CASE REPORT

Testosterone-secreting gonadotropin-responsive adrenal adenoma and its treatment with the antiandrogen flutamide

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ABSTRACT. A 55-year-old woman with virilization had an appreciably elevated testosterone level, which was not suppressed by dexamethasone, but was increased by stimulation with human chorionic gonadotropin (hCG). Ultrasonography and computed tomography revealed an adenoma 2.5-3.0 cm in diameter in the right adrenal gland. The patient was treated with the antiandrogen flutamide in a daily dose of 500 mg for 4 months. A substantial regression of her hirsutism was observed during flutamide administration, but the serum testosterone level remained high. Right adrenalectomy was performed. Histologically, the tumor proved to be an adrenocortical adenoma of *zona reticularis* type. The adenoma tissue contained specific hCG receptors (187 fmol/g). The steroid concentration in the tumor tissue was examined by means of high pressure liquid chromatography-radioimmunoassay (HPLC-RIA). A significantly increased testosterone content was detected, and the levels of its precursors, androstenedione and dehydroepiandrosterone, were also elevated. Following adrenalectomy, serum testosterone concentration decreased to the normal level. The mechanism of the inappropriate regulation in the testosterone production of the adrenal tumor has not been fully elucidated.

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INTRODUCTION

A testosterone-secreting adrenocortical adenoma with pure virilization is a rare disease in the adult female (1, 2). The tumor tissue, which synthesizes sexual steroid hormones, is generally independent of trophic hormone control from a functional aspect (3, 4). This feature of the autonomous adrenal adenomas is extremely useful in the clinical differentiation of virilizing disorders caused by tumors and adrenocortical hyperplasias. In a few cases, however, these tumors have responded to human chorionic gonadotropin (hCG) by increasing testosterone production (5-11). The functional basis for the unusual character of the testosterone-secreting gonadotropin-responsive adrenocortical adenoma is conjectural. The exact mechanism of the gonadotropin sensitivity of the adrenal adenomas has not yet been explained.

Key-words: Adrenal adenoma, testosterone secretion, hCG-dependency, flutamide treatment, hCG-binding.

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We report here a post-menopausal woman virilized by an adrenocortical adenoma with hCG-responsive testosterone secretion, in whom we studied the hormonal contents and hCG binding capacity of the tumor and the effect of the antiandrogen flutamide on the androgen steroid production.

MATERIALS AND METHODS

Case report

A 55-year-old woman was referred to the Kiskunfélegyháza hospital in May 1996 because of virilization. At the age of 22 and 25, she had given birth to a daughter and a son, respectively, without complications. Her menstrual cycles had been regular until the age of 40 years. Fifteen years before her admission, her uterus was removed due to an extensive myomatous disorder of the uterus; the ovaries remained intact. During the last 2 years, she had gradually developed extensive hirsutism and a deepening of her voice. Hypertension had been present for 7 years, for which she received β-blockers, captopril and diuretics.

Gynecological examination revealed clitoromegaly, transabdominal hysterectomy and slightly atroph-

ic ovaries. The patient was moderately obese; her blood pressure was 140/90 mmHg (during antihypertensive treatment). The voice was moderately deep and the hair growth, especially on the face, was pronounced, as assessed on the basis of the description by Ferriman *et al.* (12). Routine laboratory studies, including urine protein and sugar, blood analysis, glucose tolerance, liver and kidney functions, serum lipids and electrolytes, gave normal results.

Hormone assays

Serum gonadotropins were assayed with commercially available RIA kits. Serum steroids were determined by means of specific RIA developed in our laboratories (13, 14) and performed on the basis of World Health Organization principles (15).

Dexamethasone suppression and hCG stimulation tests

Dexamethasone suppression testing was carried out by the oral administration of 0.5 mg oradexon (Organon, Oss, The Netherlands) every 6 h for 12 days, followed by 2.0 mg oradexon every 6 h for 3 days, while hCG stimulation testing was performed by means of intramuscular injections of choriogonin (Richter, Budapest, Hungary) in daily doses of 3000 IU for 3 days. Blood samples were obtained the next morning.

