

Gonadotrophin secretion patterns in testicular cancer patients with greatly increased human chorionic gonadotrophin serum concentrations

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Abstract

Despite the fact that a number of alterations of the hypothalamic–pituitary–gonadal hormone axis have been identified in patients with testicular cancer, little is known about the gonadotrophin secretion pattern in such patients who have greatly increased human chorionic gonadotrophin (hCG) serum concentrations. The aim of this study was to assess this issue in detail using a longitudinal study design and a panel of highly sensitive and specific immunoassays.

Eleven patients with non-seminomatous ($n=11$), and one with seminomatous testicular cancer with pretreatment hCG serum concentrations exceeding 10^5 pg/ml (>1000 mIU/ml) were selected and followed for a mean of 166 days (mean of 14 serum samples/patient) after initial diagnosis. Serum concentrations of hCG, its free α - (hCG α) and β - (hCG β) subunits, human follicle-stimulating hormone (hFSH) and human luteinizing hormone (hLH) were determined by highly sensitive and specific enzymometric immunoassays based on a panel of monoclonal antibodies (MCA) established in our laboratory. A potential FSH-like activity (FSA) of hCG in the respective sera was determined by radioreceptor assays (RRA) for LH/CG and FSH. Specificity of FSA at the level of the receptor was assessed by MCA-based immunoabsorption studies.

At diagnosis, hCG ($9.8 \times 10^7 \pm 4.84 \times 10^7$ pg/ml; range 1.1×10^5 – 5×10^8 pg/ml) was greatly increased and

serum hFSH was undetectable (<9 pg/ml) in 11 patients, and one patient had very low, albeit detectable (approximately 30 pg/ml) hFSH concentrations. hLH was below the limit of detection (<2 pg/ml) in five individuals. During successful chemotherapy, hCG rapidly declined to physiological concentrations and hFSH/hLH returned to normal or even reached suprphysiological values. There was a highly significant negative correlation between hCG and hFSH ($P=0.0001$) and, to a lesser extent, hLH ($P=0.0265$). The ability of serum hCG to block the binding of [¹²⁵I]rFSH (rat FSH) to its receptor was found to be 0.01–0.1% compared with the FSH standard; this could be reversed by an anti-hCG MCA. Addition of a specific MCA against hFSH blocked 3 μ g/ml of the hFSH standard, but had no effect on the FSA of serum hCG in the FSH RRA.

As observed during pregnancy, secretion of gonadotrophin – particularly that of FSH – is substantially or completely suppressed in patients with testicular cancer when serum hCG concentrations exceed 10^5 – 10^6 pg/ml ($\approx 10^3$ – 10^4 mIU/ml). As determined by RRA, the intrinsic FSA of tumour-derived hCG is most probably responsible for the suppression of hFSH in this group of patients with testicular cancer.

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Introduction

Human chorionic gonadotrophin (hCG) is secreted in high concentrations by the fetoplacental unit during pregnancy, and occasionally under pathological conditions, such as testicular tumours, molar pregnancy and choriocarcinoma (Madersbacher *et al.* 1992, Gerl *et al.* 1993, Mann *et al.* 1993, de Wit *et al.* 1997). The recent characterisation of the crystal structure of hCG revealed that this molecule is a member of the structural family of cystine knot growth

factors that includes nerve growth factor, platelet-derived growth factor and transforming growth factor β (Lapthorn *et al.* 1994). hCG, human follicle-stimulating hormone (hFSH), human luteinizing hormone (hLH) and human thyroid-stimulating hormone (hTSH) form the human glycoprotein hormone family (Berger *et al.* 1996). The biochemical similarities of these four hormones, all consisting of a common α - and hormone-specific β -subunits, are the structural basis for the suggested intrinsic cross-reactivity of hCG at the glycoprotein hormone receptor

