Effects of oestradiol on sex hormone binding globulin

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Abstract. Sex hormone binding globulin (SHBG) levels were studied for possible effects of oestradiol-17β on SHBG. No change in SHBG plasma was recorded during normal menstrual cycles or during treatment with oestradiol-17β to menopausal women. However, gonadotrophin treatment to amenorrhoeic women to induce ovulation resulted in high oestradiol concentrations and a pronounced increase in SHBG was found during the luteal phase of these cycles. A marked increase of SHBG was also recorded in a woman with pronounced fluctuations of oestradiol during treatment with levonorgestrel sc implants for contraception. In conclusion, effects on SHBG were only found when extraordinarily high levels of plasma oestradiol were recorded.

Sex hormone binding globulin (SHBG) is the specific plasma binding protein for testosterone and oestradiol. The plasma concentration of SHBG reflects the balance between androgens and oestrogens (Anderson 1974). Thus, androgens decrease the concentration of SHBG in plasma (Anderson 1974) whereas synthetic oestrogens increase SHBG concentrations (Briggs 1975; van Kammen et al. 1975). Apart from binding these endogenous hormones, SHBG has been shown to carry certain progestins, such as levonorgestrel (Victor et al. 1976). Levonorgestrel per se has a lowering effect on SHBG concentrations (Victor et al. 1977). During pregnancy high concentrations of SHBG in plasma are found, which has been attributed to high circulating levels of oestradiol (Anderson et al. 1976). Unchanged SHBG concentrations during the menstrual cycle have been reported (Anderson 1974; Motohashi et al. 1969). However, little is known about the effects of oestradiol on SHBG.

The aim of the present investigation was to study the influence of different oestradiol levels on SHBG.

Methods

Sex hormone binding globulin

SHBG-capacity was determined according to the method described by Rosner (1972) with some modifications. Dihydrotestosterone (DHT) (1,2[^3]H(N)-5α-androstan-17β-ol-3-one) from New England Nuclear with a specific activity of 50.6 Ci/mmol was used for the tracer solution. A mixture of tritiated and non-labelled DHT was prepared in ethanol and adjusted to give 17 000 cpm and 2 ng DHT per sample. Of the ethanol-DHT solution a volume sufficient for all tubes in one assay (5 µl per sample) was evaporated and re-dissolved in phosphate buffered saline (PBS), pH 7.0. The volume of PBS added was 250 µl per sample. Unknown samples were assayed on two volumes of plasma: 25 µl and 50 µl. The plasma was diluted with PBS and the final solution was always adjusted to be 250 µl. When the dilution of all samples was completed 250 µl of the DHT[^3]H[PBS] solution was added to each sample. In each assay two total activity tubes were run. They contained the same mixture of tritiated and non-labelled DHT, as the other tubes, in 50 µl of plasma, obtained from healthy, non-pregnant, fertile women. To each of these tubes 100 ng of DHT was added to saturate the binding sites. All samples were incubated at room temperature for 15 min and thereafter placed in an ice bath for another 15 min. 0.5 ml of icecold ammonium sulphate — diluted to 50% (w/v) in distilled water — was added to each sample during constant vortex mixing of the assay tubes. Each tube was replaced in the ice bath after the precipitation. All tubes were centrifuged at 0°C and at 8000 g for 10 min. The
supernatant was decanted into glass counting vials containing 6 ml of scintillation fluid (Riafluor, New England Nuclear) and counted after 2 h for 5 min. Calculation of the SHBG binding capacity was performed according to the method of Rosner (1972).

Evaluation of the method. Three plasma pools were utilized. They were all obtained from healthy non-pregnant women. One pool had a high SHBG capacity, (130 nmol/l), one had a medium high, (80 nmol/l), and one had a low SHBG capacity (20 nmol/l). Saturation of the pools was tested by adding increasing amounts of DHT to constant volumes (25 µl) of plasma. The addition of 2 ng DHT saturated all pools. When 2 ng DHT was added to increasing volumes of plasma from each of the three pools it was found that constant values for DHT-binding (= SHBG) were obtained when 30–80 per cent of the added DHT was recovered in the supernatant. When less than 30 per cent of the added DHT was recovered in the supernatant the SHBG was not saturated. When more than 80 per cent of the added DHT was recovered the results appeared unstable. If the values obtained with the standard assay procedure (25–50 µl of plasma) fall outside the range 30–80 per cent, a smaller or larger volume of plasma should be used.

