J Steroid Biochem Molec Biol Vol 43, No 5, pp 431–437, 1992
Printed in Great Britain

0960-0760/92 $5 00 + 0 00

STEROIDAL AND NON-STEROIDAL FACTORS IN PLASMA SEX HORMONE BINDING GLOBULIN REGULATION

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Summary—Sex hormone binding globulin (SHBG) is a specific steroid-binding plasma glycoprotein regulated by several factors Sex steroids are currently considered to be the main physiological regulators of this protein SHBG levels, in fact, increase during estrogen treatment and decrease after androgen administration It is well known, however, that in many physiological and pathological conditions SHBG concentrations cannot be explained only on the basis of steroidal control mechanisms The regulation of SHBG levels is in fact more complex and other factors can also affect its plasma values Between the steroidal factors our attention was focused on the role of androgens, of glandular and peripheral origin, in their capacity to lower SHBG plasma levels We studied hyperandrogenic conditions in prepubertal (65 subjects with precocious adrenarche and 16 girls with prepubertal hypertrichosis, aged between 4 and 8 years) and in adult age (51 hirsute patients aged between 14–35 years and 51 acneic patients aged between 15–40 years) The effects of dexamethasone and ACTH administration on SHBG plasma levels were also evaluated The results obtained showed that in adult hyperandrogenic patients SHBG levels, significantly lower than in controls, were not always inversely correlated with androgen levels, which, on the contrary, were higher than in controls In patients with precocious adrenarche we found an inverse correlation only between SHBG, which was significantly lower than normal, and body mass index or bone age but not with androgens, suggesting that in this condition other factors may be more relevant than steroids in SHBG regulation Between the non-steroidal factors our attention focused on insulin We studied 40 non-obese hyperandrogenic patients with or without ultrasonographic evidence of polycystic ovaries, aged 18–39 years, and 35 obese patients, aged 19–37 years, with or without hyperandrogenism or evidence of PCO Low levels of SHBG were found not only in hyperandrogenic obese patients but also in obese patients with normal androgens

It is possible to conclude that (1) several factors (calorie intake, energy balance and growth factors), other than steroids, may be involved in the regulation of SHBG levels in plasma, and (2) each regulating factor may act to a different extent depending on the various periods of the life cycle

INTRODUCTION

Sex hormone binding globulin (SHBG) is a specific steroid-binding plasma glycoprotein, which is mainly synthesized in the liver [1–5] This protein reversibly and with high affinity binds biologically-active circulating androgens [testosterone (T), dihydrotestosterone (DHT) and 3α androstanediol (3Ad)], and the active estrogen, estradiol (E2) although to a lesser degree [6, 7]

In a paper published in 1974 Anderson [1] described SHBG as a fulcrum whose position moves in response to estrogens and androgens, thus regulating the androgen–estrogen balance According to Anderson's view any other factor altering the SHBG concentration may tip this balance in favour of either T or E2 depending on the direction of the change in the SHBG plasma concentration.

This theory, which considers SHBG plasma level as strictly dependent on androgen–estrogen plasma concentration, went unquestioned for several years At present there is much evidence that sheds doubt on the theory concerning the exclusive role of steroids in the SHBG plasma levels regulation in both physiological and pathological conditions [8]

The aim of the present study was to evaluate the actual role of steroids in SHBG plasma level

Proceedings of the XV Meeting of the International Study Group for Steroid Hormones, Rome, Italy, 28–30 November 1991
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regulation For this purpose SHBG, steroid pattern and their correlation in some physiological and pathological conditions which were characterized by markedly increased plasma steroids, in particular androgens, were evaluated both in adults and in prepubertal subjects

PHYSIOLOGICAL CONDITIONS

The fact that mean serum SHBG levels are higher in women than in men, and SHBG levels increase during pregnancy may lead to the supposition that gonadal steroids play an important role in SHBG regulation In many other physiological conditions however, SHBG concentrations cannot be explained exclusively by this classical control mechanism

