Modulation of immunoreactive somatomedin-C levels by sex steroids

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Abstract. Among 28 menstruating women tested once randomly during the cycle, somatomedin-C (Sm-C) values were lower in the 10 women in normal follicular phase than in the 10 women in normal luteal phase or the 8 women with hyperandrogenism. Among these 28 subjects, Sm-C showed a positive correlation with testosterone and a positive correlation of borderline significance with oestriadiol. A positive correlation was also evidenced between Sm-C and ln progesterone among the 20 women of this group who were not hyperandrogenic. In 5 other normal women investigated daily throughout an entire menstrual cycle, Sm-C concentrations were higher during days +4 to +9 of this cycle (luteal phase) than during days −3 to −8 (follicular phase). In another group of 21 healthy women, Sm-C values were increased during medroxyprogesterone acetate (150 mg trimestrially) treatment. In 7 normal men, Sm-C decreased during ethinyl-oestradiol (1 mg daily for 5 days) administration. These findings suggest that circulating Sm-C levels are modulated by variations of sex steroids which occur during the menstrual cycle as well as by pharmacological doses of oestrogens and progestagens.

The effects of sex steroids on serum somatomedin (Sm-C) concentrations are still controversial. It has been previously reported that administration of high doses of ethinyl-oestradiol decreased circulating Sm-C concentrations in acromegaly (Clemmons et al. 1980). In contrast, administration of relatively low doses of ethinyl-oestradiol to girls with Turner’s syndrome was followed by an increase in serum Sm-C (Ross et al. 1983; Cuttler et al. 1985) and during normal puberty in girls the rise in Sm-C concentration was related to increasing oestradiol and androgens levels (Rosenfield et al. 1983). Similarly, intramuscular testosterone therapy has been shown to increase Sm-C levels in GH-sufficient prepubertal males (Parker et al. 1984). However, in adults intramuscular testosterone in pharmacological doses had no significant effect on Sm-C, while medroxyprogesterone acetate was found to increase Sm-C levels (Meyer et al. 1982). The purpose of the present study was to investigate the effect of variations in sex steroids during menstrual cycle and in pharmacological conditions (administration of ethinyl-oestradiol and of medroxyprogesterone acetate) on serum Sm-C levels in healthy adults.

Materials and Methods

Subjects
All subjects investigated were healthy adults. Informed consent was obtained from each of them. In all investigations, serum samples were drawn at 09.00 h

1. Cross-sectional study
Three groups of normally menstruating women were investigated. Feeding habits and nutritional status were normal in all subjects. None of them had received contraceptive pills for at least 6 months.

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Group A. Normal follicular phase: 10 women, 23–48 y.o. (mean 30 years), investigated randomly at days 2–15 of a 27–30 day-cycle, with concomitant progesterone values <0.8 ng/ml and testosterone values <0.6 ng/ml.

Group B. Normal luteal phase: 10 women, 19–41 y.o. (mean 30 years), investigated randomly at days 16–31 of a 26–31 day-cycle, with concomitant progesterone values >2.0 ng/ml and testosterone values <0.6 ng/ml.

Group C. Hyperandrogenism: 8 women, 20–34 y.o. (mean 26 years), investigated randomly at days 2–35 of a 22–35 day-cycle, with concomitant testosterone values >0.6 ng/ml. Six of them (group C) were in follicular or anovulatory phase (progesterone levels <0.8 ng/ml) and two in luteal phase (progesterone values >2.0 ng/ml).

2. Longitudinal study

Five normal women, 26–32 y.o., off contraceptive pills for at least 6 months, were investigated daily during an entire menstrual cycle.

3. Medroxyprogesterone treatment

In 21 women, 24–48 y.o., treated with medroxyprogesterone acetate (MPA) (Depo-Provera 150) im, blood was obtained 10 days and (in 10 of them) 30 days after the last trimesteral injection.

4. Ethinyl-oestradiol treatment

Seven men, 21–32 y.o., were given 1 mg ethinyl-oestradiol p.o. daily for 5 days. Blood was obtained daily for 2 days immediately before, during and for 2 days after the test.

Hormone assays

Serum LH (Robyn et al. 1971), FSH (Odell & Hescox 1971), oestradiol, progesterone (Delvoye et al. 1978), testosterone (Delbeke & Lejeune-Lenain 1982) were measured by previously described radioimmunoassays. Normal follicular progesterone values are <0.8 ng/ml; normal testosterone values are <0.6 ng/ml. Sm-C was measured on non-acidified, unextracted serum using a modification of a previously described method (Furlanetto et al. 1977, 1982). This modified assay is performed in polyethylene tubes and the assay buffer contains 0.5 U/ml sodium heparin. All serum samples were assayed in duplicate at two dilutions and samples from the same subject were analyzed in a single assay. The intra- and inter-assay coefficients of variation averaged 10% and 19%, respectively. A pool of sera from 20 normal adult subjects (arbitrarily considered to contain 1 U/ml) was used as standard. The normal range for adults in the assay is 0.4–1.5 U/ml.