Antiandrogen treatment with flutamide

Because of the virilization and high serum testosterone level, we treated the patient with 2 x 250 mg flutamide (Fugerel, Schering-Plough, Kenilworth, USA) daily for 4 months. The liver function was controlled during and after the flutamide treatment; the results of functional tests remained normal.

Imaging

Ultrasonography and computed tomography revealed a right adrenal tumor, estimated to measure 2.6 x 2.5 x 3.0 cm.

Surgery and histology

In view of the positive imaging finding, surgical intervention was performed. The right adrenal gland was found to be in contact with an ovoid, encapsulated yellow mass, which was removed together with the adjacent uninvolved, but small right adrenal gland. There were no enlarged lymph nodes in the surrounding tissues. A small part of the tumor was examined histologically, and proved to consist of acidophilic cells with scant cytoplasmic pigment. No histologic signs of malignancy were seen. The histology suggested an adrenocortical origin for the tumor, mainly resembling zona reticularis cells. The major part of the adenoma was used for determination of the steroid content and hCG receptors of the tumor tissue.

Measurement of adrenal hCG receptors

The hCG binding capacity of the adrenal tumor was measured as reported earlier (16). Briefly summarized, the method is as follows. The tumor tissue was homogenized in Dulbecco's phosphate-buffered saline (pH 7.4) containing 0.1% bovine serum albumin (PBS-BSA). After centrifugation for 10 min at 600 g, the supernatant was sedimented for 30 min at 20,000 g. The membrane preparation was resuspended in PBS-BSA solution and was used for receptor binding determination. [^{125}I] hCG (New England Nuclear, Boston, USA) in increasing concentrations was added to the membrane suspensions and the mixtures were incubated overnight at 23 C. The bound and free hormone fractions were separated by centrifugation after 15-fold dilution of the samples with ice-cold PBS-BSA. Subsequently the bound radioactivity was measured. Non-specific binding was determined in the presence of an excess (50 IU/tube) of unlabelled hCG. The specific hCG binding was measured by Scatchard method (17). Following linear regression analysis, the apparent association constant (K_a) and binding capacity were determined.

Determination of tissue steroids

Tissue steroid concentrations were determined by high-pressure liquid chromatography (HPLC)-RIA methods described previously (18, 19) with some modifications. In brief, the steroid hormones from the tumor sample were extracted with ethyl acetate, using ultrasonic homogenization. The solvent was then evaporated off under nitrogen, and the extract was purified on a 500 mg C18 column (Supelclean LC-18, Supelco, Bellefonte, USA) with methanol-water as eluent. Steroids were isolated by reversed-phase HPLC on a 250x4.0 mm C18 column (Eurospher 100-C18-7; Knauer, Berlin, Germany) with 55 v/v% methanol-water as eluent. Fractions were collected and the eluted steroids were detected via the radioactivity of tritiated steroids added as internal standards to the tissue samples before the extraction procedure. Fractions of isolated steroids were dried and dissolved in the RIA buffer and the assays were performed as above. The steroid contents of the tissue sample were calculated with consideration of the recovery of the labeled internal standards and the methodological background (blank). Results are given in µg/g wet weight tissue.

RESULTS

Following flutamide treatment, hirsutism regressed: the virile hair growth on the trunk area disappeared, but the facial hair growth persisted. Four months after the tumor removal, the hair growth had normalized; the total hirsutism scores (12) had decreased from 16 to 6.

The serum hormone concentrations before the operation are listed in Table 1. The testosterone level and the ratio testosterone/SHBG were high. The FSH and LH concentrations were also increased because of the post-menopausal state. The other hormone values were in the normal ranges. During the antiandrogen treatment with flutamide, the serum testosterone level remained high. Four months after the surgical intervention, the testosterone level and the ratio testosterone/SHBG had normalized. Before the operation, dexamethasone suppression testing and hCG testing were applied (Table 2). Following dexamethasone administration (2 or 8 mg/day), the cortisol level decreased by about one-third. The testosterone concentration remained elevated, while the DHEA and DHEAS levels were unchanged. After 3 days of hCG treatment, the testosterone level was significantly increased.

Specific hCG binding capacity was detected in the membrane fraction of the adrenal tumor. Linear regression analysis indicated an equilibrium association constant (K_a) of $1.28x10^{-10}$ M^{-1} and a specific hCG receptor binding capacity of 187 fmol/g of original adrenal tumor tissue.