level. This intrinsic activity of hCG might become clinically relevant when serum concentrations are high, such as during pregnancy or under the pathological circumstances indicated above. *In vitro*, hCG has a weak intrinsic TSH-like activity (TSA) and can stimulate iodine uptake, generation of cAMP, secretion of 3,5,3'-tri-iodothyronine, and even growth of rat thyroid cells (Mann *et al.* 1986, Yoshikawa *et al.* 1989, Kennedy *et al.* 1990). This *in vitro* TSA, however, is in contrast to *in vivo* observations that hyperthyroidism in patients with high circulating levels of hCG (pregnancy or testicular cancer) is only rarely observed (Norman *et al.* 1985, Madersbacher *et al.* 1993a). In addition to TSA, an intrinsic FSH-like activity (FSA) of hCG has been proposed for many years (Albert 1969, Siris *et al.* 1978, Simoni *et al.* 1989). FSA was demonstrated in extracts of pregnancy urine and serum samples and in hCG preparations (Albert 1969, Siris *et al.* 1978, Padmanabhan *et al.* 1989). Whether FSA is due to FSH cross-contamination in hCG standards or represents 'true' FSA remains a matter of debate (Simoni *et al.* 1991). Clinical data indicate that this *in vitro* FSA of hCG might be of clinical relevance. During pregnancy, there is a complete suppression of pituitary secretion of hFSH after the 4th week of gestation, as confirmed by a highly sensitive time-resolved fluoroimmunoassay (Simoni *et al.* 1989). Suppression of FSH secretion has been occasionally observed in patients with testicular cancer with increased serum concentrations of hCG (Cochran *et al.* 1975, Joos *et al.* 1993), but the exact mechanism leading to these phenomena are still unknown. With respect to hLH, no valid data are available, as previously published reports are compromised by the fact that the hLH-immunoassays yielded a high cross-reactivity with hCG (Morrish *et al.* 1990).

During the past decade, our laboratory has produced and characterised a large panel of monoclonal antibodies (MCA) directed against hCG, naturally occurring metabolic variants thereof, and hFSH, which enabled us to develop highly specific two-site MCA-based immuno-metric assays for the respective molecules (Kofler *et al.* 1982, Berger *et al.* 1988, 1990, 1996, Madersbacher *et al.* 1992, 1993b). The present study was undertaken to assess patterns of pituitary gonadotrophin secretion in those patients with testicular cancer who have excessively increased hCG concentrations at diagnosis, and subsequently to determine whether LH/FSH secretion is fully suppressed by tumour-derived hCG, as occurs in pregnancy. Thus we retrospectively analysed patterns of gonadotrophin secretion in patients with testicular cancer who had greatly increased serum concentrations of hCG before treatment and who were followed for a mean of 6 months. hCG, hLH and hFSH were determined by using the MCA-based immunoassays previously established in our laboratory (Madersbacher *et al.* 1992, 1993b). The potential FSA of hCG in the respective sera was determined by radioreceptor assay (RRA) for LH/CG and

FSH. Specificity of this FSA at the receptor level was assessed by MCA-based immunoabsorption studies.

Materials and Methods

Patients

Serum samples ($n=168$) from patients with seminomatous (SGCT; $n=1$) and non-seminomatous (NSGCT; $n=11$) germ-cell tumours were collected and kept frozen at -20°C until required for analysis. Only patients with greatly increased pretreatment serum hCG concentrations ($>10^5$ pg/ml; ≈ 1000 mIU/ml) were included. The patients were followed over a mean of 166 days after diagnosis, and several serum samples were collected during this time period (mean: 14 samples/patient). Serum samples were diluted in the immunoenzymometric assay (IEMA) buffer and were analysed in duplicate.

Hormone quantification

The two-site MCA-based IEMAs for highly specific and sensitive quantification of hCG (pg/ml), hCG α (pg/ml), hCG β (pg/ml) and hFSH (pg/ml) were performed as described previously (Madersbacher *et al.* 1992, 1993b). As hormone standards, we used hCG (highly purified, kindly provided by Dr V C Stevens, Ohio State University, Columbus, OH, USA), hCG α (CR-mix; NIADDK, Bethesda, MD, USA), hCG β (CR 125, NIADDK) and hFSH (hFSH-I-3, NIADDK) to calibrate these four IEMAs (Madersbacher *et al.* 1992, 1993b). The respective pairs of MCA chosen in the four IEMAs were selected on the basis of selectivity and epitope compatibility (Kofler *et al.* 1982, Schwarz *et al.* 1986, Berger *et al.* 1988, 1990). These four IEMAs were extensively validated. The respective sensitivities, defined as the smallest dose outside the 97% confidence limit of the zero standard, were in the range between 8–10 pg/ml (Madersbacher *et al.* 1992, 1993b). Cross-reactivities of immunoaffinity-purified homologous human glycoprotein hormones (GPH) and their free subunits were less than 0.01% in all cases, except for hFSH, hTSH, and hLH in the IEMA for the free α -subunit, in which cross-reactivities were 0.3% (Madersbacher *et al.* 1992, 1993b). The within-run coefficients of variation (CV) of the four IEMAs at 16, 125 and 500 pg/ml were between 4% and 8% (Madersbacher *et al.* 1992, 1993b). The interassay CV of these IEMAs were in the range 5–12% (Madersbacher *et al.* 1992, 1993b). Serial dilutions of sera containing high amounts of the respective analyte showed linearity ($r=0.99$) over a range of five dilution steps (Madersbacher *et al.* 1992, 1993b).

