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GROWTH HORMONE AND THE STEROID BINDING β-GLOBULIN OF HUMAN PLASMA

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SUMMARY

The possible influence of growth hormone on the binding activity of the steroid-binding β-globulin (SBβG) in human plasma has been studied. Growth hormone has a *chronic effect* on SBβG binding. Acromegalic patients with evolutive disease have an SBβG activity about 40% lower than normal. Daily intramuscular injections of 5 mg human growth hormone in 5 growth-retarded children did, after 2–3 days, depress SBβG activity to about three-quarters of the original value.

Other experiments give the impression that growth hormone also has an *acute effect* on SBβG binding. Indeed, significant but short-lived decreases in SBβG activity are noted 2–3 h after the beginning of sleep and these decreases correlate significantly with increases in serum growth hormone levels. Similar decreases are found 30 min after the end of a short period of violent exercise or 30–90 min after an arginine infusion. But, after intravenous injection of insulin or after oral administration of glucose, where changes in growth hormone are also noted, no clearcut variations in SBβG activity occur. Moreover, intramuscular injections of human growth hormone, although inducing marked increases in the immunoassayable growth hormone of serum, do not alter immediately SBβG activity. The decrease in plasma binding after an arginine infusion was paralleled in 4 out of 5 cases by an acute fall in serum testosterone.

INTRODUCTION

SEVERAL lines of evidence point to an association between growth hormone and androgen metabolism. Acromegalic patients often show acne, hirsutism and discrete signs of virilisation[1]. On the other hand, findings in patients with isolated growth hormone deficiency seem to imply that growth hormone either increases the responsiveness of end organs to gonadal hormones or affects gonadotropin secretion[2]. It was thought, therefore, worthwhile to study the influence of growth hormone on the activity of the steroid-binding β-globulin (SBβG).

MATERIAL AND METHODS

The subjects studied were patients hospitalized in the Department of Internal Medicine except when stated otherwise. As a rule patients treated with steroids were excluded. Blood was taken on heparin and the plasma kept at −20°C until use.

Assessment of SBβG activity

The binding of estradiol was estimated by means of competitive adsorption [3, 4]. The diluted plasma sample, treated with charcoal to remove the endogenous steroids, was incubated at 0°C in the presence of a tracer amount of [^{3}H]-estradiol. A constant amount of Sephadex G 10, an adsorbent for estradiol, was added to each sample. After equilibration, the concentration of [^{3}H]-estradiol in the supernatant was measured. As demonstrated previously, the ratio of non-

adsorbed to adsorbed estradiol is quantitatively related to the ratio of bound (B) to unbound (U) estradiol[3, 4]. This B/U ratio measured at a tracer concentration of steroid, corresponds to the sum of the products of capacity and apparent affinity of the different binding proteins in the system. By subtracting from this global binding index the B/U ratio measured at a concentration of estradiol which saturates SBβG but not albumin, a better estimate of the binding activity of SBβG for estradiol is obtained, which is called the *estradiol binding index* (EBI)[5].

The estimation of testosterone or dihydrotestosterone (DHT) binding was based on the property of SBβG to be quantitatively precipitated at a lower concentration of ammonium sulphate than albumin or than transcortin[6]. The *testosterone or DHT-precipitation index* was measured as follows. The charcoal treated plasma sample was diluted with a solution of bovine albumin and incubated at 25°C with a tracer amount of [^{3}H]-testosterone or [^{3}H]-DHT. After precipitation of SBβG by ammonium sulphate the concentration of radioactivity in the supernatant was measured. The ratio of [^{3}H]-steroid in the precipitate to [^{3}H]-steroid in the supernatant was called "precipitation index". This index is proportional to the binding activity of the precipitated proteins (SBβG). Indeed, the binding of labeled steroid in the supernatant is due for the largest part to the added bovine albumin and therefore nearly constant[7].

For the estimation of the *DHT-binding capacity* the plasma sample was incubated with an excess of [^{3}H]-DHT, which saturated almost completely the SBβG in the sample. After repeated precipitation at 0°C the amount of DHT in the precipitate was measured and was used as an estimate of the binding capacity of SBβG. Indeed, it was shown[7] that the recovery of SBβG-bound DHT was satisfactory and that interference by aspecifically bound DHT was negligible.

Assessment of serum growth hormone (GH) levels

Human growth hormone in serum was determined by radioimmunoassay using talcum to precipitate the free hormone[8]. The antiserum used was a gift from N.I.H. (Bethesda, Maryland).