The steroid concentrations in the tumor tissue are reported in Table 3. After HPLC isolation, the cortisol concentration was much less than in the normal adrenal tissue and a very high testosterone level was detected, but the DHEA and androstenedione contents of the adenomous tissue were also high. Unfortunately, the mass of normal adrenal tissue removed was not sufficient for our HPLC-RIA determination. Thus, we can not directly compare the steroid concentrations of the normal and tumorous adrenal tissues. In this respect, we had to take the normal steroid values for intact adrenal tissue from the relevant literature (11).

DISCUSSION

A 55-year-old post-menopausal woman with virilization had a markedly increased testosterone level; this was not suppressed by dexamethasone, but

Table 1 - *Serum hormone concentrations before surgery, following flutamide treatment and after surgery.*

Steroids (nmol/l)	Before operation		After operation	Normal range
	Basal	Following flutamide treatment		
Cortisol	371.0	467.0	286.0	150.0-550.0
Estradiol	0.38	0.32	0.26	0.25-0.40
Testosterone	7.6	8.0	1.2	0.9-2.8
DHEA	10.01	10.4	5.6	7.9-24.8
DHEAS	220.0	370.0	120.0	220.0-650.0
Progesterone	0.7	1.2	2.0	0.6-3.2
17α-hydroxyprogesterone	3.6	3.2	2.9	1.2-4.2
Androstenedione	8.6	10.03	3.9	1.9-8.9
Androstenediol	6.0	7.1	0.4	2.4-8.3
Others				
FSH (IU/l)	75.5	76.2	67.8	30.0-96.0*
LH (IU/l)	45.2	49.4	51.3	28.0-100.0*
PRL (IU/l)	0.24	0.27	0.31	0.04-0.47
SHBG (nmol/l)	42.3	50.0	36.2	25.0-65.0
Testosterone/SHBG ($x10^{-2}$)	17.9	16.0	3.3	2.0-7.0
CBG (nmol/l)	264.0	-	258.0	250.0-450.0
Cortisol/CBG ($x10^{-2}$)	140.0	-	111.0	60.0-122.0

*Post-menopausal level.

Table 2 - *Serum steroid concentrations (nmol/l) following dexamethasone suppression and human chorionic gonadotropin (hCG) administration.*

Reference range	Cortisol 150-550	Testosterone 0.9-2.8	DHEA 7.9-24.8	DHEAS 220-650
Basal	357	7.1	10.6	210
Dexamethasone 2 mg	242	6.5	11.7	221
Dexamethasone 8 mg	233	6.2	10.5	218
hCG	348	25.4	16.2	273

was further elevated by stimulation with hCG. Ultrasonography and computed tomography revealed an adenoma in the right adrenal gland, and right adrenalectomy was performed. Histologically, the tumor proved to be an adrenocortical adenoma of zona reticularis type. The steroid concentrations in the tumor tissue were measured by means of HPLC-RIA. A smaller cortisol concentration was detected and a significantly increased testosterone content was found, and the concentrations of its precursors, androstenedione and dehydroepiandrosterone, were also elevated. These latter two results and the normalization of the serum testosterone level following surgical intervention clearly prove the origin of the virilization, which was caused by the autonomous adrenocortical adenoma. Before the adrenalectomy, we treated the patient with the non-steroid antiandrogen flutamide: a substantial regression of her virilization was observed during the 4-month flutamide treatment, but the serum testosterone level remained increased.

Unusual features of this case were the significantly increased serum testosterone level induced by the administration of hCG and the demonstration of the presence of ectopic hCG receptors in the adrenal tumor tissue. Many years ago Reifenstein *et al.* (20) postulated that adrenal androgen secretion was influenced by gonadotropin treatment. In stark contrast, it was later described that normal healthy women did not exhibit increases in serum testosterone concentrations following gonadotropin administration (21, 22), but an elevation may develop in women with Stein-Leventhal syndrome or idiopathic hirsutism (22). Increased 17-ketosteroid excretion was reported in oophorectomized women after hCG administration (23-25). Lloyd *et al.* (26), however, found no increase in plasma testosterone concentrations following hCG injections in two oophorectomized women. In connection with the hCG responsiveness of testosterone-secreting adrenal adenoma, many contradictory data are to be found in the literature on this subject. Increased serum testosterone levels have been reported in patients with virilizing adrenal adenoma after hCG treatment (2, 7, 9, 10). On the other hand, this stimulation test has also yielded negative results in patients with testosterone-secreting adrenal adenoma (1, 27).