To exclude artefact caused by interferences of high hCG concentrations with the coating or detection MCA in the hFSH IEMA, we diluted the hFSH standard in a

buffer containing 5 µg hCG/ml. The resulting standard curve was compared with that of a conventional IEMA. Both standard curves were identical, indicating that even 5 µg hCG/ml did not interfere with the hFSH IEMA.

The following physiological values were calculated by using these IEMAs: holo-hCG: 0–180 pg/ml; free hCGβ: 0–32 pg/ml; free hCGα: 16–190 pg/ml; hFSH: 182–1056 pg/ml (Madersbacher *et al.* 1992, 1993c).

In the IEMA for hLH, a capture MCA specific for the hLH β-subunit (kindly provided by Boehringer Mannheim GmbH, Germany) was combined with a high-affinity MCA recognising the common α-subunit of GPH (Berger *et al.* 1990). This IEMA was performed as previously reported (Madersbacher *et al.* 1992, 1993b). In brief, 2 µg highly purified coating antibody was adsorbed to a flat-bottom microtitre plate (Virion GmbH, Würzburg, Germany) overnight at 4 °C. After blocking the remaining sites in each well with 200 µl IEMA assay buffer (PBS supplemented with BSA (10 g/l)) for 45 min at 37 °C, we incubated the hormone standard (hLH-I-1, NIADDK) diluted in 100 µl assay buffer for 90 min at 37 °C. Preparation of horseradish peroxidase-labelled detection MCA was performed as described elsewhere (Madersbacher *et al.* 1992). The detection MCA was diluted to 2 mg/l in assay buffer, added to the microtitre plate (100 µl/well), and incubated for 30 min at 37 °C. The substrate was 3,3',5,5'-tetramethylbenzidine and the colorimetric quantification was read at 450 nm. The sensitivity of this hLH-IEMA was 2 pg/ml and no cross-reactivity was observed with hFSH, hCG and hTSH at concentrations of 10 µg/ml. The within-run coefficients of variation (CV) of the hLH IEMAs at 16, 125 and 500 pg/ml were between 5% and 9%. The interassay CV of the IEMA were 10%, 8% and 7% at the lower/mid and upper assay range respectively. 17β-Oestradiol (E2) was quantified by a commercially available radioimmunoassays (RIA; DRG-Instruments GmbH, Marburg, Germany).

Radioreceptor assay

Before the RRA, the respective sera and hormone standard preparations were dialysed against 0.05 M Tris-HCl (pH 7.4) for 16 h at 4 °C. hCG (highly purified) was kindly provided by V C Stevens (Ohio State University, Columbus, OH, USA); hFSH-I-3 (NIADDK), hLH-I-1 (NIADDK) and rat FSH (rFSH-I-7) were generously supplied by the National Pituitary Agency (Baltimore, MD, USA).

Decapsulated testes from adult Sprague-Dawley rats were cut into small pieces and homogenised in 10 ml ice-cold 0.05 M Tris-HCl with an Ultra turrax tissue homogeniser (3 × 5 s), followed by three washing steps (3200 × g, 20 min, 4 °C). The pellet was diluted to 100 or 200 mg tissue wet weight (tww) per ml 0.05 M Tris-HCl supplemented with 1% bovine serum albumin (BSA) and 30 mM MgCl₂.

The RRA for hCG with radioiodinated hLH-I-1 was performed as described previously (Schwarz *et al.* 1988). The RRA for hFSH was performed with slight modifications: aliquots of the testis homogenate (250 µl, 200 mg tww/ml) were incubated with 50 µl hormone standard or serum (diluted in 0.05-M Tris-HCl/1%BSA), and 100 µl [¹²⁵I]rFSH (rat FSH) (250 000 c.p.m./tube) for 16 h at 20 °C.