To verify these conditions we have reviewed SHBG patterns during various periods in the life cycle SHBG levels are higher in children than in adults At puberty SHBG levels decrease in both males and females In boys [9] this decrease can be justified by increased T production (even if the decrease in SHBG is not strictly correlated to the increase in T) However SHBG also showed lower values in girls [10], despite the rise of estrogen In addition, the pubertal fall in boys has been shown to occur even in those with isolated gonadotropin deficiency or with complete androgen insensitivity, as Cunningham *et al* [11] have demonstrated, suggesting that other factors besides androgens may induce this fall

Very recently Holly *et al* [12] suggested that the main factor responsible for the fall of SHBG plasma levels at puberty might be the pubertal rise in insulin In both sexes, in fact, insulin correlated with SHBG plasma levels better than androgens did The increased pancreatic insulin output during puberty may be responsible for the decreased hepatic SHBG production In the same paper Holly sustained that insulin-like growth factor-1 (IGF-1) was also negatively related to SHBG, at least in boys This suggests that GH may also play a role in the regulation of SHBG plasma levels, as was previously suggested by von Shoultz and Carlstrom [8] In addition, Rudd *et al* [13] described a negative relationship between SHBG and GH when evaluating SHBG plasma levels in GH deficient children In fact in these cases SHBG plasma levels are higher than in controls and in short normal children Treatment with GH in prepubertal subjects, with or without GH defect, lowers circulating SHBG as was demonstrated by Belgorosky *et al* [14] However, Balducci *et al* [15], a member of our group, demonstrated that in some cases with combined GH and gonadotropin (Gn) deficiencies the addition of GH to Gn treatment significantly increased T production and parallely decreased SHBG production, the difference between SHBG plasma levels in the two groups was not statistically significant however on account of the small number of patients studied These results, however, are particularly interesting because they lead to the consideration that in some cases the effects of GH on plasma SHBG may be mediated by the increased T production However the results recently published by the Cutler group [16] support the hypothesis that GH may play a direct role not mediated by androgens In fact Cutler and co-workers showed that GH secretion increases during puberty, both in normal boys and girls, thus the decrease in SHBG, observed in this period of life, can be well correlated to the increase in GH

After decreasing during puberty SHBG plasma levels remain unchanged until the 4th or 5th decade of life Then SHBG levels gradually increase until one reaches his or her mid-80s At that time the average levels of both sexes are approx twice those observed in the early 20s [17] In men this rise is not related to the decline of the testicular function, since the decrease in T levels is not parallel to the increase in plasma SHBG [18] Moreover SHBG levels are not affected by orchidectomy [19] Similarly in women plasma SHBG are virtually unaffected by the dramatic reduction of estrogen following menopause [17] The SHBG plasma pattern, therefore, does not seem to be correlated with the steroid pattern during the life cycle thus suggesting that other factors such as GH may be responsible for these modifications Indeed during the life cycle of both men and women GH declines, especially because of the reduction of the nocturnal pulses [20, 21] Insulin and IGF-1, parallel to GH, also gradually decline with advancing age so that IGF-1 plasma levels reach values lower than 0 35 U/ml in about 30% of the healthy men over 60 years of age [20–21]

On the basis of these findings we may, therefore, conclude that endogenous gonadal steroids are not the main regulators of SHBG production Thus serious consideration should be given to the hypothesis that other factors such as insulin, IGF-1 and GH which are usually involved in protein synthesis may play a more

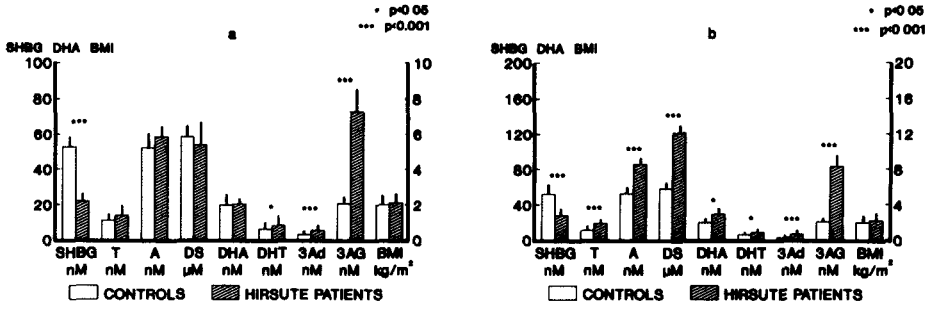

Fig 1 SHBG, T, androstenedione (A), DHA, DHA sulphate (DS), DHT, 3Ad, 3AG and BMI pattern in hirsute patients with normal glandular androgens (panel a) or with high glandular androgens (panel b)

