RELATION BETWEEN SEX HORMONE BINDING GLOBULIN AND D-NORGESTREL LEVELS IN PLASMA

By
Arne Victor, Erik Weiner and Elof D. B. Johansson

ABSTRACT

In order to investigate the effect of changes in sex hormone binding globulin (SHBG) levels on d-norgestrel (d-Ng) levels in plasma, the plasma levels of SHBG and d-Ng were studied during one treatment cycle in 6 women on oral contraceptives containing d-Ng and ethinyloestradiol (EOe2) and in 3 women using subcutaneous silastic rods containing d-Ng concomitantly taking EOe2 for three weeks. A significant positive correlation between the SHBG and d-Ng levels was found in 7 of the 9 subjects studied. The results provide evidence for an in vivo binding of d-Ng to SHBG, a SHBG influence on the metabolic clearance rate of d-Ng and consequently a dependence of the plasma levels of d-Ng on the SHBG concentrations in the plasma. These findings support the concept that the clinically and biochemically observed anti-oestrogenic/androgenic effects observed in women on d-Ng containing medication are due to a displacement of testosterone from SHBG by d-Ng.

Changes in the level of a specific high affinity binding protein for steroid hormones affect the metabolic clearance rate of these hormones (Anderson 1974). An increase in the binding protein leads to a reduction in the metabolic clearance rate which will be reflected in higher plasma levels of the steroid when administered at a constant rate. We have found that d-norgestrel (d-Ng) binds strongly to the sex hormone binding globulin (SHBG) in vitro (Victor et al. 1976). Ethinyloestradiol (EOe2) administration leads to an increase in the SHBG levels in the plasma (Anderson 1974; Briggs 1975a; van Kammen et al. 1975). The aim of the present study was to investigate if the EOe2 induced changes in SHBG affect the plasma levels of d-Ng. This would provide evidence for an in vivo binding of d-Ng to SHBG.

Department of Obstetrics and Gynaecology, University Hospital, S-750 14 Uppsala, Sweden
Plasma levels of SHBG and d-Ng in 3 women using oral contraceptives containing 150 μg d-Ng and 30 μg E0e2.

**MATERIALS AND METHODS**

The plasma levels of d-Ng and SHBG were studied in 3 groups, each consisting of 3 women. Two groups received oral contraceptives containing 150 μg d-Ng + 30 μg E0e2 or 250 μg d-Ng + 50 μg E0e2, respectively, for three weeks. Blood samples were collected before treatment started and 12 h after the regular intake of each pill during treatment. This time was chosen to avoid the period of absorption after pill intake when the plasma levels are more variable (Weiner et al. 1976). The third group consisted of women treated with d-Ng containing subdermal silastic rods. Their plasma levels of d-Ng had been constant for several months before the start of concomitant treatment with 50 μg of E0e2 for 21 days. Details of all 3 groups and their plasma d-Ng levels have been published elsewhere (Weiner et al. 1976; Weiner & Johansson 1976). The plasma levels of d-Ng were determined by radioimmunoassay (Weiner et al. 1976). This method measures all unconjugated d-Ng, both bound and unbound. In this study the values have been given in nmol/l (1 ng/ml = 3.22 nmol/l). SHBG levels in plasma were determined by the method of Rosner (1972) and are expressed as the dihydrotestosterone binding capacity of plasma in nmol/l. The precision and reproducibility of the method is good with inter- and intra-assay coefficients of variation of 10 per cent or below. The addition of large amounts of cold d-Ng to plasma does not influence the results of the SHBG determinations. A reference distribution of SHBG levels was calculated from the results of SHBG determinations in 68 healthy women, ages ranging from 18 to 43 years, with regular menstruations using an IUD for contraception and in 35 healthy men, with ages ranging from 23 to 40 years.
RESULTS

