Adrenergic innervation and steroidogenic activity of cystic porcine ovaries

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Abstract

We studied both morphology and steroidogenic activity of porcine ovaries after dexamethasone (DMX)-induced polycystic status. In the polycystic-changed ovaries, an increase in the number of DßH-IR and/or NPY-IR nerve terminals was found in the wall of follicles, cysts and blood vessels. After DXM injections, we observed changes in the mean contents of progesterone, androstendione, estradiol-17ß, as well as noradrenaline, dopamine and adrenaline in the studied ovarian structures. The obtained data revealed that, in the polycystic ovaries of gilts, an increase in the number of adrenergic nerve terminals was associated with changes of the steroidogenic activity, what may suggest an important role of the adrenergic innervation in the ovarian cyst formation in the gilts.

Key words: ovarian cysts, catecholamines, steroidogenesis, gilts.

Introduction

The role of the nervous system in the control of normal ovarian function has already been documented in part. It is generally known that sympathetic nerves influence ovarian steroidogenesis and follicular development [1]. However, only little attention has, so far, been paid to the possibility that the derangement in neurogenic inputs may be an underlying component of some ovarian pathologies, such as cystic ovarian disease (COD). It has been suggested that an alternation in the activity of the sympathetic neurons, innervating the ovary, may contribute to etiopathogenesis of cysts in women [2] and rats [3]. COD is a common reproductive disorder in female domestic animals, leading to temporal or permanent infertility. According to our knowledge, there is still no available information on the role of sympathetic nerves in the etiopathogenesis of the ovarian cysts in pigs. Therefore, the purpose of our study was to determine the morphology and steroidogenesis of polycystic ovaries in pigs.

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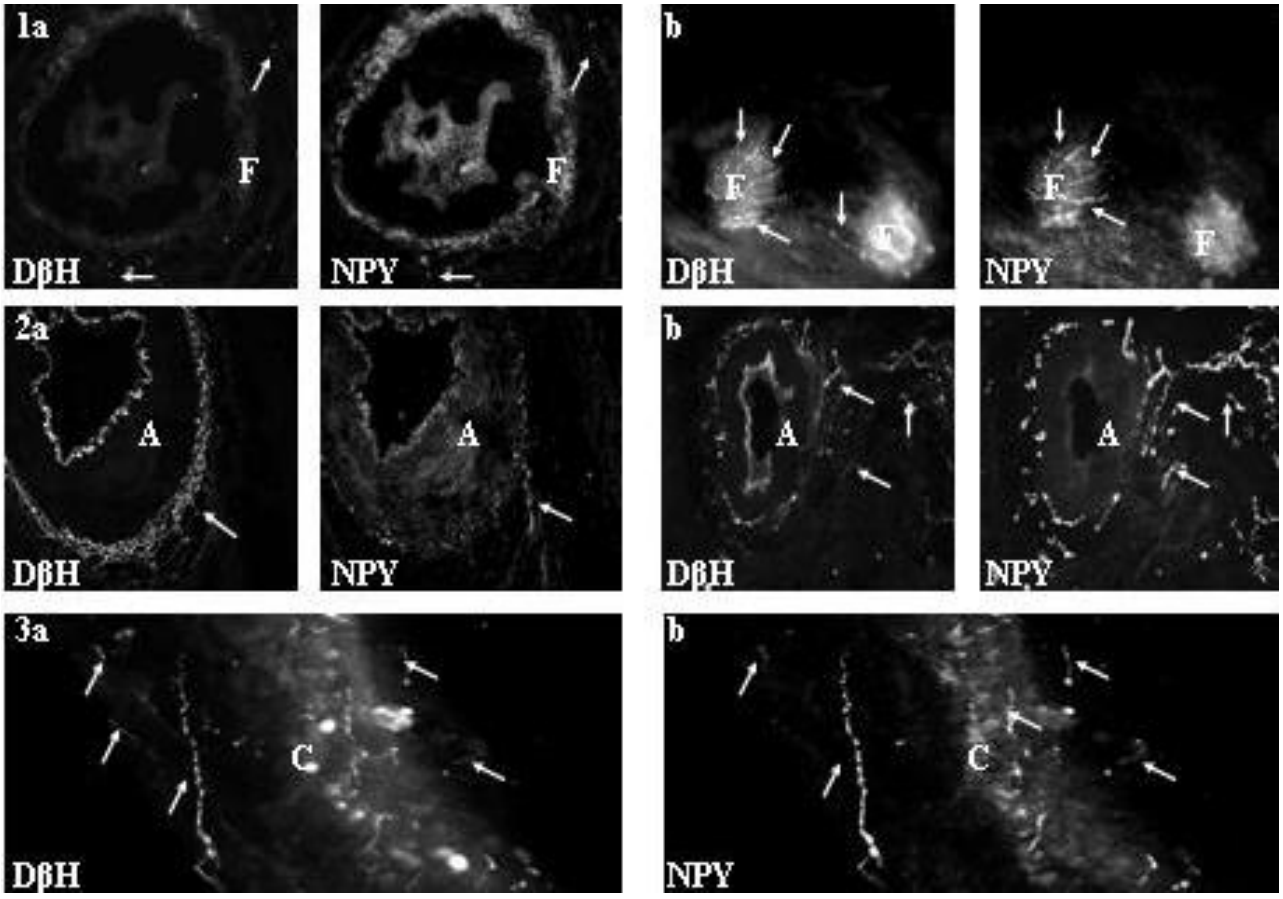
Material and Methods