active role in regulating SHBG, while steroids may have an indirect effect

PATHOLOGICAL CONDITIONS

If physiological factors other than steroids regulate SHBG synthesis, it would be interesting to verify if it is possible to find or not a correlation between plasma steroids and SHBG levels in pathological conditions such as those characterized by low plasma SHBG and high androgen levels

For this purpose we have reviewed our findings in the following hyperandrogenic conditions, both in prepuberty and in adulthood

—hirsutism and acne, including either the cases with increased glandular androgens or those with all secreted androgens within the normal range Moreover in hirsute females we have re-evaluated the effects of ACTH and dexamethasone administration on SHBG and androgen plasma values,
—morbidly obese women with or without clinical signs of hyperandrogenism, and
—idiopathic precocious adrenarche and prepubertal hypertricosis, two conditions of hyperandrogenism studied during the prepubertal age

Hirsutism and acne

The plasma hormonal pattern in 16 young hirsute patients aged 14–27 years with a body mass index (BMI) within the control range and presenting plasma levels of secreted androgens comparable to control subjects of matched age are reported in Fig 1(a), peripheral androgens [DHT, 3Ad and 3α-androstanediol glucuronide (3AG)] are all significantly increased, in particular 3Ad and 3AG Plasma SHBG is significantly ($P < 0\,001$) decreased showing values negatively correlated ($r = -0\,58$, $P < 0\,05$) with 3AG values, but not with DHT or 3Ad Similarly another group of 35 hirsute patients [Fig 1(b)] aged 15–35 years with a BMI within the control range showed glandular and peripheral androgens which were significantly increased with respect to controls We found a negative correlation ($r = -0\,54$, $P < 0\,01$) between the decreased values of plasma SHBG and the increased values of plasma 3AG, leading to suppose that peripheral androgens may play a role in SHBG regulation, at least in these pathological conditions

The plasma SHBG and androgen patterns in 25 patients aged 15–40 years with acne and without hirsutism is reported in Fig 2(a) The only glandular androgen in these patients which

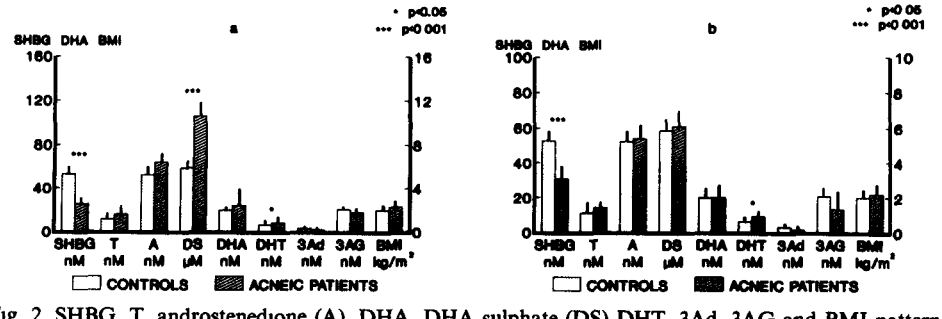

Fig 2 SHBG, T, androstenedione (A), DHA, DHA sulphate (DS) DHT, 3Ad, 3AG and BMI pattern in acneic patients with high glandular androgens (panel a) or with normal glandular androgens (panel b)

