Prenatal dexamethasone treatment affects gonadotropic cells in adult male and female rats

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Developmental responses to environmental challenges during pregnancy may permanently alter fetal structure, physiology and/or metabolism. The responses to environmental challenges usually assist immediate fetal survival, but later in life these developmental changes are often shown to be disadvantageous. Link between adverse environmental signals during prenatal development and greater incidence of pathophysiological conditions in postnatal life, such as cardiovascular, metabolic and neuroendocrine disorders, is implied by concept of developmental programming. Adverse environmental conditions are usually signalled by increase of glucocorticoid levels, which results in fetal glucocorticoid overexpression. Hence, synthetic glucocorticoids such as dexamethasone (Dx), are used in numerous experimental protocols to induce developmental programming. Development of reproductive axis can also be affected by prenatal glucocorticoids, which may be associated with impaired reproductive function. Undisturbed functioning of pituitary gonadotropic cells that produce follicle-stimulating (FSH) and luteinizing hormone (LH), are essential for healthy reproduction. We have previously shown that prenatal Dx treatment evokes developmental programming of pitutary gonadotropic cells, which is apparent in neonatal, infantile and peripubertal females [1]. Wheather the changes of gonadotropic cells, caused by glucocorticoid overexposure in fetal period life, will persist till adulthood in female and male rats, is the aim of present study. To that end, relative intensity of fluorescence (RIF), as a measure of intracellular FSH and LH content, and the number of gonadotropic cells per mm^2 were determined.

Pregnant female Wistar rats subcutaneously received 0.5 mg Dx per kg/b.w. on 16th, 17th and 18th day of pregnancy. Control gravid females received the same volume of saline vehicle. Upon weaning, female and male offsprings were divided into four groups: control females (CF, n=6), control males (CM, n=6), and females (DxF, n=6) and males (DxM, n=6) prenatally exposed to Dx. Animals were sacrificed in adult period of life. Pituitary sections from dorsal, middle and ventral portion of *pars distalis*, were double immohistochemically stained using guinea pig anti-rat βFSH and rabbit anti-rat βLH primary antibodies. For visualization, Alexa-488 and -555 secondary antibodies were used, respectively. Images were obtained using a confocal laser scanning microscope (Leica TCS SP5 II Basic; Leica Microsystems CMS GmbH, Mannheim, Germany). An Ar-488 nm and HeNe-543 nm lasers were used for excitation of fluorescence. RIF in the cytoplasm of pituitary gonadotropic cells was evaluated according to previously described procedures [2]. Additionally, the number of gonadotropic cells *per* unit area was determined.

Gonadotropic cells in pituitaries of control animals were almost all bihomonal, i.e. both βFSH and βLH were present in most of the analysed cells. RIF of βFSH was not different between the sexes. However, in gonadotropic cells of CM rats, RIF of βLH was higher comparing to CF rats (Fig. 1a and 1b), by 32.9% ($p<0.05$). This is probably caused by the low content of LH in the female pituitaries during diestrus, when all females were sacrificed. After prenatal Dx exposure, the most prominent fluorescence was that of βLH, giving impression that only LH is present in gonadotropic cells. However, after quantification of the intensity of fluorescence signal, it was observed that βFSH intracellular content was dramatically decreased in both sexes, but still present (Fig. 1a). In DxF group, content of βFSH in gonadotropic cells was decreased by 69.7% ($p<0.05$) comparing to the control females. In males the same parameter was lowered by 58.4% ($p<0.05$) (Fig. 1a and 1b). Interestingly, the number of gonadotropic cells was changed only in females. Namely, comparing to corresponding controls, in pituitaries of females prenatally exposed to Dx, gonadotropic cells were decreased by 35.3% ($p<0.05$)(Fig. 1c).

