THE INFLUENCE OF HORMONAL CONTRACEPTIVES ON SEX HORMONE BINDING GLOBULIN (SHBG) CAPACITY

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ABSTRACT

The sex hormone binding globulin (SHBG) capacity was measured by ammonium sulfate precipitation in the plasma of women using various progestational steroids and combined hormonal contraceptives. For reference purposes, determinations were done in the plasma of normal males, normal menstruating women, women at different stages of pregnancy, post-menopausal women, and women using ethinylestradiol (EE_2) 0.050 mg daily.

The progestational compounds, d-norgestrel (0.030 mg daily), lynestrenol (0.5 mg daily), and medroxyprogesterone acetate (150 mg three-monthly intramuscularly), caused a decrease of about 30% below the level found in normal menstruating women. With lynestrenol 5 mg daily, a further decrease of SHBG was observed.

EE_2 (0.050 mg daily) alone increased SHBG. This effect of EE_2 was (partly) neutralized by its combination with d-norgestrel or lynestrenol. The combination of EE_2 with megestrol acetate gave rise to high SHBG capacity values, comparable to those attained during pregnancy.

The response of SHBG to the use of hormonal contraceptives is different from that of transcortin-binding capacity as found by other authors.

Accepted for publication November 7, 1974

CONTRACEPTION

INTRODUCTION

The plasma level of steroid hormone binding protein is (partly) dependent on the androgen/estrogen balance (1). Schwartz and Hammerstein (2) recently reported on the transcortin-binding capacity (TC-BC) during the use of hormonal contraceptives. Little is known about the influence of these agents on SHBG capacity (3, 4, 5).

In this study the SHBG capacity was measured in the plasma of women using hormonal contraceptives for at least two months. The values obtained were compared with those of women with normal menstrual cycles. SHBG capacity was also measured in the plasma of women at various stages of pregnancy, in that of post-menopausal women, and in plasma of normal healthy males.

MATERIALS AND METHODS

The SHBG capacity was measured with ammonium sulfate precipitation after a preliminary comparison of this technique with equilibrium dialysis. Both techniques were performed with 1-2-^{3}H-dihydrotestosterone (DHT) as ligand (47 Ci/mM, TRK 295, batch 4, Radio Chemical Centre Amersham, Great Britain).

The ammonium sulfate precipitation technique was done as described by Rosner (6): 40,000 dpm of 1-2-^{3}H-DHT with a specific activity of 18,000 dpm/ng for normal females, and 4,000 dpm/ng for pregnant females, were added to 0.5 ml of 1:20 saline diluted plasma samples; 24,000 dpm/ng were added to 0.5 ml of 1:15 diluted plasma samples of normal males. After shaking on a vortex mixer, the tubes were kept for 15 minutes at room temperature followed by 15 minutes at 0°C. Then 0.5 ml of ice cold saturated ammonium sulfate was added to every tube during mixing on the vortex mixer. After spinning (3,000 rpm) during 10 minutes, 0.5 ml of the supernatant was counted in a liquid scintillation counter. All plasma samples were assayed in triplicate. In each sample the radioactivity added was determined according to Heyns and De Moor (7).

Equilibrium dialysis was performed in Visking tubing with plasma diluted 1:5 with saline against saline containing albumin in a concentration identical to that of the diluted plasma (3).

The values of the binding capacity are expressed in 10^{-9}M/L or nM/L.

Plasma samples were drawn from women (18-45 years) with normal menstrual cycles, who used no hormonal contraceptives; from women in the 12-14th, 24-26th, or 38-40th week of pregnancy, from post-menopausal women (age 51-62 years) and from normal healthy males (age 19-50 years). Plasma samples were collected between 1:00 and 3:00 P.M. and were stored at -20°C.