Immunoabsorption of serum samples

To analyse the basis of the FSA in serum, the respective sera were preincubated with MCA specific either for the free and the combined β-subunit of hCG (β-MCA, code: INN-hCG-2), or for intact hFSH molecule (c-MCA; code: INN-hFSH-117) (Berger *et al.* 1988). The ascites-derived MCA were purified by ammonium salt precipitation and subsequently dialysed in parallel with the patient sera (see above). Before RRA, 100-µl aliquots of the MCA preparations (1 mg IgG/ml) were incubated with 50 µl serum (diluted 1 : 10) for 1 h at room temperature on an orbit shaker.

Statistical analyses

Serum concentrations of hCG, hFSH and hLH before and after multimodal therapy were compared by Spearman Rank Correlation test. In parallel, crosswise correlations of hCG, its free non-assembled subunits, hLH and hFSH were assessed by the Spearman Rank Correlation test and linear regression analyses after logarithmical transformation.

Results

Gonadotrophin secretion pattern

At diagnosis, serum concentrations of hCG ($9.8 \times 10^7 \pm 4.84 \times 10^7$ pg/ml; mean ± s.e.m.), hCGα ($6.7 \times 10^5 \pm 5.4 \times 10^5$ pg/ml) and hCGβ ($9.4 \times 10^5 \pm 5.4 \times 10^5$ pg/ml) were greatly increased in all cases. Serum hFSH was undetectable in 11 patients (<9 pg/ml), and the 12th exhibited a substantially decreased value of 30 pg/ml. hLH was undetectable (<2 pg/ml; 0.02 mIU/ml) in five individuals, six had physiological concentrations and one had increased hLH concentrations. To determine the correlation of hLH/FSH to hCG and its free subunits in more detail, the patients were followed-up for several months (Fig. 1). All patients underwent inguinal semicastration and subsequent multimodal polychemo- and radiotherapy using various treatment protocols. The patients were subsequently divided according to treatment success: seven experienced full remission. At diagnosis, hFSH had been undetectable (<9 pg/ml) in all these seven patients and hLH suppressed in three of them. Successful