Table 3 - *Steroid concentrations in virilizing adrenal tumor tissue.*

Steroids	Tumorous adrenal tissue µg/g wet weight	Normal adrenal tissue*
Cortisol	2.37	17.10
Testosterone	2.69	0.16
Dehydroepiandrosterone	0.58	0.27
Progesterone	0.17	-
Androstenedione	1.00	0.14
Androstenediol	1.31	-
Dihydrotestosterone	0.49	0.36

*Werk, *et al.* (1973)

It is very difficult to explain the mechanism of the inappropriate response to hCG and the aberrant regulation in the testosterone production of the adrenal tumor. First, it should be mentioned that the adrenal gland and ovary share a common anlage (28). Ovarian thecal metaplasia has been reported within the adrenals of postmenopausal women (29), while a metaplasia of embryologically competent mesenchymal cells is subject to extended stimulation by increased levels of gonadotropin (30). On the other hand, in accord with our observation, Leinonen *et al.* (2) revealed the presence of hCG receptors in the tissue of testosterone-secreting virilizing adrenal adenoma. The characteristics of the hCG-binding sites were similar to those present in the testosterone-secreting ovarian androblastoma examined by the same method (31) and to those in normal human ovaries (32). The presence of ectopic hCG receptors was suggested by the tissue culture experiments reported by Leinonen *et al.* (2). Steroid hormone secretion was studied in primary tissue cultures of the virilizing adrenal tumor and

the normal adjacent cortex, and the effects of ACTH and hCG on testosterone production were compared. ACTH increased the testosterone secretion by the normal adrenocortical cells, but hCG had no effect on it. In the adenoma tissue culture, however, both ACTH and hCG stimulations maintained the testosterone production at the previous level; without such stimulation, the testosterone concentration in the tissue culture medium decreased. These data can be compared with the findings of Schorr and Ney (33), who observed inappropriate trophic hormone stimulation of adenyl cyclase in experimental tumors and human endocrine tumors *in vitro*. The unusual hCG stimulation does not refer only to the adrenal sexual steroidogenesis. LH-dependent cortisol or corticosterone production was recently reported in a case with ACTH-independent Cushing's syndrome (34) and likewise in transgenic mice (35).

In our case, the LH level was high because of the post-menopausal state, which may have a positive influence on the testosterone level, as a permanent gonadotropin stimulus. This finding is in accordance with recent experimental data showing that inappropriate or prolonged elevation of gonadotropins can induce LH receptors in the adrenal gland (36, 37). It was reported that gonadectomy or the direct action of LH can trigger adrenocortical tumorigenesis in mice transgenic for the mouse inhibin α-subunit promoter/Simian virus 40 T-antigen fusion gene. A permanent high LH level in itself, however, is not sufficient to induce a testosterone-secreting LH-dependent adrenal tumor. Other factors may be needed for the development of such a tumor with a special hormonal activity. Further observations will be required to clarify this unusual and interesting hormonal regulatory phenomenon.