showed significantly increased levels was dehydroepiandrosterone sulphate (DHA-S) (10 6 ± 1 4 vs 5 8 ± 1 3 μM in controls, $P < 0\ 001$) and the only peripheral androgen with high values was DHT (0 83 ± 0 07 vs 0 63 ± 0 06 nM in controls, $P < 0\ 05$) The significantly decreased plasma values of SHBG (25 5 ± 8 9 nM, $P < 0\ 001$) correlated negatively with both DHA-S ($r = -0\ 5$, $P < 0\ 05$) and DHT ($r = -0\ 78$, $P < 0\ 01$) If we consider, however, another group of 26 acneic patients [Fig 2(b)] of matched age (15–36 years), who showed similarly decreased levels of SHBG (28 5 ± 9 7 nM) with respect to controls, but normal levels of DHA-S and only slightly increased levels of DHT (0 92 ± 0 06 nM, $P < 0\ 05$), plasma SHBG levels do not correlate with any of the androgens considered DHT included These results seem to exclude that a cause–effect relationship between peripheral androgens and plasma SHBG must always exist This observation confirms what is already shown in androgen resistant patients in whom SHBG declines at puberty even if DHT does not exert any action [11], as androgen receptors are absent These data strongly support previous data reported by von Schoultz and Carlstrom [8]

The conditions which have been examined always showed an inverse tendency between plasma values of SHBG and one or more androgens even if it was not always possible to point out a negative correlation However, there are some other conditions in which this tendency was not present as can be seen in the following In Fig 3(a) the effects of acute (Synacthen 0 25 mg/bolus i v) and chronic (Synacthen depot 1 mg/die i m for 2 days) ACTH stimulation in two groups consisting of 35 and 21 hirsute patients between the ages of 19–31 and 14–36 years, respectively, are reported In these patients the ACTH tests were performed as part of the clinical investigation, because their basal hormonal pattern was suggestive of hirsutism of adrenal origin

Acute administration of ACTH induced an increase in the levels of several androgens but SHBG plasma values remained unchanged The short period of time between these two evaluations, however, may justify these results Comparable results [Fig 3(b)] were obtained following chronic stimulation In fact the dramatic increase in the adrenal fraction of androgens is not followed by a parallel decrease in SHBG plasma values which, on the contrary, remained unchanged

The effects of dexamethasone administered at the dosage of 0 25 mg/die (Protocol 1) or at the dosage of 0 5 every 2 days (Protocol 2) for at least 2 months in women between the ages of 14 and 36 years who are affected by an hirsutism of adrenal origin are reported in Fig 3(c and d),

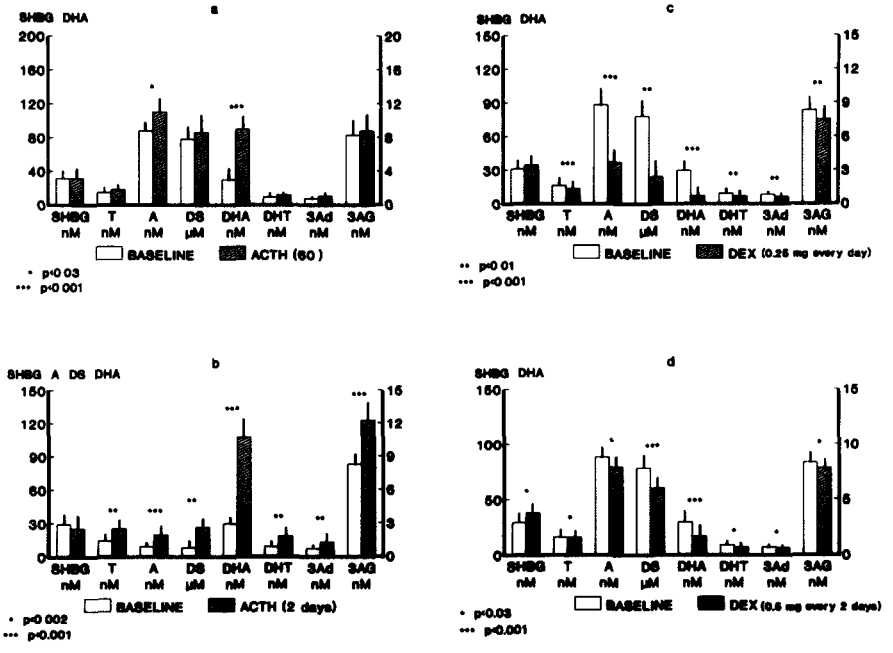

Fig 3 SHBG, T, androstenedione (A), DHA, DHA sulphate (DS), DHT, 3Ad and 3AG pattern in hirsute patients following acute (panel a) or chronic (panel b) ACTH tests or following dexamethasone treatment (every day, panel c, every 2 days, panel d)