Other techniques

Plasma testosterone levels were measured by means of competitive protein binding[9]. Serum insulin levels were determined according to Yalow and Berson[10].

RESULTS

(a) *Chronic effects of growth hormone on SBβG activity*

1. *SBβG activity in acromegalics.* Eight acromegalic men with active disease and elevated growth hormone levels had an EBI of 75 ± 33 (SD), a value significantly lower ($P < 0{\cdot}001$) than normal (176 ± 90; mean ± SD). Similarly, 9 acromegalic women had an EBI of 198 ± 138 (SD), significantly lower ($P < 0{\cdot}05$) than normal (308 ± 173; mean ± SD). In fact 3 of these women had normal or slightly elevated values (234, 351 and 487) while the 6 others had definitely low values. The cortisol binding, measured in 2 acromegalics with a low EBI, was normal.

The decrease of SBβG activity in acromegalics was confirmed by measurement of the DHT-binding capacity. In 4 acromegalic men values of 0·34, 0·52,

0·59 and 0·97 μg per 100 ml were found. Their mean value (0·60 ± 0·26; SD) was significantly ($P < 0·025$) lower than the mean DHT-binding capacity of normal men (1·07 ± 0·37; SD). In 2 acromegalic women DHT-binding capacities of 0·65 and 0·80 μg per 100 ml were measured. These values are low in comparison to the value of 1·76 ± 0·59 (SD) μg per 100 ml, found in normal women. A third woman with non-active acromegaly had a DHT-binding capacity of 1·01 μg per 100 ml.

2. *Chronic effects of intramuscular injections of human growth hormone in growth retarded children.* Growth hormone was injected in 5 growth-retarded children in daily injections of 5 mg. Three of these children (2 boys and a girl) had a documented growth hormone deficiency; the two other children (boys) were Silver Dwarfs. The testosterone precipitation index after 3 daily injections had decreased respectively to 67, 87, 66, 80 and 83% as compared to the pretreatment value (Table 1). After 5 daily injections the children received further 5 mg of human growth hormone intramuscularly twice a week. In the 4 boys the SBβG activity was tested again just before the next injection of growth hormone. Three days after the last injection the SBβG activity was still low in 3 out of the 4 boys when compared with the pretreatment levels; the values of the testosterone precipitation index being respectively 1·81 vs. 1·74 (G.E.), 2·53 vs. 3·12 (N.A.), 1·55 vs. 1·90 (S.K.) and 1·01 vs. 1·44 (V.M.). The low levels found in 3 of the boys 3 days after the last injection of growth hormone were similar to the values obtained after the third daily injection.

(b) *Acute changes in SBβG activity*

1. *Spontaneous variations during the day.* Blood was taken from 10 hospitalized women at 8 a.m. just before breakfast and again at 9 a.m. The DHT-precipitation indices were not significantly different, the values being respectively 97·5 and 102·5 ± 5·7% of the individual mean value. In another series of 20 hospitalized women blood was taken at 8 a.m. and 8 p.m. and the EBI was measured. Again no significant changes were recorded (103·6 vs. 96·4 ± 16·5%).

2. *Decreases in SBβG activity in test situations where increases in serum growth hormone are noted.* Shortly after the onset of sleep growth hormone levels

Table 1. SBβG activity in growth-retarded children injected daily intramuscularly with 5 mg human growth hormone. The SBβG activity was assessed before the daily injection and, for all cases except for R.R., 1 h after the injection (second line)

			Testosterone-precipitation index on day					DHT-precipitation index on day				
Name	Sex	Diagnosis	1	2	3	4	5	1	2	3	4	5
G.E.	M	GH+TSH	1·74		1·21			2·06		1·83		
		deficiency	1·77		1·15			1·98		1·57		
N.A.	M	GH	3·12		2·75			2·98		2·43		
		deficiency	3·16		2·72			3·27		3·11		
R.R.	F	GH+TSH	2·97	2·38	1·96	2·01	1·93	3·12	2·58	2·43	2·32	2·05
		deficiency										
S.K.	M	Silver	1·90		1·52			2·12		1·73		
		dwarf	1·92		1·55			2·18		1·89		
V.M.	M	Silver	1·44		1·21			1·26		1·01		
		dwarf	1·40		1·15			1·18		1·08		