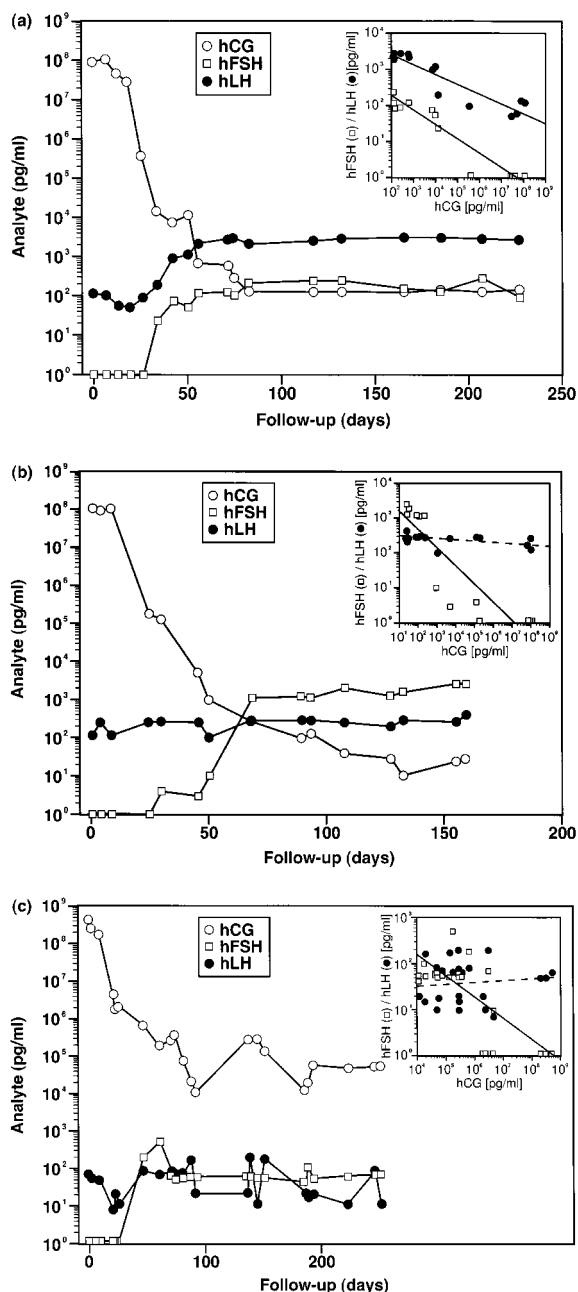


Figure 1 hCG, hFSH and hLH serum concentrations in patients with testicular cancer with successful (a, b) and unsuccessful (c) treatment. Note the logarithmic scale on the y-axis. The three parts (a,b,c) represent the data of individual patients; each dot represents a single hormone determination during the respective follow-up. The inserts indicate the correlation between hCG, hFSH and hLH, as assessed by linear regression analysis. In all three patients there was a highly significant negative correlation (r =correlation coefficient) between hCG and hFSH: a, $r = -0.94$; b, $r = -0.9$; c, $r = -0.76$). A statistically significant negative correlation between hCG and hLH was observed only in patient L.G. (a, $r = -0.96$); in the remaining two patients there was no significant correlation between hCG and hLH. Regression lines of statistical significance are indicated as continuous lines, those lacking significance as dashed lines.

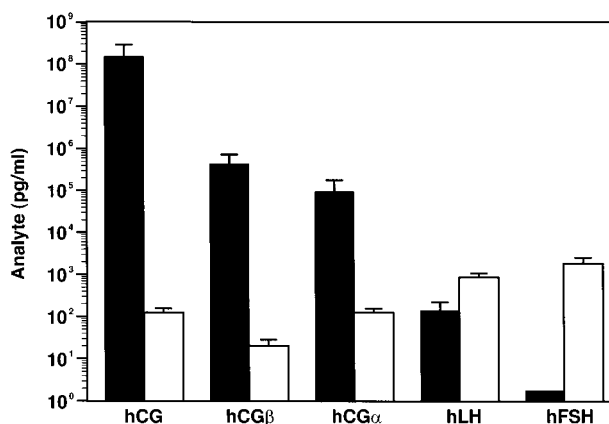


Figure 2 Pre- and post-treatment serum concentrations of hCG, its free non-assembled subunits, hLH and hFSH in the seven patients with full tumour remission. At diagnosis (■), hCG and its free subunits were significantly increased, whereas FSH was consistently undetectable (<9 pg/ml). After successful treatment (□), hCG and its free subunits were within normal ranges, paralleled by an increase of hLH and hFSH. Mean (bar) and S.E.M. are given. Note the logarithmic scale on the y-axis.

multimodal therapy (inguinal semicastration/polychemotherapy/irradiation) led to a rapid decline in hCG, and its free subunits rapidly declined to physiological values parallel to increased hFSH in all patients (Fig. 1a,b). At the last control visit, hFSH was in the normal range in two patients and increased in five (range 1020–4400 pg/ml). The correlation of hCG to hLH and hFSH in two representative patients are presented in Fig. 1a,b. Figure 2 shows hCG, its free subunits, hLH and hFSH serum values at diagnosis and the last control visit, in the seven patients who experienced full tumour remission. There was no evidence of tumour recurrence at this last visit and the levels of tumour markers were within normal limits.

Five patients experienced no complete tumour remission as defined by (i) increased hCG serum concentrations and (ii) clinical signs of residual tumour during follow-up. At diagnosis, hFSH was undetectable in four of five patients, and the remaining patient had very low hFSH concentrations (30 pg/ml). hLH was undetectable (<2 pg/ml) in one patient, in the normal range in two patients and the remaining two exhibited significantly increased concentrations (3600 and 9000 pg/ml). Figure 1c shows hCG and serum gonadotrophin concentrations of a representative patient with no complete tumour remission.