The precision of the method calculated as coefficient of variation within and between assays (range 10 to 135 nmol/l) was 8 per cent (n = 34) and 13 per cent (n = 100), respectively. The method described can be used for SHBG values obtained from healthy, non-pregnant women.

Steroid determinations
Oestradiol-17β was determined by a radioimmunoassay described by Edqvist & Johansson (1972) and modified by Lindberg et al. (1974). No separations on columns were performed. The oestradiol antibody cross-reacts 11 per cent with oestriol and less than 0.1 per cent with oestriol (Lindberg et al. 1974).

Progesterone was determined by a radioimmunoassay described by Thornycroft & Stone (1972) and modified as described by Bosu et al. (1976).

Material and Results

SHBG during the menstrual cycle
In 3 healthy women 22–33 year old, with a previous history of regular menstruations and on no medication, venous blood samples were taken daily in the morning during two complete menstrual cycles each. The samples were centrifuged and the plasma kept frozen until analysis. SHBG and oestradiol were determined in all samples. Progesterone was determined to assess ovulation. All cycles were ovulatory as judged by the height and duration of the progesterone rise. The results are shown in Fig. 1. The early follicular phase oestradiol concentrations were around 300 pmol/l. The pre-ovulatory oestradiol peak ranged between 800 and 1500 pmol/l (mean = 1200 pmol/l). Luteal phase oestradiol levels ranged between 300 and 1000 pmol/l. The mean SHBG concentration varied between 30 and 40 nmol/l throughout the cycles. No difference in SHBG was found when pre- and post-ovulatory values were compared. None of the women showed any deviation from this pattern.

![Fig. 1.](image)

SHBG, oestradiol and progesterone concentrations during two consecutive menstrual cycles in 3 healthy women. Mean values, and for SHBG, SEM, are given.
**SHBG and oestradiol concentrations during eight ovulatory cycles in 8 amenorrhoeic, but otherwise healthy women, during induction of ovulation with gonadotrophins. Mean values and for SHBG, SEM, are given.**

**SHBG during gonadotrophin stimulation**

Eight anovulatory and amenorrhoeic, but otherwise healthy women, scheduled for ovulation induction with gonadotrophins, were studied during one induction period each. They were treated with human menopausal gonadotrophin (Humegon®, Organon), resulting in increasing levels of oestradiol. Ovulations were induced with 9000 IU hCG (Pregnyl®, Organon). All cycles were ovulatory as judged by progesterone values during the luteal phase and two of the women conceived. The results are shown in Fig. 2. Initial oestradiol levels were below 100 pmol/l and increased within 10–12 days to peak values of 1500 to 4000 pmol/l (mean: 3095 pmol/l). During the luteal phase the mean oestradiol levels increased from 800 to around 3000 pmol/l. In the beginning of the treatment SHBG varied between 23 and 48 nmol/l (mean: 31 nmol/l) and remained within that range until 2 days after the pre-ovulatory oestradiol peak. Then, an increase in SHBG was found in all women with a maximum 14 days after the first oestradiol peak, levels varying between 35 and 100 nmol/l (mean: 64 nmol/l; \( P < 0.001 \), Student's *t*-test, paired data, when compared to SHBG during the follicular phase).

**SHBG during anovulatory oestradiol peaks**

One patient participating in a study of a new delivery system for contraceptive steroids was treated with sc implants releasing levonorgestrel (30 μg/day). During treatment repeated oestradiol peaks without subsequent ovulation (constant baseline progesterone) occurred (Fig. 3). The oestradiol peak levels were usually above 2800 pmol/l and elevated oestradiol plasma concentrations lasted for 10 to 14 days. SHBG determinations were performed from plasma obtained three times weekly for more than 12 months. The SHBG levels increased in parallel with the oestradiol peaks with 1 or 2 days' delay. The basal SHBG was around 60 nmol/l and the peaks amounted to around 150 nmol/l. Levonorgestrel concentrations fluctuated with the SHBG variations, probably due to binding of levonorgestrel to SHBG (VICTOR et al. 1977).
SHBG and oestradiol concentrations in one woman, treated with sc implants releasing levonorgestrel for contraception.