REFERENCES

1. Gabrilove J.L., Seman A.T., Sabet R., Mitty H.A., Nicolis G.L.
   Virilizing adrenal adenoma with studies on the steroid content of the adrenal venous effluent and a review of the literature.
   Endocr. Rev. 1981, *2:* 462-470.
2. Leinonen P., Ranta T., Siegberg R., Pelkonen R., Heikkila P., Kahri A.
   Testosterone-secreting virilizing adrenal adenoma with human chorionic gonadotrophin receptors and 21-hydroxylase deficiency.
   Clin. Endocrinol. (Oxf.) 1991, *34:* 31-35.
3. Bertagna C., Orth D.N.
   Clinical and laboratory findings and results of therapy in 58 patients with adrenocortical tumors admitted to a single medical center (1951 to 1978).
   Am. J. Med. 1981, *71:* 855-875.
4. Freeman D.A.
   Steroid hormone-producing tumors in man.
   Endocr. Rev.1986, *7:* 204-220.
5. Blichert-Toft M., Vejlsted H., Hehlet H., Albrechtsen R.
   Virilizing adrenocortical adenoma responsive to gonadotrophin.
   Acta Endocrinol. 1975, *78:* 77-85.
6. de Lange W.E., Pratt J.J., Doorenbos H.
   A gonadotrophin responsive testosterone producing adrenocortical adenoma and high gonadotrophin levels in an elderly woman.
   Clin. Endocrinol. (Oxf.) 1980, *12:* 21-28.
7. Givens J.R., Andersen R.N., Wiser W.L., Coleman S.A., Fish S.A.
   A gonadotropin-responsive adrenocortical adenoma.
   J. Clin. Endocrinol. Metab.1974, *38:* 126-133.
8. Larson B.A., Vanderlaan W.P., Judd H.L., McCullough D.L.
   A testosterone-producing adrenal cortical adenoma in an elderly woman.
   J. Clin. Endocrinol. Metab. 1976, *42:* 882-887.
9. Smith H.C., Posen S., Clifton-Bligh P., Casey J.
   A testosterone-secreting adrenal cortical adenoma.
   Aust. NZ J. Med.1978, *8:* 171-175.
10. Takahashi H., Yoshizaki K., Kato H., Masuda T., Matsuka G., Mimura T., Inui Y., Takeuchi S., Adachi H., Matsumoto K.
    A gonadotrophin-responsive virilizing adrenal tumour identified as a mixed ganglioneuroma and adreno-cortical adenoma.
    Acta Endocrinol. 1978, *89:* 701-709.
11. Werk E.E.J., Sholiton L.E., Kalejs L.
    Testosterone-secreting adrenal adenoma under gonadotropin control.
    N. Engl. J. Med. 1973, *289:* 767-770.
12. Ferriman D., Gallway J.D.
    Clinical assessment of body hair growth in women.
    J. Clin. Endocrinol. Metab. 1961, *24:* 1440-1447.
13. Faredin I., Tóth I., Janáky T., Tekeres A., Faluhelyi S., Szabó I., Antal T., László F.
    A direct radioimmunoassay of progesterone in bovine serum and milk.
    Acta Vet. Hung. 1987, *35:* 373-382.
14. Julesz J., Tóth I., Faredin I., Szarvas F.
    Human serum testosterone and sex hormone binding globulin levels as a function of age and in various diseases. Advances in steroid analysis '93.
    Akadémiai Kiadó, Budapest, 1994, p. 445.
15. Sufi S., Donaldson A., Jeffcoate S.L.
    WHO matched reagent programme method manual. 1999.
16. Huhtaniemi I.T., Catt K.J.
    Effects of gonadotropin-releasing hormone receptor blockade on rat testicular gonadotropin and lactogen receptors, steroidogenesis, and responses to human chorionic gonadotropin stimulation.
    Endocrinology 1985, *116:* 281-287.

17. Scatchard G.
The attraction of proteins for small molecules and ions.
Ann. NY Acad. Sci. 1949, *51:* 660-672.

18. Szécsi M., Tóth I., Rengei B., Scultéty S., Faredin I.
Androgenic steroids in normal and hyperplastic tissue samples from human prostates. Advances in steroid analysis '93.
Akadémiai Kiadó, Budapest, 1994, p. 469.

19. Szécsi M., Tóth I.,Faredin I.
The levels of androgens in rat prostate and serum after castration.
Endocrine Regulation (Endocrinologica Experimentalis) 1995, *29:* 107-113.

20. Reifenstein E.C., Forbes A.P., Albright F.
Effect of methyl testosterone on urinary 17-ketosteroids of adrenal origin.
J. Clin. Invest. 1945, *24:* 416-434.

21. Ettinger B., Von Werder K., Thenaers G.C., Forsham P.H.
Plasma testosterone stimulation-suppression dynamics in hirsute women.
Am. J. Med. 1971, *51:* 170-175.