respectively These patients were assigned randomly to the first or the second protocol The results of Protocol 1 [Fig 3(c)] show a dramatic decrease of both 5ene and 4ene androgens, without a change in SHBG plasma levels In Protocol 2 blood samples were taken the second morning after the end of treatment Their hormonal pattern showed a less evident inhibition of 4ene steroids, as was previously reported by other authors [22, 23], whereas 5ene steroids remain significantly lower with respect to basal values, even if at levels slightly higher than those of the previous group Unexpectedly, plasma SHBG showed a slight but significant increase with respect to basal values ($37\,5 \pm 11$ vs 29 ± 9 nM, $P < 0\,03$) and were irrespective of androgen modifications

All these results taken together are in agreement with other authors' [8, 12, 13] hypothesis that steroids do not play a central role in SHBG regulation even in the pathological conditions characterized by high androgen plasma levels Other factors therefore may be implicated and, among these, insulin seems to be very important considering the *in vitro* findings of Playmate and coworkers [24, 25] who demonstrated that insulin is able to decrease SHBG production in cultured hepathoma cells

The role of insulin

Figure 4 (left panel) shows the hormonal pattern in 40 non-obese hirsute women aged 18–36 years with or without the ultrasonographic evidence of polycystic ovaries (PCO) and with different degrees of androgenization Plasma SHBG was low, the PCO group showing values lower than those of the non-PCO (NPCO) group Fasting insulin and integrated areas of insulin following a glucose challenge showed higher values in the PCO compared to the NPCO group and a high significant negative correlation with SHBG considering either the two groups together or separately (fasting insulin $r = -86$ $P < 0\,001$ considering both groups, $r = -75$ $P < 0\,01$ considering PCO group, and $r = -66$ $P < 0\,01$ considering NPCO group, insulin area $r = -79$ $P < 0\,001$, considering both groups, $r = -65$ $P < 0\,01$ considering PCO group, and $r = -58$ $P < 0\,01$ considering NPCO group) Moreover the hormonal pattern in a group of 35 obese patients, aged 19–37 years, with or without clinical signs of hyperandrogenism or evidence of PCO were also reviewed (Fig 4, right panel) All three groups showed comparable low plasma levels of SHBG with respect to lean controls and there were no differences between PCO and NPCO patients However, insulin (fasting and area) which was higher in all 3 groups in comparison to lean controls showed comparable levels between obese NPCO and obese controls, the obese PCO showing the highest values [26] Considering all patients together no correlation could be found between SHBG and insulin, similar frustrating results were also obtained taking each group separately Analogous results were obtained by Conway *et al* [27] However, this group of patients were very heterogeneous as far as the BMI and degree of obesity is concerned Therefore, we tried to see if there was a correlation between fasting insulin, the insulin area and SHBG plasma levels, subgrouping the patients according to their BMI Using this approach, a negative correlation between SHBG and fasting insulin ($r = -0\,73$, $P < 0\,02$) is found, but only in a selected group with a BMI ranging between 25 and 29 kg/m^2, but not in obese patients with a higher BMI Probably in severe obesity other factors, particularly lipids and lipoproteins, play a more important role in SHBG level regulation, as was already observed by Gorbach *et al* [28] Studies

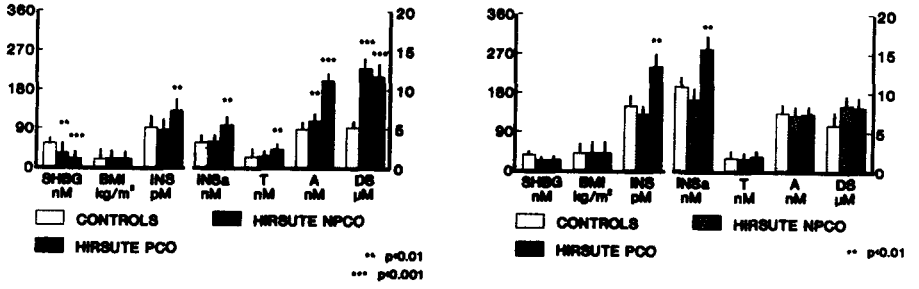

Fig 4 SHBG, fasting insulin (INS), BMI, insulin area following glucose challenge, T, androstenedione (A), DHA. DHA sulphate (DS) pattern in lean patients with or without PCO (left panel) and in obese patients with or without PCO (right panel)

