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Endogenous nandrolone production: Studies in granulosa cells from patients with polycystic ovary syndrome (PCOS)

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Introduction

Human in vivo production of nandrolone (17 β -hydroxy-4-estren-3-one) has been reported in the ovarian follicle at the time of ovulation and during pregnancy [1-4]. Endogenous nandrolone appears to be a by-product of the enzymatic conversion of androgens to estrogens [5]. Patients with the diagnosis polycystic ovary syndrome (PCOS) have higher levels of androgens than normally found in women [6]. The objective of this study was to find out if women with PCOS have an increased production of nandrolone compared to women who do not suffer from this syndrome. Granulosa-lutein cells obtained from women with PCOS were cultured. Nandrolone production was measured in cell culture media with and without stimulation with testosterone. The results were compared to a control group of cell cultures from women without the diagnosis PCOS.

Experimental

Granulosa-lutein cells have a high amount of aromatase and are thus a well suited medium for studies of the aromatase system. Granulosa-lutein cells were obtained from women undergoing treatment for infertility. Follicle fluid was collected during oocyte aspiration and granulosa-lutein cells were isolated. Viability was tested to ensure in-vitro survival of the cells. The cells were cultured in Dulbeccos Modified Eagle Medium (DMEM) as described in [7]. Parallel cultures were incubated with or without additions of 4000 nmol/l testosterone at 37 °C in a humidified atmosphere of 5 % CO₂ in air.

After liquid-liquid extraction with *tert*-butyl-methyl ether at pH=8 and derivatisation with MSTFA:NH₄I:ethanethiol, the culture medium was analysed by GC-HRMS. The concen-

tration of nandrolone, norandrostendione, androstendione, testosterone, estradiol, estrone and progesterone was determined down to a limit of detection of about 0,1 ng/ml (0,35 nmol/l).

Results

The results are presented in Figure 1. Endogenous nandrolone production was observed when the cell cultures were not stimulated with testosterone. The highest concentration of nandrolone was 0.2 ng/ml. Nandrolone production appeared in 9 out of 20 cell cultures from PCOS patients. The mean concentration was 0.13 ng/ml (± 0.05 ng/ml). In the control group, endogenous nandrolone production was observed in 4 out of 18 cell cultures. The mean concentration was 0.10 ng/ml (± 0.06 ng/ml). Cell cultures from PCOS patients seem to have a higher endogenous nandrolone production, but the difference is not significant ($p < 0.05$, Students t-test).

Stimulation of the cell cultures with 4000 nmol/l testosterone resulted in a considerable increase in the production of nandrolone, both in cell cultures from PCOS patients and from the control group. No significant difference in the synthesis rate of nandrolone between cell cultures from PCOS patients and cell cultures from the control group can be observed ($p < 0.05$, Students t-test).

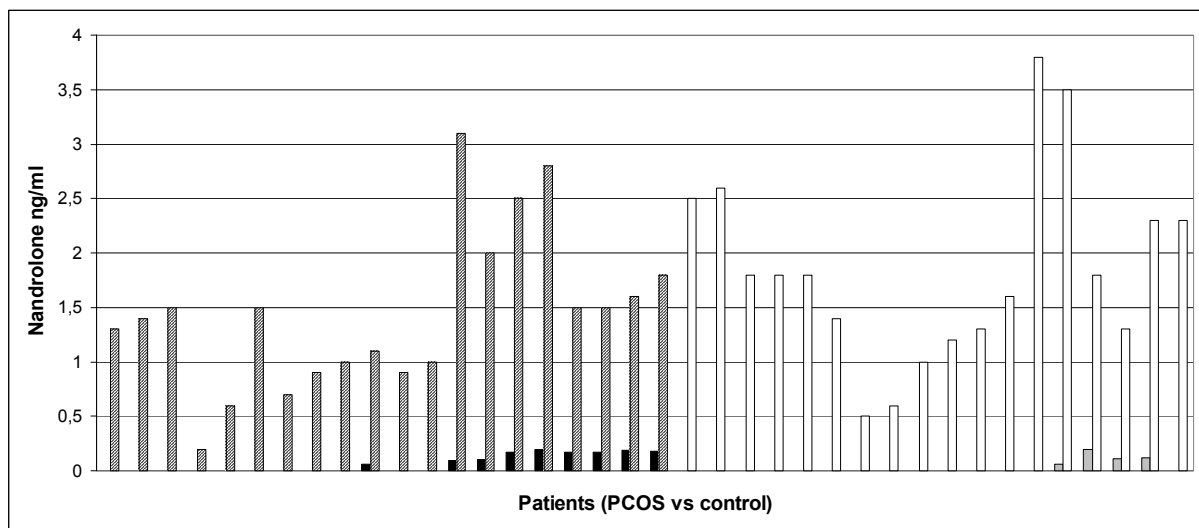


Figure 1. Nandrolone production in granulosa-lutein cell cultures obtained from PCOS / control patients.

- PCOS without stimulation
- ▨ PCOS stimulated with 4000 nmol/l testosterone
- Control without stimulation
- Control stimulated with 4000 nmol/l testosterone

Discussion

Even though PCOS is associated with high androgen levels, the level of estradiol remains low, which could be a result of suppressed aromatase activity due to inhibitors of aromatase activity in follicular fluid in PCOS patients [8]. Theca cells from PCOS-women have increased basal androgen synthesis, these cells were however neither isolated nor cultured in this work. Androgen levels will therefore be similar in all cell cultures. This may perhaps explain why there is no significant difference in the production of nandrolone in cell cultures from PCOS patients compared to the control group. We may also consider the limitations with cell culture systems because of their non-physiological nature where the spatial organisation of ovarian cells, cell-to-cell contact and microcirculation are not present, and the heterogeneity of cell population is often not accounted for [9].

Conclusion

Endogenous nandrolone production was observed, but the synthesis of nandrolone occurred mainly when the cells were exposed to testosterone. This confirms that norsteroids are synthesised as a by-product of the aromatisation of androgens to estrogens. No difference in the rate of synthesis of nandrolone between the PCOS-patients and the control group could be observed.

Acknowledgement

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