Figs. 1 and 2 show the plasma levels of SHBG and d-Ng in women taking oral contraceptives containing both EOe2 and d-Ng. There is no uniform pattern in the SHBG changes. An increase is seen in the 4 subjects with the initially lowest SHBG levels (AT and BMJ, Fig. 1 and GW and MH, Fig. 2). In 2 subjects, AT and BMJ, a decrease is seen during the last week of treatment after the SHBG level has reached values close to 50 nmol/l. In subject GW a plateau is reached at about the same level. Subjects CE (Fig. 1) and RMÅ (Fig. 2) who have the highest SHBG levels initially show a decrease during treatment. This decrease changes to an increase during the last week of treatment in the case of subject RMÅ. After reaching nadir levels of about 25 nmol/l SHBG rises again to a level around 40 nmol/l. In subject MH (Fig. 2) the initial
SHBG levels are below the range of the reference group of women but increase up to the normal range. In all the other cases the levels remain within the range of the reference material. With the exception of subjects CE and RMA the d-Ng plasma levels show a pattern parallel to the changes in SHBG levels.

The plasma levels of d-Ng and SHBG in the subjects using subdermal silastic rods containing d-Ng are shown in Fig. 3. As can be seen EOe2 administration results in increases of SHBG from subnormal levels of 10 to 20 nmol/l to levels that are above the upper limit of the reference material (68.9 nmol/l) in 2 subjects (EL and IL) and to levels in the upper range of the reference material in 1 subject (LB). In subject LB no further increase is seen after day 8 of treatment, whereas in the other 2 subjects SHBG seems to increase almost continuously throughout the treatment period with EOe2. After stopping EOe2 administration the SHBG levels slowly return to values close to the pretreatment values during the following two weeks. The plasma levels of d-Ng increase and decrease in close parallelism to the SHBG levels.

Table 1 shows that there is a significant positive correlation between the plasma SHBG binding capacity and the plasma levels of d-Ng in 7 of the 9 subjects studied.

---

**Fig. 3.**

Plasma levels of SHBG and d-Ng in 3 women using subcutaneous rods containing d-Ng taking 50 µg EOe2 daily for 21 days.
Table 1.
Correlation between the plasma levels of SHBG and d-Ng.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number of samples</th>
<th>Coefficient of correlation (r)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMJ*</td>
<td>22</td>
<td>0.37</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>CE*</td>
<td>17</td>
<td>-0.40</td>
<td>n. s.</td>
</tr>
<tr>
<td>AT*</td>
<td>21</td>
<td>0.76</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RMÄ**</td>
<td>21</td>
<td>0.07</td>
<td>n. s.</td>
</tr>
<tr>
<td>GW**</td>
<td>21</td>
<td>0.43</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>MH**</td>
<td>20</td>
<td>0.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EL***</td>
<td>23</td>
<td>0.80</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL***</td>
<td>19</td>
<td>0.81</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LB***</td>
<td>21</td>
<td>0.74</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Drug used

* 150 μg d-Ng + 30 μg EOe2.
** 250 μg d-Ng + 50 μg EOe2.
*** d-Ng rods + 50 μg EOe2.

DISCUSSION

The results of the present study indicate a close in vivo relationship between the plasma levels of unconjugated d-Ng and the plasma levels of SHBG. In an in vitro study of the binding of Ng to human plasma proteins Uniyal & Laumas (1976) found that the binding of Ng to SHBG was of minor importance. They claimed that the major binding protein of Ng in plasma is a protein different from the human serum albumin, corticosteroid binding globulin and SHBG, possibly being an $α_1$-acid glucoprotein. The major objection raised against their study is that they used concentrations of Ng several times higher than ever found in plasma in studies with pharmacological doses of d-Ng (Weiner et al. 1976). This probably results in a saturation of SHBG allowing a considerable amount of Ng to be bound to other proteins with a lower affinity but a higher capacity for Ng. The use of dl-Ng in their experiments further disturbs the picture since l-Ng is only weakly bound to SHBG (Victor et al. 1976) and has no significantly biological activity (Edgren et al. 1963) probably due to its low affinity for progesterone receptors (Briggs 1975b).