We followed the principles of animal care (NIH publication No. 86-23, revised in 1985), as well as the specific national law on animal protection. The experiment was carried out on 12 crossbred gilts, aged 7-8 months, with controlled oestrous cycle. In the gilts of Group I (n=6), cysts were induced by i.m. injections of dexamethasone (DXM; Dexasone®, Norbrook Lab., Newry, UK, 3.3 µg/kg b.m., in a total volume of 6 ml), every 12 h, in the period from day 16th of the first studied oestrous cycle to day 9th of the second studied cycle. The control group (II, n=6), received similarly 6 ml of saline. The gilts were slaughtered on day 20th of the second cycle. The ovaries were dissected and the number of ovarian structures of interest (follicles, cysts) was counted. Cryostat ovarian sections were studied by means of a routine double-immunofluorescence technique, used to visualize the distribution of dopamine-ß-hydroxylase (DßH) and neuropeptide Y (NPY). The concentrations of progesterone (P4), androstendione (A4) and estradiol-17ß (E2) in follicular and cystic fluid, as well as in the wall was estimated by radioimmunoassay. The content of noradrenaline (NE), dopamine (DA) and adrenaline (A) was estimated in ovarian tissue and in the follicular or cystic fluid by high performance liquid chromatography. The expression of cytochrome $P450_{scc}$ and $P450_{arom}$ in follicular and cystic wall was determined by Western blot. Imm-

Figures 1 and 2. Single (a) DβH/NPY-IR nerve fibres (control animals) and numerous nerve terminals (b) in a DXM-treated pig, running in a close vicinity to follicles (Figure 1) and blood vessels (Figure 2). A - artery, F - follicle, (↑) - nerves, x 200.
Figure 3. Numerous DβH/NPY-IR nerve terminals in the vicinity of the cyst. C - cyst, (↑) - nerves, x 200.



munoblots were quantified by scanning on the Kodak 1D Image Analysis Software (USA). The mean (±SEM) number of ovarian structures, as well as the content of hormones and catecholamines, were calculated for the control and DXM-treated groups. The data were compared by one-way analysis of variance (ANOVA).

Results

DXM injections resulted in an increase ($p<0.001$) in the number of follicles with the diameter of 3-6 mm (7.4±1.6 vs. 2.33±0.84; respectively) and in a formation of follicular cysts with the diameter of 1-3 cm (1.8±0.5). In both groups, the control and DXM-treated, the number of follicles with the diameter of 1-3 mm were not significantly different (6.7±0.9 vs. 7.4±1.2; respectively). Neither the follicles, measuring 6-10 mm in diameter, nor the corpora lutea were found after the administration of DXM.

In the gilts, receiving DXM, DβH-IR and/or NPY-IR nerves, located in the vicinity of the follicles (Fig. 1), and blood vessels (Fig. 2) were more numerous, compared to respective values in the control animals. The most numerous nerve terminals were found near the cysts (Fig. 3). DXM injections lead to a decrease ($p<0.05$-0.001) in the concentrations of P4 and A4 in the fluid from follicles (3-6 mm in diameter; 159.2±5.5 vs. 359.2±4.7 ng/ml, 3.8±0.5 vs. 34.5±2.6 ng/ml; respectively), as well as A4 and E2 in the cystic fluid (1.7±0.08 vs. 11.5±1.8 ng/ml, 0.8±0.1 vs. 4.8±0.4 ng/ml; respectively), compared to the follicles, measuring 6-10 mm in diameter, studied in the control animals. The content of P4 in the cystic wall was higher ($p<0.001$) than that found in follicles (6-10 mm in diameter) of the control gilts (4223±164.2 vs. 1651±287.2 ng/g of tissue; respectively). In the DXM-treated animals, an increase in the content of A in cystic wall and NA in cystic fluid was found, as compared to the follicles (6-10 mm in diameter) of animals of the control group (2.18±0.02 vs. 1.3±0.009 ng/g of tissue, 8.3±0.5 vs. 1.4±0.02 ng/ml; respectively). In gilts with the DXM-induced polycystic status, the content of NA was higher in the follicular wall (of the follicles, measuring 3-6 mm in diameter), when compared to that in the control animals (11.73±1.2 vs. 1.9±1.9 ng/g of tissue; respectively). After DXM treatment, the content of cytochrome $P450_{scc}$ and $P450_{arom}$ protein was lowered by 22.3% and 41.5%, respectively, in the follicular wall, whereas it was elevated by 77.4% and 6.3%, respectively, in the cystic wall, when compared to the values, found in the follicles of the control gilts.