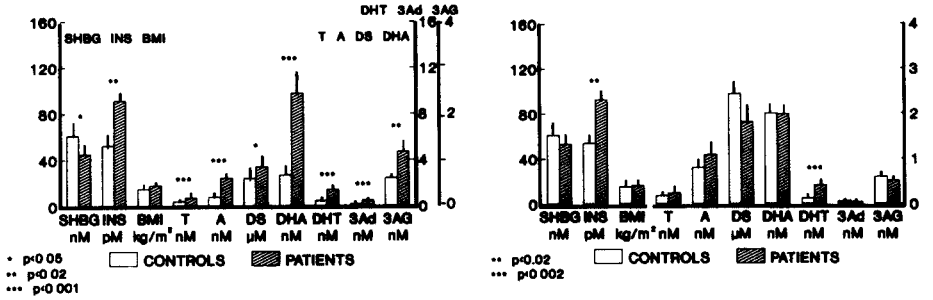

Fig 5 SHBG, fasting insulin (INS), BMI, T, androstenedione (A), DHA, DHA sulphate (DS), DHT, 3Ad and 3AG pattern in boys and girls with precocious adrenarche (left panel) or in girls with prepubertal hypertrichosis (right panel)

are in progress to ascertain the validity of this hypothesis

Prepubertal hyperandrogenism

In prepubertal subjects we studied 2 particular conditions characterized by clinical signs of hyperandrogenism (1) precocious adrenarche, and (2) simple prepubertal hypertrichosis

Precocious adrenarche The results in 65 patients aged between 4 and 8 years with precocious adrenarche are reported in Fig 5 (left panel) The hormonal results obtained in male and female subjects are comparable and therefore the data are pooled Plasma SHBG shows a slight decrease at the limit of significance with respect to controls, whereas the values of 5ene, 4ene and of peripheral steroids were significantly increased Insulin values were higher ($P < 0\ 02$) than in controls Plasma SHBG was negatively correlated with BMI ($r = -0\ 36$, $P < 0\ 05$), with advanced bone age ($r = -0\ 40$, $P < 0\ 01$) and with insulin ($r = -0\ 46$, $P < 0\ 01$), but no correlation was observed with the increased levels of steroids

Prepubertal hypertrichosis In another condition of prepubertal hyperandrogenism—prepubertal hypertrichosis—16 girls aged 5–8 years were studied (Fig 5, right panel) We found glandular secreted androgens perfectly comparable to controls and, among peripheral androgens, isolated high levels of DHT ($0\ 42 \pm 0\ 04$ nM, $P < 0\ 001$) [29] SHBG plasma levels, although slightly but not significantly decreased, with respect to controls showed a negative correlation ($r = -0\ 67$, $P < 0\ 03$) with the insulin levels These are significantly increased with respect to controls, but not with respect to BMI or bone age, which, on the other hand, were not advanced with respect to the chronological age

Therefore, even if in adults with pathological conditions characterized by increased androgens, SHBG plasma levels correlate with circulating steroids, we are unable to show any correlation between steroids and SHBG plasma levels, during prepuberty, even in clinical conditions similarly characterized by increased androgen production In this period of life, on the contrary, metabolic factors nutritional status and growth seem to play the major role in SHBG regulation [8, 27]

CONCLUSIONS

We can conclude that SHBG probably recognizes multifactorial regulation both in prepuberty and in adulthood Steroids may not always play a central role either in physiological or in pathological conditions Indeed insulin and other growth factors, may be involved

It is probable that all these factors may act together and this would explain the difficulties in establishing a correlation between SHBG plasma levels and each factor considered singularly Moreover each regulating factor may act to a different extent depending on the various period of the life cycle More studies are therefore necessary to completely clarify the fine mechanisms which may be involved in SHBG plasma level regulation

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