Statistical analysis was performed using the Student’s t-test for paired or unpaired groups and linear regression analysis. All group values are expressed as the mean ± SEM.

Results

Physiological conditions

1. Cross-sectional study

As expected, LH and LH/FSH ratio were significantly higher (P < 0.02 at least) in women with hyperandrogenism (30.1 ± 7.9 mIU/ml and 4.36 ± 0.73, respectively). Oestradiol values were also significantly higher (P < 0.05 at least) in hyperandrogenic women (117 ± 18 pg/ml) and in normal luteal phase (150 ± 12 pg/ml) than in normal follicular phase (67 ± 8 pg/ml).

Results of Sm-C determinations are shown in Table 1 and in Fig. 1. Sm-C values were significantly lower in normal follicular phase (group A) compared to normal luteal phase (group B) and hyperandrogenism (group C). They were similar in these last two groups. These results were not altered if the 2 subjects of group C with elevated progesterone values were excluded from the analysis.

When all three groups were considered together (n = 28), a significant positive correlation (r = 0.45, P = 0.02) was found between Sm-C and testosterone (Fig. 2). This correlation was enhanced (r = 0.67, P = 0.004) if only subjects in follicular or anovulatory phase were considered (n = 16, groups A + C). Progesterone values were log-normally distributed in the 28 subjects of this cross-sectional study. A significant positive correlation was disclosed between Sm-C and luteal progesterone (r = 0.45, P = 0.04) among the 20 subjects in follicular or luteal phase without hyperandrogenism (groups A + B). This correlation did no more reach significance when all 28 subjects (groups A + B + C) were considered together (r = 0.34, P = 0.07).

A weak positive correlation (r = 0.37, P = 0.05) was found between Sm-C and oestradiol only when all three groups were considered together.

Table 1.

<table>
<thead>
<tr>
<th>Sm-C (U/ml)</th>
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<tr>
<td>A. Normal follicular phase (n = 10)</td>
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<tr>
<td>B. Normal luteal phase (n = 10)</td>
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<tr>
<td>C. Hyperandrogenism (n = 8)</td>
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<td>C. Hyperandrogenism in follicular or anovulatory phase (n = 6)</td>
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*P < 0.02 vs A; **P < 0.005 vs A; ***P < 0.001 vs A.
2. Longitudinal study

Ovulatory cycle was evidenced in each of the 5 subjects investigated during an entire menstrual cycle. During the follicular phase, serum levels of LH, FSH, Prl, oestradiol and progesterone were all in the normal range. A normal sequence of oestradiol, FSH and LH peaks was followed by a progesterone elevation lasting at least 12 days. The mean progesterone peak value was $14.4 \pm 2.1$ ng/ml. We considered the day of the preovulatory LH surge as day 0 of the cycle. The overall Sm-C levels averaged $0.79 \pm 0.15$ U/ml; mean Sm-C levels during days +4 to +9 ($0.83 \pm 0.15$ U/ml) were significantly higher ($P < 0.02$, paired t-test) than during days −8 to −3 ($0.72 \pm 0.13$ U/ml) (Fig. 3). During those periods, progesterone values averaged $10.2 \pm 1.6$ and $0.5 \pm 0.1$ ng/ml, re-
5.0
3.0
1.0
0.5
0.3
0.1
1.00
0.75
0.50

ETHINYL-OESTRADIOL: 1mg/DAY

DAYS

Fig. 4.

Sm-C (●—●) and testosterone (○—○) patterns in 7 men before, during and after administration of 1 mg ethinyl-oestradiol daily. Values given are mean ± sem. B: basal. The asterisks denote statistically significant differences vs basal value (* P < 0.05; ** P < 0.01; *** P < 0.001). The cross denotes significant differences (P<0.025) vs day 5.

spectively. On the entire cycle, positive correlations were disclosed between daily Sm-C and ln progesterone in 3 out of the 5 subjects (r = 0.65, df = 23, P < 0.001; r = 0.36, df = 27, P = 0.05; r = 0.53, df = 26, P = 0.003). No correlation was evidenced between daily Sm-C and oestradiol.

Pharmacological conditions
1. Effects of MPA
In women receiving injections of MPA, progesterone values averaged 0.2 ± 0.05 and 0.2 ± 0.03 ng/ml 10 and 30 days after the injection, respectively; corresponding testosterone levels averaged 0.23 ± 0.02 and 0.27 ± 0.03 ng/ml. Individual Sm-C levels were widely scattered. Mean Sm-C values 10 days (1.14 ± 0.10 U/ml) and 30 days (1.07 ± 0.17 U/ml) after the injection were significantly (P < 0.005 and P < 0.02, respectively) higher than those recorded in normal follicular phase (group A) but not different from those in luteal phase (group B). Individual data are shown in Fig. 1.