22. Korenman S.G., Kirschner M.A., Lipsett M.B.
Testosterone production in normal and virilized women and in women with Stein-Leventhal syndrome or idiopathic hirsutism.
J. Clin. Endocrinol. Metab. 1965, *25:* 798-803.

23. Pauerstein C.J., Solomon D.
LH and adrenal androgenesis.
Obstet. Gynecol. 1966, *28:* 692-699.

24. Perloff W.H., Jacobson G.
Effect of human chorionic gonadotropin (HCG) on urinary 17-ketosteroids in a patient with Stein-Leventhal syndrome before and after oophorectomy.
J. Clin. Endocrinol. Metab. 1963, *23:* 1177-1178.

25. Plate W.P.
The stimulating effect of chorionic gonadotrophin on the adrenal cortex.
Acta Endocrinol. 1952, *11:* 119-126.

26. Lloyd C.W., Lobotsky J., Segre E.J., Kobayashi T., Taymor M.L., Batt R.E.
Plasma testosterone and urinary 17-ketosteroids in women with hirsutism and polycystic ovaries.
J. Clin. Endocrinol. Metab. 1966, *26:* 314-324.

27. Schteingart D.E., Woodbury M.C., Tsao H.S., McKenzie A.K.
Virilizing syndrome associated with an adrenal cortical adenoma secreting predominantly testosterone.
Am. J. Med. 1979, *67:* 140-146.

28. Goldzieher J.W.
The interplay of adrenocortical and ovarian function.
In: Mack H.C. (Ed.), The ovary.
Charles C. Thomas, Springfield, 1968, p. 106.

29. Wont T.W., Warner N.E.
Ovarian thecal metaplasia in the adrenal gland.
Arch. Pathol. 1971, *92:* 319-328.

30. Rosenfield R.L., Eberlein W.R., Bongiovanni A.M.
Measurement of plasma testosterone by means of competitive protein binding analysis.
J. Clin. Endocrinol. Metab.1969, *29:* 854-859.

31. Siegberg R., Rantala M.L. , Stenman U.H., Wahlstrom T., Ylikorkala O., Huhtaniemi I., Ranta T.
Characterization of gonadotrophin receptors in a testosterone-producing ovarian androblastoma. Case report.
Br. J. Obstet. Gynaecol. 1986, *93:* 1104-1107.

32. Rajaniemi H.J., Ronnberg L., Kauppila A., Ylostalo P., Jalkanen M., Saastamoinen J., Selander K., Pystynen P., Vihko R.
Luteinizing hormone receptors in human ovarian follicles and corpora lutea during menstrual cycle and pregnancy.
J. Clin. Endocrinol. Metab. 1981, *52:* 307-313.

33. Schorr I., Ney R.L.
Abnormal hormone responses of an adrenocortical cancer adenyl cyclase.
J. Clin. Invest. 1971, *50:* 1295-1300.

34. Lacroix A., Hamet P., Boutin J.M.
Leuprolide acetate therapy in luteinizing hormone-dependent Cushing's syndrome.
N. Engl. J. Med. 1999, *341:* 1577-1581.

35. Kero J., Poutanen M., Zhang F.P., Rahman N., McNicol A. M., Nilson J.H., Keri R. A., Huhtaniemi I.T.
Elevated luteinizing hormone induces expression of its receptor and promotes steroidogenesis in the adrenal cortex.
J. Clin. Invest. 2000, *105:* 633-641.

36. Kananen K., Markkula M., Mikola M., Rainio E.M., McNeilly A., Huhtaniemi I.T.
Gonadectomy permits adrenocortical tumorigenesis in mice transgenic for the mouse inhibin alpha-subunit promoter/simian virus 40 T-antigen fusion gene: evidence for negative autoregulation of the inhibin alpha- subunit gene.
Mol. Endocrinol. 1996, *10:* 1667-1677.

37. Rilianawati, Paukku T., Kero J., Zhang F.P., Rahman N., Kananen K., Huhtaniemi I.
Direct luteinizing hormone action triggers adrenocortical tumorigenesis in castrated mice transgenic for the murine inhibin alpha-subunit promoter/simian virus 40 T-antigen fusion gene.
Mol. Endocrinol. 1998, *12:* 801-809.