in serum increase markedly; this increase persists for 1·5–3·5 h and is not accompanied by changes in glucose, insulin or cortisol levels[11]. In our study SBβG activity was evaluated about 2–3 h after the clinical onset of sleep and compared with the values obtained in the same subject at 10 p.m. This was done in 12 women using the EBI as parameter of SBβG activity; plasma growth hormone levels were determined in the same samples. A significant decrease in SBβG activity was noted, the figures being respectively 119 vs. 81 ± 26 (SD) per cent of the individual mean value. Furthermore, the decrease in EBI was related to the measured increase in serum growth hormone levels, the correlation coefficient being 0·663 ($P < 0·01$). Two of the women in this group were taking oral contraceptives and had EBI values about 10 times higher than normal. Both of them displayed clear increases in serum growth hormone during sleep paralleled with profound decreases in the estradiol binding index.

The SBβG activity of plasma was also studied before and respectively 30, 60, 90 or 120 min after the intravenous administration over 30 min of 30 g arginine hydrochloride in 5 per cent glucose solution, by measuring either the estradiol binding index, the DHT-precipitation index or the DHT-binding capacity. The values obtained were expressed as a percentage of the individual mean value (Table 2). As compared to the initial values, significant decreases in SBβG activity were noted from the end of the arginine infusion to 90 min later. These variations were much less in diabetics of the maturity-onset type; and in one women with panhypopituitarism (not included in Table 2) no variation in the DHT-precipitation index was recorded. Serum growth hormone levels in the latter woman were of course uniformly low, but increased markedly after arginine

Table 2. SBβG activity in hospitalized subjects receiving an intravenous injection of 30 g arginine in 5% glucose solution over 30 min

Subjects	Number Sex	Parameter of SBβG activity	Results expressed as a percentage of the individual mean value ± SD				
			0	30	60	90	120
"sick"	10 F	EBI	139·9 ± 39·3	90·4 ± 24·1*	90·4 ± 17·3*	79·8 ± 20·5*	99·3 ± 19·1*
"sick"	14 F	DHT-precipitation index	111·2 ± 10·7	96·7 ± 12·4*	98·3 ± 6·9*	95·8 ± 5·6*	98·0 ± 10·8*
"sick"	3 M	EBI	121·0 ± 10·8	87·0 ± 7·2	95·7 ± 3·8	102·2 ± 22·1	94·0 ± 11·4
"sick"	7 M	DHT-precipitation index	116·4 ± 6·6	96·7 ± 10·6*	94·6 ± 10·0*	99·1 ± 4·7*	97·5 ± 14·0*
"sick"	4 M 2 F	DHT-binding capacity	114·0 ± 21·6	89·0 ± 7·3	95·8 ± 6·5	98·5 ± 5·6	102·7 ± 6·7
maturity-onset diabetics	4 F	EBI	98·0 ± 8·4	83·0 ± 17·8	102·8 ± 19·1	108·8 ± 4·7	106·5 ± 14·5

*value significantly ($P < 0·01$) different from the beginning level.

infusion in 12 other subjects where these levels were measured. In these experiments, however, no correlation was found between the increase in growth hormone levels and the decrease in SBβG activity. In fact the latter decrease sometimes preceded the increase in growth hormone levels. In 6 men plasma testosterone levels were measured before and during arginine infusion; they were compared to the DHT-precipitation index and/or the DHT-binding capacity measured concomitantly. The results are given in Table 3. In 3 cases a good parallel was found between the changes in both parameters.

Five young (21–25 y) normal women were submitted to a short (10–12 min) period of violent exercise on an ergometer bicycle. As compared to the values obtained at the end of the exercise, the testosterone precipitation index declined significantly ($P < 0.05$) 30 min later, although at that time the growth hormone levels, already high at the end of the exercise period, had not gone up significantly. Sixty and 90 min after the end of the exercise the SBβG activity rose again while the serum growth hormone levels reverted to normal (Fig. 1).

3. *No changes in SBβG activity in patients receiving insulin or glucose.* Intravenous administration of insulin elicits a brisk rise in serum growth hormone levels in most subjects[12]. However, no significant changes in SBβG activity were observed 30, 60, 90 or 120 min after the intravenous injection of 0·05 or 0·1 U/kg body weight of insulin to 7 hospitalized women.

After oral administration of glucose a decrease in serum growth hormone levels is to be expected; this is followed more or less rapidly by a rise[12]. SBβG activity and growth hormone levels were studied before and after oral administration of 1 g/kg body weight of glucose. No clearcut pattern of SBβG activity emerged in 25 tests.