2. Effects of high doses of ethinyl-oestradiol
Results are shown in Fig. 4. During ethinyl-oestradiol administration, Sm-C decreased rapidly (within 24 h) in all subjects from a basal value of 0.92 ± 0.10 U/ml to a minimum level of 0.65 ± 0.09 U/ml after 3 days (P < 0.001, paired t-test), then remained fairly stable, averaging 0.70 ± 0.10 U/ml, significantly less than the basal level (P < 0.001, paired t-test), at the end of the treatment period. After the treatment, Sm-C increased towards basal values, reaching 0.81 ± 0.11 U/ml in 2 days. This value was significantly higher (P < 0.025, paired t-test) than on the last day of treatment. Testosterone concentrations also decreased to reach a minimum value after 3 days (P < 0.001, paired t-test), then remained fairly stable until the end of the treatment.

Discussion
In the present study we found that, in healthy women, elevated progestin concentrations during
a normal ovulatory cycle as well as in pharmacological conditions (injections of MPA) were accompanied by small but consistent increases of Sm-C values. These findings are in agreement with the pharmacological experiments of Meyer III et al. (1982) who used much higher MPA doses (400 mg weekly). To our knowledge, modulation of Sm-C concentrations by physiological variations of progesterone was never described before. The finding that Sm-C and progesterone levels correlated only in women without hyperandrogenism might be due to the fact that in hyperandrogenic women, the testosterone effect on Sm-C overwhelmed a possible progesterone effect. Indeed, we found a positive correlation between Sm-C and testosterone concentrations in the cross-sectional study of the menstrual cycle. Such relationship, never disclosed in adult women, is consistent with the data of Parker et al. (1984) and Jasper et al. (1985) who described a Sm-C increase after intramuscular testosterone treatment in GH-sufficient boys, with delayed puberty and with hypogonadism, respectively. In the chimpanzee, Copeland et al. (1985) also showed in both sexes a positive correlation between Sm-C and testosterone at puberty. In girls, Rosenfield et al. (1983) demonstrated a clearcut positive relationship between the rises of testosterone and oestradiol and the rise of Sm-C during normal puberty. These findings are consistent with the experiments of Copeland et al. (1984) in castrate and intact female baboons, Cassorla et al. (1984) and Caruso-Nicolletti et al. (1985) in normal boys, Ross et al. (1983) and Cuttler et al. (1985) in Turner’s syndrome, who showed an increase of Sm-C concentrations after treatment with small doses of oestrogens. Copeland et al. (1984) also reported, in intact female baboons, elevation of Sm-C levels after administration of large doses of oestrogens. These stimulatory effects of testosterone (Craft & Underwood 1984; Parker et al. 1984; Rosenfield & Furlanetto 1985) and of oestrogens (Copeland et al. 1984; Rosenfield & Furlanetto 1985) on Sm-C appear to be mediated by growth hormone. In the present investigation in menstruating adult women, we found a positive correlation of borderline significance between Sm-C and oestradiol in the cross-sectional study. However, no correlation was disclosed during the longitudinal study; a possible correlation may well have been obscured by the biphasic pattern of oestradiol during menstrual cycle (Speroff & Vande Wiele 1971), and/or by prevailing influence of testosterone and/or progesterone.

In adult male to female transsexuals, Meyer III et al. (1982), using various doses of different oestrogens, did not find any significant effect on Sm-C at the group level. In contrast, Clemmons et al. (1980) reported that extremely high doses of ethinyl-oestradiol (1 mg daily) reduced circulating levels of Sm-C — but not consistently of GH — in acromegalic patients. In normal young adult males, we found, in the present experiment, that the same very large doses (1 mg daily) of ethinyl-oestradiol consistently reduced Sm-C. In constitutionally tall girls, Gourmelen et al. (1984) observed a progressive decline of Sm levels throughout several months of therapy with 250—300 µg/day of ethinyl-oestradiol. Finally, in postmenopausal women, Duursma et al. (1984) also reported a decrease of serum Sm-C after a 3-week period of substitutive therapy with smaller doses (20 µg/day) of ethinyl-oestradiol. Thus, very high doses of oestrogens consistently reduce Sm-C levels, while discrepant data have been reported with lower amounts, suggesting that the effect of oestrogens on Sm-C might depend on several factors including dose, duration of the treatment and age related factors such as GH secretion (which declines with age; Zadik et al. 1985). Moreover, treatment with oestrogens may induce alterations in Sm-C binding to its carrier proteins (Copeland et al. 1984). Therefore modifications in plasma levels of immunoreactive Sm-C do not always necessarily reflect similar variations in biologic activity.

In conclusion, the present study strongly suggests that during menstrual cycle in healthy adult women, Sm-C concentrations, while depending mainly on other factors, may be modulated by sex steroids. Pharmacological doses of progestagens increase Sm-C levels. Despite discrepancies in effects of pharmacological doses of oestrogens, it appears that extremely high doses of ethinyl-oestradiol decrease Sm-C values.

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References


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