Hormonal preparations used in this study are listed below:
150 mg medroxyprogesterone acetate (MPA)(depo-Provera^{R}, Upjohn),
0.030 mg d-norgestrel (WL 17^{R}, Wyeth),
0.5 mg lynestrenol (Exluton^{R}, Organon),
5.0 mg lynestrenol (Orgametril^{R}, Organon),

0.050 mg ethinylestradiol (EE_2)(Lynoral®, Organon),
0.050 mg EE_2 + 0.125 mg d-norgestrel (71144®, Schering),
0.050 mg EE_2 + 0.250 mg d-norgestrel (Stediril-d®, Wyeth or Neogynon®, Schering),
0.050 mg EE_2 + 0.500 mg dl-norgestrel (Stediril®, Wyeth or Eugynon®, Schering),
0.050 mg EE_2 + 1.0 mg lynestrenol (Pregnon®, Organon),
0.050 mg EE_2 + 2.5 mg lynestrenol (Lyndiol®, Organon),
0.050 mg EE_2 + 4.0 mg megestrol acetate (Planovin®, Novo).

MPA was injected intramuscularly every three months. Progestational compounds were given continuously. Combined preparations were given cyclically according to the instructions by the manufacturers.

The Wilcoxon test was used for statistical analysis.

RESULTS

Table I demonstrates the good correlation (r= +0.93) between the data obtained by ammonium sulfate precipitation and those obtained by equilibrium dialysis. This is in agreement with the data of others (7).

Table I. Correlation between SHBG capacity measured with ammonium sulfate precipitation (A) and with equilibrium dialysis (B).

	A	B
normal men (n=9)	28 ± 3 nM/L	26 ± 3 nM/L
normal women (n=10)	59 ± 8 nM/L	53 ± 12 nM/L
women on oral contraceptives (n=15)	138 ± 20 nM/L	126 ± 20 nM/L
coefficient of variation	8.6%	11.1%
correlation coefficient: r= +0.93		

Figure 1 shows the values of SHBG capacity found in normal men, normal menstruating women, post-menopausal women, and women at different stages of pregnancy. The results are similar to those found by other authors (2, 6, 7, 8).

Progestational steroids in dosages as used for contraceptive purposes depressed the SHBG capacity of plasma by approximately 30% (Figure 2). With lynestrenol 5 mg daily, there was a further decrease in SHBG capacity. Addition *in vitro* of medroxyprogesterone acetate or lynestrenol (concentration range 0.005 to 0.025 mg/L) had no influence on SHBG determinations.

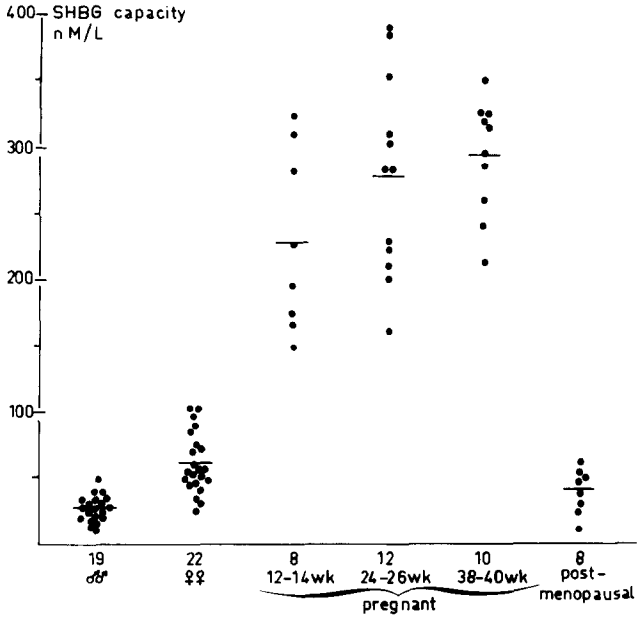

Figure 1. SHBG capacity under different physiological conditions.

As could be expected (1), SHBG capacity was elevated by the administration of 0.050 mg EE_2 (Figure 2). The combination of 0.050 mg EE_2 with either 0.125 mg d-norgestrel or 1.0 mg lynestrenol caused less elevation of SHBG capacity than EE_2 did alone. Addition of 0.250 mg d-norgestrel, or 2.5 mg lynestrenol, to EE_2 even neutralized its effect on SHBG capacity.