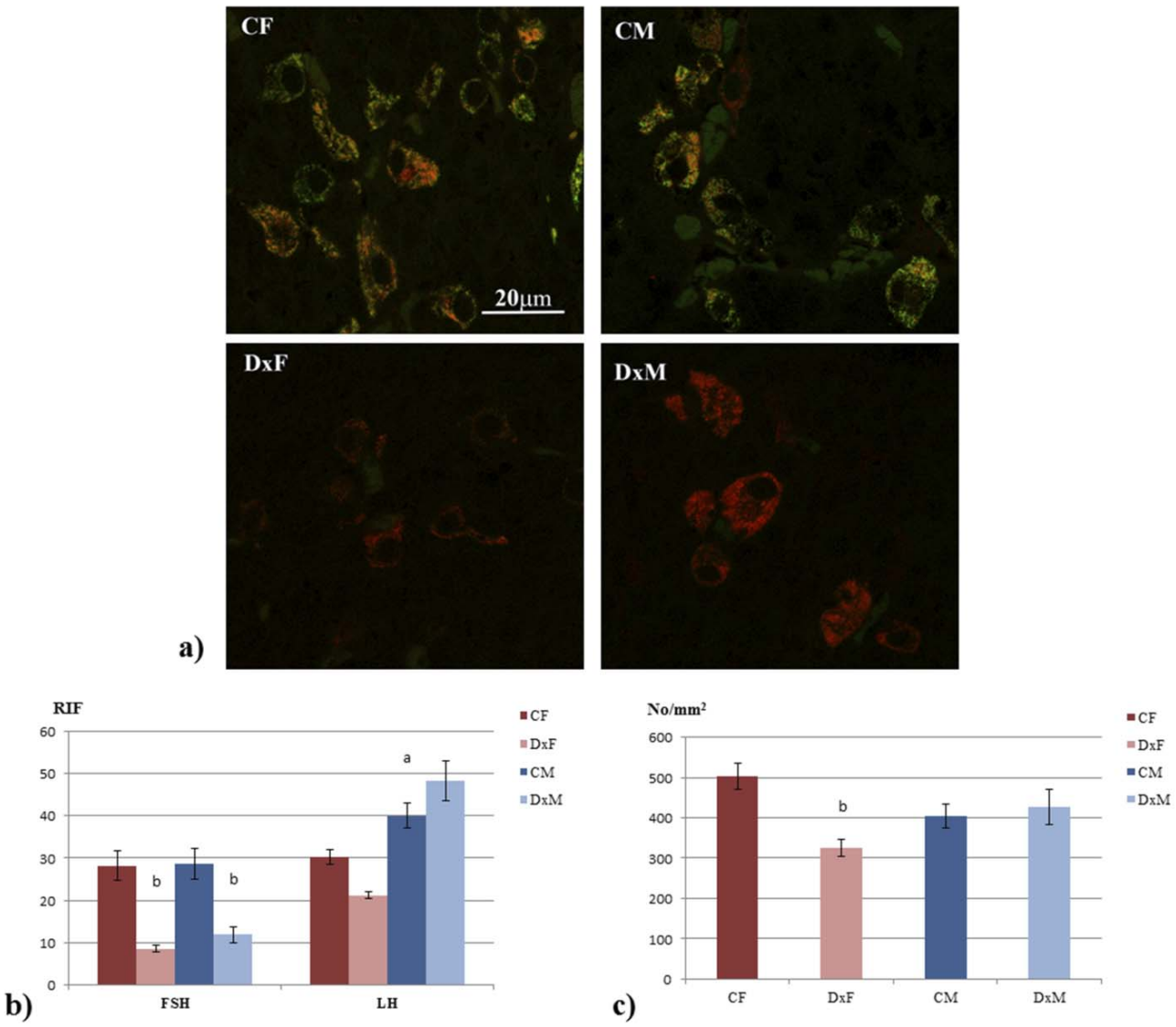

Figure 1. Immunofluorescence of pituitary gonadotropic cells. a) Representative micrographs of double immonostained gonadotropic cells; immunofluorescence for βFSH-green and βLH-red, bar-20µm; b) The relative intensity of fluorescence (RIF) of βFSH- and βLH- labeled cells; c) Number of gonadotropic cells per unit area (No/mm^2) in the pituitary *pars distalis* of control females (CF), control males (CM) and females (DxF) and males (DxM) prenatally exposed to Dx. All values are provided as the mean±SD; n=6. $^a p<0.05$ CM vs. CF, $^b p<0.05$ Dx vs. C.

On the basis of result presented, it can be concluded that prenatal dexamethasone exposure affects gonadotropic cells in females and males and that changes originated in fetal life persist till adulthood. The most prominent change observed is diminution of intracellular FSH content. Additionally, it appears that females are more affected, having in mind that the number of gonadotrops *per* unit area is decreased, while in males reduction in number did not occur.

References

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