons of fluorescence intensity between gonadotropic cells of control and treated females was enabled. The FSH and LH cells of middle-aged females were polygonal, oval or polyhedral in shape with prominent, often eccentrically located nuclei with strong immunofluorescence located in the cytoplasm (Fig. 1 A, D). After estradiol treatment both types of gonadotropes were smaller and the intensity of FSH and LH fluoroscence was significantly decreased by 43.24% and 27.69%, respectively compared to the controls ($P < 0.05$; Fig. 1B, E; Fig. 2). After genistein treatment FSH and LH immunoreactive cells were larger in size, irregularly shaped, and with unhomogenously stained cytoplasm. RIF was unchanged (Fig. 2A). In conclusion, genistein changed morphology of gonadotrophic cells without changing the intracellular content of gonadotropic hormones in the pituitaries of middle-aged female rats.

1. M. de Lima Toccafondo Vieira et al., Phytomedicine. 15 (2008) p31.
2. S. Ramos. J Nutr Biochem. 18 (2007) p427.
3. G. Kuiper et al., Endocrinology. 138 (1997) p863.
4. J.Waters and J. Swedlow. Cell Press. (2008) p37.
5. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 173009.

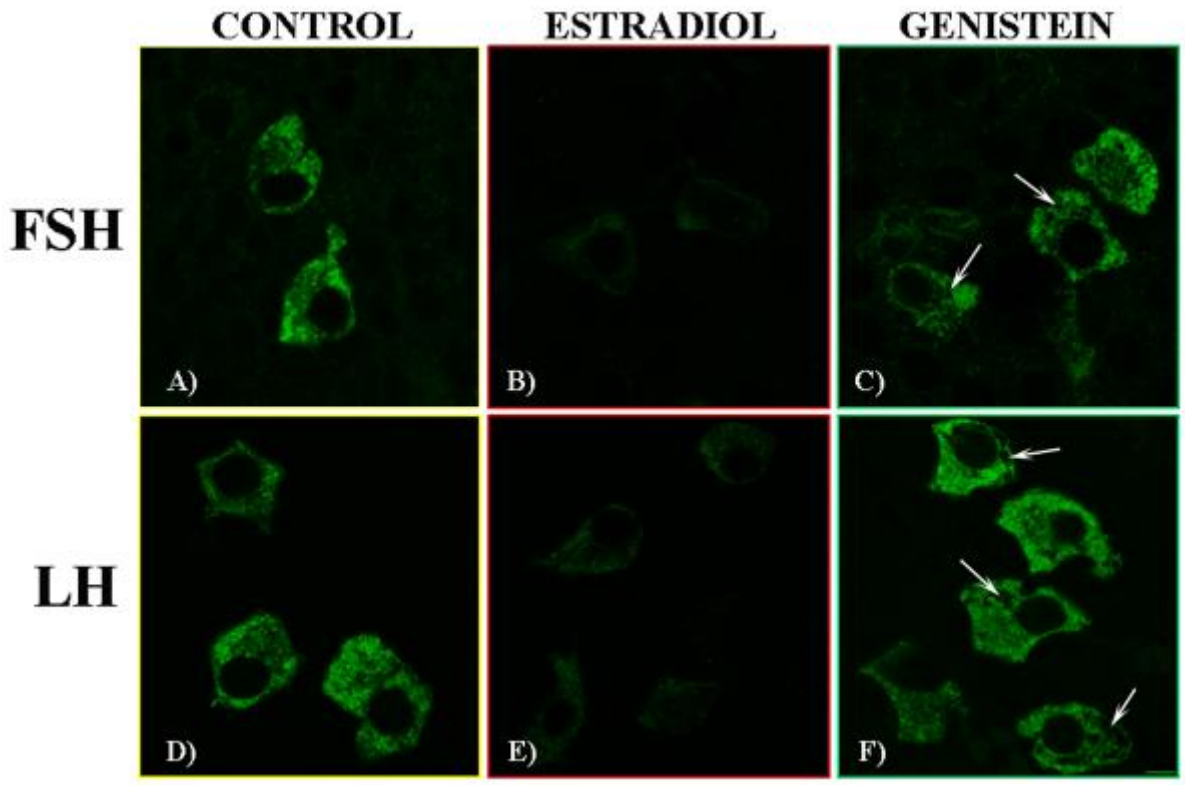

Figure 1. Representative micrographs of immunofluorescently labeled FSH (A-C) and LH (D-F) cells in the pituitary *pars distalis* of control (A, D), estradiol- (B,E) and genistein-treated (C,D) middle-aged female rats. After estradiol (B, E) treatment, FSH and LH cells were smaller in size and irregularly shaped. After genistein (C, D) treatment, FSH and LH cells were larger and with unstained parts of cytoplasm (arrows). Scale bar 50μm.

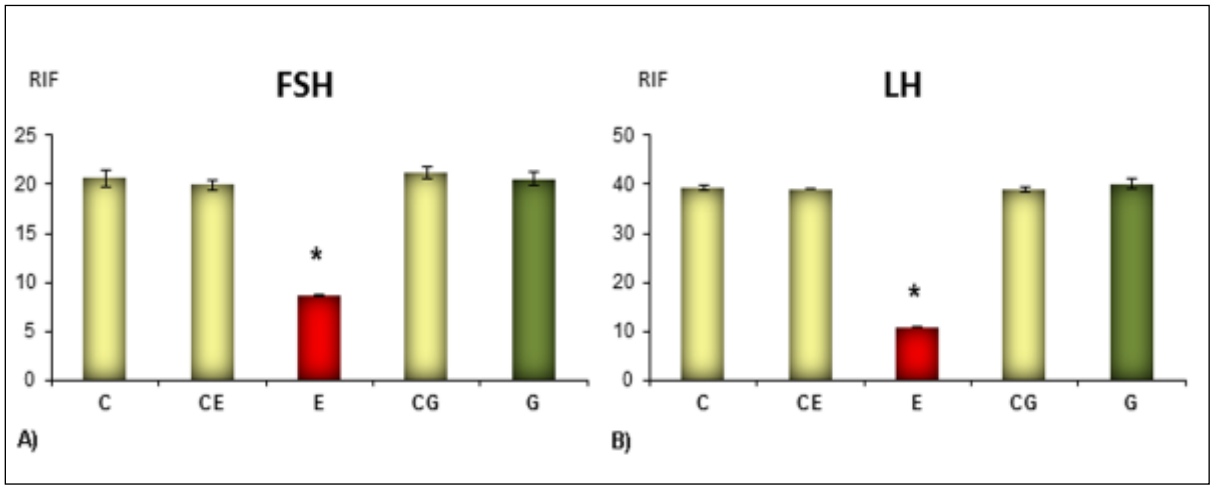

Figure 2. Relative intensity of fluorescence (RIF) of FSH (A) and LH (B) cells in the pituitary *pars distalis* of control, estradiol- and genistein-treated middle-aged female rats. All values are given as means ± SD; *Significantly different from corresponding control (C, CE and CG) (P< 0.05).