4. *No acute changes in SBβG activity in patients treated with human growth*

Table 3. SBβG activity and plasma testosterone levels in men receiving an arginine infusion. The DHT-precipitation index was used to assess SBβG activity in the first 4 cases and the DHT-binding capacity in the 2 last ones. The actual values and the percentage of the mean individual values are given

Name Age	Diagnosis	SBβG activity 0	30	60	90	120	Plasma testosterone ng/100 ml 0	30	60	90	120
R.E.	acromegalic	0·62	0·47	0·52	0·51	0·60	117	139	111	113	127
59		114·0	86·4	95·6	93·8	110·2	96·4	114·5	91·4	93·1	104·6
M.R.	juv. diabetic	2·10	1·69	1·53	1·71	1·52	486	—	354	293	266
19		122·8	98·8	89·5	100·0	88·9	139·0	—	101·2	83·8	76·0
G.R.	normal	0·65	0·48	0·46	0·48	0·48	949	642	817	843	839
25		127·5	94·1	90·2	94·1	94·1	116·0	78·5	99·9	103·1	102·5
L.L.	normal	0·52	0·48	0·50	0·51	0·54	796	660	821	760	764
25		102·0	94·1	98·0	100·0	105·9	104·7	86·8	108·0	100·0	109·5
H.A.	normal	0·66	0·62	0·68	0·62	0·71	305	219	221	242	234
29		100·3	94·2	103·3	94·2	108·0	124·9	89·7	90·5	99·1	95·8
P.K.	normal	1·14	0·95	1·03	1·02	1·09	315	298	332	276	325
39		109·0	90·8	98·5	97·5	104·2	101·9	96·4	105·4	87·6	103·2

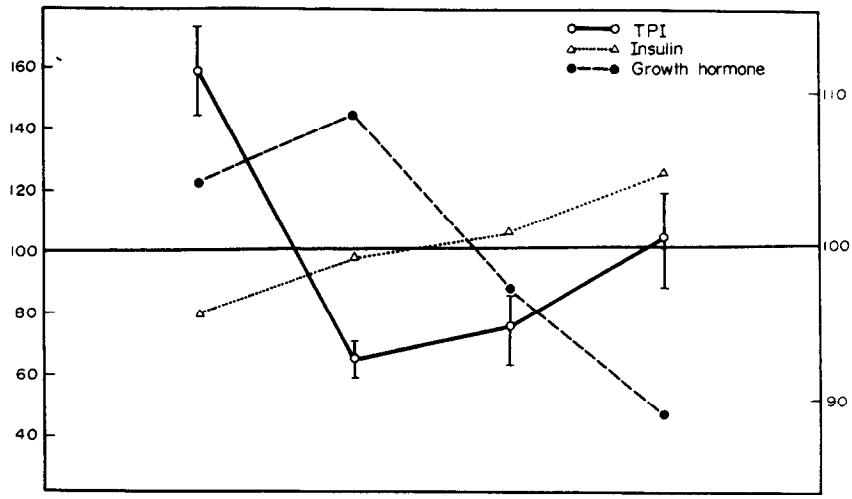

Fig. 1. SBβG binding at the end of and 30, 60 or 90 min after a short period of violent exercise in 5 normal fasting women; serum growth hormone levels, serum insulin levels and testosterone precipitation index expressed as a percentage of the individual mean value (mean ± S.E.M.).

hormone. A single dose of human growth hormone, prepared according to the Raben procedure by Organon (Holland), was injected intramuscularly in 2 healthy women (age 20 and 21); one of them got 5 mg and the other 10 mg. Blood was taken before the injection as well as 12 h later and afterwards twice a day (8 a.m. and 6 p.m.) for 3 days. No changes in SBβG activity were recorded.

In another experiment two healthy men, 19 and 25 yr old, got respectively 10 and 20 mg human growth hormone intramuscularly. Blood was taken 60 and 5 min before the injection, as well as 30, 60, 120, 240 and 360 min later. Again no changes in SBβG activity were noted.

In the 5 growth-retarded children treated chronically with human growth hormone blood was taken on two occasions, before and 1 h after the growth hormone injection. SBβG activity was assessed with the testosterone precipitation index and the DHT-precipitation index. As can be seen in Table 1, the growth hormone injection had no *acute* effects on SBβG activity.