The finding in this study of a positive correlation between the plasma levels of SHBG and d-Ng supports the concept that SHBG is the high affinity binding protein for d-Ng in vivo (Victor et al. 1976). The significantly positive correlation between SHBG and d-Ng plasma levels found in the subjects using sub-
cutaneous silastic d-Ng rods, where a constant release of d-Ng throughout the treatment period can be assumed, and in the 4 subjects on combined pills showing increasing SHBG levels, indicates that the metabolic clearance rate of d-Ng is decreased with increasing SHBG levels.

In subjects CE (Fig. 1) and RMÅ (Fig. 2) there was no positive or even a slightly negative correlation between the SHBG and d-Ng levels. This finding is difficult to explain but it is worth noting that these subjects had the highest SHBG levels at the beginning of treatment and both showed decreasing levels during treatment.

It has been shown that d-Ng, probably in a dose dependent manner, antagonizes the stimulant effects on SHBG production by E0e2 (Briggs 1975a; van Kammen et al. 1975). This would explain why the increase in SHBG is greater in the subjects using subcutaneous rods compared to the subjects using oral contraceptives. In the subjects on oral contraceptive, the plasma levels of d-Ng are much higher during the first few hours after ingestion of a pill (Weiner et al. 1976), whereas in the subjects using rods the plasma levels of d-Ng are constant throughout the day. Thus the “bioavailable” amount of d-Ng is much higher in subjects on oral contraceptives.

The mechanism for the oestrogen antagonistic effect of d-Ng on SHBG production is not yet clear. Testosterone has been shown to reduce the SHBG levels (Anderson 1974). A direct effect of d-Ng, similar to that of testosterone, is one possible mechanism for the anti-oestrogenic effect of d-Ng, which is a 19-nortestosterone derivate. The finding that Ng, in the rat, exerts androgenic effects (Edgren et al. 1968) only in very high doses seems to contradict such a mode of action of d-Ng on SHBG production. Another possibility is that d-Ng exerts its effect on SHBG indirectly through a competition for the binding sites on SHBG with testosterone, resulting in an increase in the free, biologically active, amount of testosterone. This hypothesis is supported by the in vivo finding by Tremblay & Dube (1974) of an increase in the non-SHBG bound testosterone in women on an oral contraceptive containing d-Ng and E0e2. The absence of an increase of free testosterone in their study could possibly be explained by their failure to time correctly blood sampling and pill intake. An increase in free testosterone in women on d-Ng containing contraceptive pills would most likely be found about 2 h after the pill intake when peak plasma levels of d-Ng are reached (Weiner et al. 1976). In an in vitro study a significant increase in the free testosterone was found on addition of 5 ng/ml of d-Ng to plasma (Tremblay & Dube 1976) which corresponds to peak concentrations of d-Ng after oral intake of 250 μg d-Ng (Weiner et al. 1976).

If displacement of testosterone from SHBG is the mechanism by which d-Ng exerts its anti-oestrogenic effects on SHBG production, this could also explain androgenic side effects like acne and hirsutism which are encountered in women on oral contraceptives containing d-Ng. However, these side effects are only
seen occasionally. This could possibly be explained by a displacement of oestradiol from SHBG by d-Ng, as d-Ng seems to have a somewhat higher affinity for SHBG than oestradiol (Victor et al. 1976). In this case the increase in non-SHBG bound oestradiol would counteract the testosterone effect. Androgenic side effects would then be expected in cases where the basal production of oestradiol is low or where the basal production of testosterone is high.

ACKNOWLEDGMENT

This study was supported by grants from the International Committee for Contraception Research of the Population Council, The Ford Foundation and the Swedish Medical Research Council.

REFERENCES

Briggs M. H.: Contraception 12 (1975a) 149.

Received on February 3rd, 1977.