**SHBG during oestradiol treatment in menopausal women**

Four healthy women, menopausal for more than 2 years, without any hormonal or other medication for several months, were treated with a silastic oestradiol releasing vaginal ring for 21 days (for details, see: Englund et al. 1981). The release of oestradiol was calculated to be 200 µg daily. Before treatment all 4 women had oestradiol plasma concentrations below 100 pmol/l. Oestradiol concentrations increased during treatment and varied

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**Fig. 3.**

SHBG and oestradiol concentrations in one woman, treated with sc implants releasing levonorgestrel for contraception.

**Fig. 4.**

SHBG and oestradiol concentrations in 4 menopausal women treated with vaginal rings releasing 200 µg oestradiol-17β daily. Absolute values of SHBG and mean values for oestradiol are given.
between 275 pmol/l and 1000 pmol/l. SHBG determinations were performed from blood samples obtained before treatment and from samples obtained at the end of treatment. Before treatment SHBG concentrations were between 25 nmol/l and 58 nmol/l. SHBG did not change in any of the women during treatment (Fig. 4).

Discussion
Alterations in the concentration of SHBG could interact with its transport capacity both of androgens and exogenous hormones that bind to SHBG, i.e. some progestins, and thus affect the rate of metabolism and biological effects of these hormones. This study indicates that the oestradiol fluctuations seen during the normal menstrual cycle do not affect SHBG levels. SHBG levels did not even change when a pronounced increase in oestradiol to fertile levels was accomplished in the menopausal women treated with intravaginal oestradiol. Increasing levels of SHBG were only found in the woman with extraordinarily high oestradiol peaks and in the women treated to induce ovulation, whose oestradiol rose from very low levels to concentrations considerably higher than those encountered during normal menstrual cycles. From the present study it seems as if oestradiol has to surpass a certain limit before it can induce hepatic synthesis of SHBG. This limit seems to be 1500 pmol/l to 2000 pmol/l.

The increase in SHBG induced by oestradiol as seen in Figs. 2 and 3 seems to occur 1–3 days after the oestradiol peak. It is reasonable to believe that the duration of the oestradiol elevation could influence the degree of SHBG increase, although the present study does not allow any conclusions. It is well known that androgens (Anderson 1974) and levonorgestrel (Victor et al. 1977) reduce SHBG levels. Conversely, a reduction in testosterone or a withdrawal of levonorgestrel treatment could increase SHBG. In the women with levonorgestrel implants the release of levonorgestrel was close to constant throughout treatment. Therefore, it is likely that the profound increases of SHBG recorded were due to the oestradiol elevations.

During the menstrual cycle, testosterone concentrations increase slightly around ovulation, but remain constant during the rest of the cycle (Aedo et al. 1977). It is possible that this peri-ovulatory testosterone increase accounts for the lack of effect on SHBG of the pre-ovulatory oestradiol rise.

In the women treated with gonadotrophins testosterone was measured throughout the cycles and in fact there was a more than two-fold increase in mean testosterone in this group of women after ovulation compared to their pre-ovulatory testosterone. Despite this marked increase in testosterone, a pronounced effect on SHBG was recorded, indicating that oestradiol caused this effect on SHBG. The rise in SHBG could explain the increase in testosterone concentrations in these women by an increased binding capacity for testosterone.

In the menopausal women it is unlikely that the administered oestradiol could have any effect on testosterone production that would explain the lack of effect on SHBG despite considerable increases from pre-treatment in oestradiol concentrations. The lack of effect could be due to the vaginal route of administration when the steroid passes through the general circulation before reaching the liver where SHBG synthesis is induced. The lack of effect could also be due to the relatively low dose of oestradiol, that did not result in plasma concentrations of oestradiol high enough to induce SHBG synthesis.

In conclusion, oestradiol fluctuations within the physiological range seen in normal, fertile, non-pregnant women do not cause any changes in basal SHBG levels. An effect on SHBG can be expected only in instances when extraordinarily high concentrations of oestradiol are encountered.

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References

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