Discussion

In polycystic-changed ovaries, we observed an increased number of follicles with diameter of 3-6 mm, paralleled by the lack of larger follicles (6-10 mm in diameter) and corpora lutea. This is in agreement with earlier studies, revealing higher numbers of follicles with diameter >6 mm, observed in sows, treated with DXM [4]. Furthermore, corpora lutea were not found in the ovaries of such treated pigs, either [5]. In addition, estradiol valerate-induced polycystic ovary syndrome in rats resulted also in a distinct reduction in the number of both the antral follicles and corpora lutea [6]. The present study showed an increase in the density of DßH- and NPY-immunoreactive nerve fibres, especially in the vicinity of the follicles, blood vessels and cysts after DXM injections. This is in line with the increased number of adrenergic nerves in the cyst wall in women [2] and rats [3], suffering from polycystic changed ovaries. Moreover, this was paralleled by an elevated content of NA in the cystic fluid. An increase in the levels of NA in the cystic fluid, as well as of A in the cystic wall, correlated with an increase in the concentration of P4 in the cystic wall. It can be explained by the stimulatory effect of NA on the production of P4, observed previously in the porcine granulosa [7] and bovine luteal cells [8]. Moreover, A elevated FSH-stimulated P4 synthesis in the human of granulosa layer [9]. Thus, it is possible that an increase in P4 production in the cysts may be associated with an increase in the content of $P450_{scc}$. In contrast to cysts, the content of P4 in the fluid from follicles of 3-6 mm in diameter was lower after DXM injections, than that, found in the control group. It can be attributed to an early atresia of follicular cells [4] and, in turn, to the drop in the expression of $P450_{scc}$, observed in our study. The decrease in the contents of A4 and E2 in the cystic fluid, as well as A4 in the follicular fluid from follicles of 3-6 mm in size, as observed in the present study after DXM administration, is in agreement with earlier observations in rats [2] and pigs [10] with cystic ovarian disease. The marked reduction of the $P450_{arom}$ content, observed by us in the follicular wall after DXM injections, was previously described in granulosa cells of pigs, treated with ACTH [11].

In conclusion, our data show that in the polycystic-changed porcine ovaries, the number of adrenergic fibres, as well as the content of catecholamines increased. Thus, as it was also accompanied by distinct changes in the steroidogenic activity, this may be indicative of an important role of adrenergic nerves in the ovarian cyst etiopathogenesis.

References

1. Lara HE, Ferruz JL, Luza S, Bustamante DA, Borges Y, Ojeda SR. Activation of ovarian sympathetic nerves in polycystic ovary syndrome. Endocrinology. 1993; 133: 2690-5.

2. Nakamura Y. Treatment of polycystic ovary syndrome: an overview. Horm Res, 1990; 33: 31.

3. Paredes A, Galvez A, Leyton V, Aravena G, Fiedler JL, Bustamante D, Lara HE. Stress promotes development of ovarian cysts in rats: the possible role of sympathetic nerve activation. Endocrine, 1998; 8: 309-15.

4. Gee CM, Geissinger HD, Liptrap RM. Morphometric and steroid hormone changes associated with experimental anovulatory follicles in the sow. Can J Vet Res, 1991; 55: 206-11.

5. Frautschy SA, Liptrap RM. Anovulation and plasma hormone concentrations after administration of dexamethasone during the middle of the luteal phase in sows undergoing oestrous cycles. Am J Vet Res, 1988; 8: 1270-75.

6. Lara HE, Dissen AG, Leyton V, Paredes A, Fuenzalida H, Fiedler JL, Ojeda SR. An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. Endocrinology, 2000; 141: 1059-72.

7. Wiesak T, Przała J, Muszynska A, Hunter MG. Effect of catecholamines and FSH on progesterone secretion by pig granulosa cells. Endocrinol Exp, 1990; 24: 449-56.

8. Kotwica J, Bogacki M, Rękawiecki R. Neural regulation of the bovine corpus luteum. Domest Anim Endocrinol, 2002; 23: 299-308.

9. Papenfuss F, Bodis J, Tinneberg HR, Schwarz H. The modulatory effect of catecholamines on gonadotropin-stimulated granulosa cell steroid secretion. Arch Gynecol Obstet, 1995; 253: 97-102.

10. Babalola GO, Shapiro BH. Sex steroid changes in porcine cystic ovarian disease. Steroids, 1990; 55: 319-24.

11. Ryan PL, Raeside JI. Steroidogenesis in granulosa cells after the induction of large cystic follicles with adrenocorticotrophin (ACTH) in the cyclic gilt. In: Proceedings of the Eleventh International Congress on Animal Reproduction and Artificial Insemination. Dublin, Ireland; 1988, p. 458.