5. *Factors which do not explain the observed acute changes in SBβG activity*. Since the acute changes in SBβG activity we observed are not directly due to changes in growth hormone levels, other possible explanations were looked for. Blood glucose levels, plasma cortisol levels and the transcortin activity of plasma were measured, but did not seem to intervene. In the 2 young men injected with human growth hormone profound changes in free fatty acid levels were documented without concomitant changes in SBβG activity. Furthermore, free fatty acids (e.g. myristic acid) added in vitro did not affect noticeably SBβG binding.

On the other hand, no changes in SBβG activity were recorded in the first hours after an injection of secretine (1 subject; 1 U/kg) or during an intravenous infusion over 8 h of 50 IU of ACTH (4 men and 1 woman).

DISCUSSION

1. *Variations in SBβG activity*. Administration of various hormones influences the binding activity of the steroid-binding β-globulin after a few days of treatment.

While estrogens or thyroxine increase its binding activity, testosterone decreases it[13]. The data presented show that a chronic excess of growth hormone also depresses the activity of this steroid carrier. Acute *in vivo* variations in SBβG capacity, as noted here, have not been described until now.

2. *Mechanism and cause of the acute variations in SBβG activity.* Although our first experiments suggested it, the acute and transient decreases in SBβG activity described in this paper are probably not due to growth hormone itself. These changes in activity involve a decrease in capacity. In view of the slow turnover of plasma proteins such a rapid decrease in capacity cannot be due to a decrease in protein synthesis. An augmented and temporary removal of the carrier protein from the plasma compartment, either real or apparent (masking of binding sites), must thus be invoked.

3. *Possible significance of the observed decreases in SBβG activity.* The level of SBβG activity is said to determine, at least in part, the metabolic clearance rate of testosterone and might influence mainly the extrahepatic part of it[14]. If this were so, a decrease in SBβG activity would be accompanied by an increase in the extrahepatic clearance of testosterone. The latter now is often considered to be due to testosterone utilization by the target cells and thus to be an index of androgenic activity[15]. Growth hormone by its effect on SBβG activity might therefore increase the responsiveness of end organs to testosterone. A few years ago this was suggested on clinical grounds by Goodman *et al.*[2] and by Laron *et al.*[16]. On the other hand, the low total serum testosterone levels observed in acromegalics[17] could be due, at least in part, to a decreased plasma testosterone binding.

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DISCUSSION

Vermeulen: Peter, do you believe that this binding protein is pooled somewhere, or do you think it's really the half-life of the protein that is so short? In 30 min it

falls sometimes to half the initial level, and I don't think that it should have such a short half-life.

De Moor: I don't think synthesis is involved, and since the change is reversed to normal so quickly, I don't think half-life is involved either. It must be that SBβG is going out of the plasma compartment, which is very unlikely, or that it is masked. and that the capacity decreases apparently and not really. It is not masked however, by free fatty acids, we've checked that, both in *in vitro* and in clinical experiments.

Vermeulen: Did you try to determine the capacity with a method where you remove the steroids or possible interfering substances prior to determination?

De Moor: We've used only the methods described. In the first method, charcoal treatment is applied; in the second also; in the third, not.

Martini: In several of the conditions which you showed, in addition to an increase of growth hormones, there is also an increase in ACTH, and consequently of cortisol, etc. Could you rule out ACTH as a possibility?

De Moor: We could rule out cortisol, not ACTH. We also ruled out insulin: We determined insulin in several of these dynamic situations without seeing any apparent correlation with SBβG activity. As far as cortisol is concerned, I can add that transcortin activity did not change either.

Vermeulen: Concerning ACTH, we found that an important decline of plasma levels of testosterone lasting about 24 hours takes place after an acute injection of ACTH. (Synacthen® depot 1 mg). The decrease was of the order of 50–60%.

Morfin: There are evidences supporting the conclusions you made on the activity of 5α-dihydrotestosterone on target tissue. After investigations on patients with testicular feminization syndrome, Dr. Mauvais-Jarvis proposed that the lack of masculinization depends upon the absence of testosterone conversion into 5α-dihydrotestosterone at the target site, this being due to either the absence of specific enzyme or to an elevated binding of testosterone to proteins which prevents the penetration of the male hormone in the target cell.

De Moor: If my memory is correct, Mauvais-Jarvis published that there was a very high binding capacity for testosterone in the blood of these patients.

Morfin: Right.