The combination of 0.050 mg EE_2 with 0.500 mg dl-norgestrel caused a rather high average SHBG capacity, but with a great variance.

SHBG capacity during the use of EE_2 with 4.0 mg megestrol acetate was uniformly high. This was the only preparation tested that caused values comparable to those found in pregnant women.

DISCUSSION

The dosage levels at which the progestational compounds were tested are those which have been found to be useful in contraceptive practice. Progestational activity, as defined in animal experiments by the Clauberg test or in human by the transformation dose, calculated for the dosages used, are very different (9) for the various compounds. Nevertheless, they give rise to a very uniform depression of SHBG capacity. This decrease of SHBG capacity is not due to interference by MPA or lynestrenol in our in vitro assay. It may be concluded, therefore, that there is no correlation between these para-

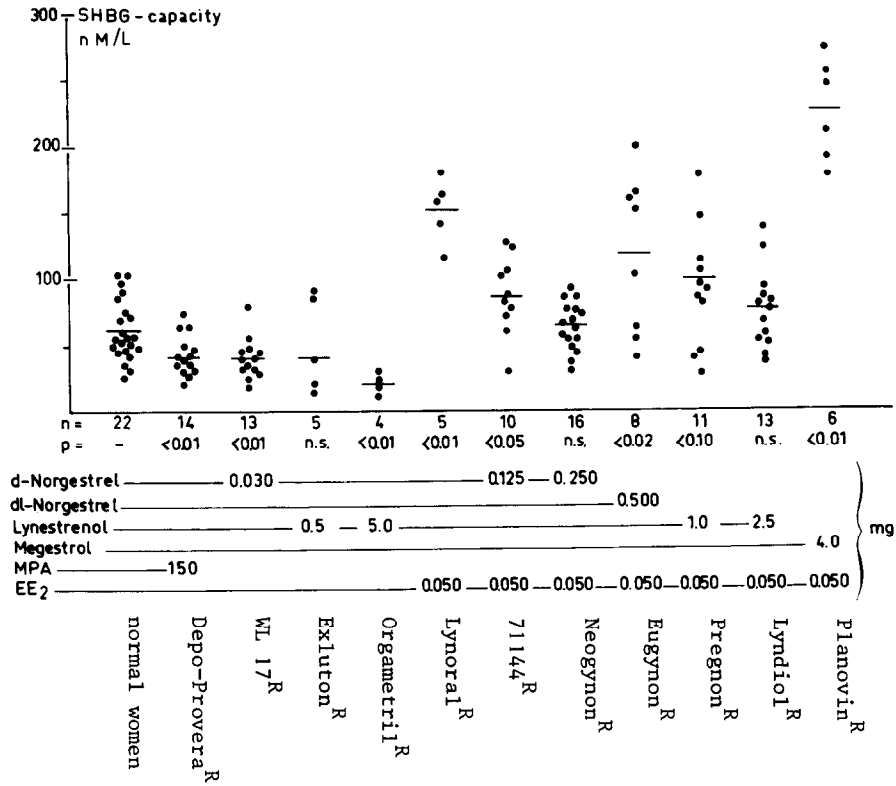

Figure 2. SHBG capacity in women using various hormonal preparations.

meters and the response of SHBG. Whether such correlation exists with, e.g.,cervical hostility, one of the goals for contraceptive purposes, can only be answered by more extensive investigation.

The question arises whether SHBG values can be used as a measure for "total estrogenicity" of combined preparations, as has been suggested for TC-BC by Schwartz and Hammerstein (2). Unfortunately, in our study, different preparations have been used. Furthermore, in their study the subjects were hypogonad, while in ours the subjects were normal women. A few conclusions, however, can be drawn from the comparison of both investigations.

It appears that the combination of 0.050 mg EE_2 and 0.250 mg d-norgestrel induces an increase in TC-BC, while SHBG capacity remains at a normal level. No decrease of TC-BC is caused by administration of progestational agents; SHBG capacity decreases. Our data are in accordance with those of Forest and Bertrand (10), who observed a decrease of DHT binding capacity in the plasma of girls under the influence of MPA (250 mg weekly). This might also explain the observed increase of the metabolic clearance rate of testosterone under the influence of

MPA in women with the polycystic ovary syndrome (11).

It was expected that no difference would be found between 0.250 mg d-norgestrel and 0.500 mg dl-norgestrel, as l-norgestrel is considered to be devoid of biological activity (12). We have no explanation for the high values found with the preparation containing 0.050 mg EE_2 and 0.500 mg dl-norgestrel.

The strong elevation of SHBG capacity in response to EE_2 in combination with 4.0 mg megestrol acetate might be of significance, as this combination is found (13) to have a relatively high risk of thromboembolic complications, which might be an expression of its "estrogenicity".

The use of the concept of "total estrogenicity" of contraceptive preparations has the advantage of simplicity. But the choice of parameters for its definition is arbitrary, as shown by the difference in response of SHBG and TC-BC. For practical purposes, it is preferential to aim at correlating changes in SHBG and TC-BC (and possibly other plasma proteins) with desirable and with untoward effects of various contraceptive preparations. Determination of SHBG capacity has the advantage that it is relatively simple and it can be done in large series.

ACKNOWLEDGEMENTS

We are indebted to Dr.G.Linthorst and Dr.J.C.Seelen, who provided the plasma samples, and to Prof.Dr.A.A.Haspels and Dr.M.M.Madlener for their advice.

REFERENCES

1. Anderson,D.C. Sex-hormone-binding-globulin. Clin.Endocrinol. 3: 69-96 (1974)

2. Schwartz,U. and Hammerstein,J. The oestrogenic potency of various contraceptive steroids as determined by their effects on transcortin-binding capacity. Acta Endocrinol.(Kbh) 76: 159-171 (1974)

3. Vermeulen,A. and Verdonck,L. Studies on the binding of testosterone to human plasma. Steroids 11: 609-635 (1968)

4. August,G.P., Tkachuk,M., and Grumbach,M.M. Plasma testosterone-binding affinity and testosterone in umbilical cord plasma, late pregnancy, prepubertal children, and adults. J.Clin.Endocrinol.Metab. 29: 891-899 (1969)

5. Pohlman,C., Hallberg,M.M., Zorn,E., Guevara,A., and Wieland,R. Conditions associated with elevated testosterone binding affinity. Clin.Res. 17: 291 (1969)

6. Rosner,W. A simplified method for the quantitative determination of testosterone-estradiol-binding globulin activity in human plasma.J.Clin.Endocrinol.Metab. 34: 983-988 (1972)

7. Heyns,W. and De Moor,P. The binding of 17β-hydroxy-5α-androstan-3-one to the steroid-binding β-globulin in human plasma, studied by means of ammonium sulphate precipitation. Steroids 18: 709-730 (1971)

8. Pearlman,W.H. and Crépy,P. Steroid-protein interaction with particular reference to testosterone binding by human serum. J.Biol.Chem. 242: 182-189 (1967)

9. Hammerstein,J. Theoretische Grundlagen der hormonalen Kontrazeption. Med.Klin. 65: 1279-1289 (1970)

10. Forest,M.G. and Bertrand,J. Studies of the protein binding of dihydrotestosterone in human plasma (in different physiological conditions and effect of medroxyprogesterone). Steroids 19:197-214 (1972)

11. Gordon,G.G., Southren,A.L., Calanog,A., Olivo,J., and Rafii,F. The effect of medroxyprogesterone acetate on androgen metabolism in the polycystic ovary syndrome. J.Clin.Endocrinol.Metab. 35: 444-447 (1972)

12. Edgren,R.A., Smith,H., Hughes,G.A., Smith,L.L., and Greenspan,G. Biological effects of racemic and resolved 13-β-ethyl-4-gonen-3-ones. Steroids 2: 731-737 (1963)

13. Inman,W.H.W., Vessey,M.P., Westerholm,B., and Engelund,A. Thromboembolic disease and the steroidal content of oral contraceptives. A report to the committee on safety of drugs. Brit. Med.J. 2: 203-209 (1970)