When all serum samples ($n=168$) were analysed, a statistically highly significant negative correlation between hCG and hFSH ($P=0.0001$) and, to a lesser extent, hCG and hLH ($P=0.0265$) was observed (Table 1). When serum hCG concentrations exceeded 10^5 pg/ml, hFSH was undetectable in 11 of 12 patients. To analyse steroidogenesis at changing hCG concentrations, E2 was measured

Table 1 Crosswise correlation of hCG, its free subunits, hLH and hFSH in the overall sample group: respective *P* values as determined by Spearman Rank correlation test and the corresponding correlation coefficients (*r*, in parentheses). Data were transformed logarithmically before statistical analysis

	hCGβ	hCGα	hLH	hFSH
hCG	0.0001 (<i>r</i> =0.764)	0.0001 (<i>r</i> =0.86)	0.0265 (<i>r</i> =-0.18)	0.0001 (<i>r</i> =-0.55)
hCGβ	—	0.0001 (<i>r</i> =0.74)	NS (<i>r</i> =-0.05)	0.0008 (<i>r</i> =-0.26)
hCGα	—	—	NS (<i>r</i> =-0.12)	0.0001 (<i>r</i> =-0.41)
hLH	—	—	—	0.0001 (<i>r</i> =0.62)

NS, not significant (*P*>0.05).

in 12 serum samples. A statistically significant positive correlation was calculated between hCG and E2 (*P*=0.01), whereas hFSH was negatively correlated to E2 (*P*=0.01) (Fig. 3).

RRA for hCG

The ability of tumour-derived hCG to interact with the LH/CG receptor (LH/CG-R) was studied by RRA using rat testes homogenates. The hCG standard and the patient's sera exhibited parallel displacement curves in the RRA for LH/CG, as shown in Fig. 4a for four representative patients. In two patients (L G/F R), hCG receptor binding capacity and immunoreactivity were almost identical as assessed by immunoassay. In the remaining two individuals, hCG immunoreactivity was substantially greater (B E, threefold; P J, tenfold) compared with the

respective RRA binding capacity. Immunoabsorption of the sera and the hormone standard with an MCA specific for hCGβ (code: INN-hCG-2) caused a 99.5% signal decrease, demonstrating that hCG was almost completely neutralised (Fig. 4a). The anti-hCGβ MCA (INN-hCG-2) itself did not interfere with [¹²⁵I] hLH receptor binding.

FSA of tumour-derived hCG

FSA of tumour-derived hCG was analysed by the RRA for hFSH. The ability of hCG to block binding of [¹²⁵I]FSH to its receptor was found to be approximately 0.01–0.1% in terms of the hFSH standard, suggesting that the intrinsic FSA of tumour-derived hCG is 10³–10⁴-fold less than that of hFSH (Fig. 4b). This FSA did not correlate to the hCG receptor-binding capacity, as the greatest FSA was seen in the two individuals (B E, P J) who had the lowest activity in the hCG RRA. This receptor activity could be totally blocked with the β-MCA specific for hCG (INN-hCG-2) (Fig. 5). The anti-hFSH-MCA (INN-hFSH-117) was shown to block 3 μg/ml of the hFSH standard, but had no effect on the tracer, [¹²⁵I]rFSH or the FSA in the patient's serum. This is in agreement with results obtained in the IEMA for hFSH indicating that these sera do not contain detectable hFSH.

Discussion

A number of steroid and glycoprotein hormone abnormalities, in addition to defects of spermiogenesis, have been identified in patients with testicular cancer (Cochran *et al.* 1975, Berthelsen & Skakkebaek 1983, Nijman *et al.* 1987, Morrish *et al.* 1990). The most prominent endocrine changes affect the hypothalamic–pituitary–gonadal hormone axis (Cochran *et al.* 1975, Berthelsen & Skakkebaek 1983, Morrish *et al.* 1990). Although endocrine disturbances, such as elevated hFSH serum concentrations, are frequently observed in patients with testicular cancer who do not have increased hCG, far more profound alterations, especially with respect to gonadotrophins and steroid hormones, are seen in individuals with increased hCG serum concentrations (Cochran *et al.* 1975, Berthelsen *et al.* 1983, Nijman *et al.* 1987, Hansen *et al.* 1989, Morrish *et al.* 1990, Joos *et al.* 1993, Botchan *et al.* 1997).

A number of authors have reported significantly increased hLH serum levels in patients with increased hCG. Cochran *et al.* (1975) reported mean hLH concentrations as great as 22318 ng/ml (250-fold above the upper normal range) in individuals with pathological hCG concentrations. Carroll *et al.* (1987) studied endocrine profiles in 15 patients with testicular cancer. In all three patients with hCG concentrations exceeding 10 ng/ml, hLH was substantially elevated (60.3–109 mIU/ml), as confirmed by others (Berthelsen *et al.* 1983, Morrish *et al.* 1990).

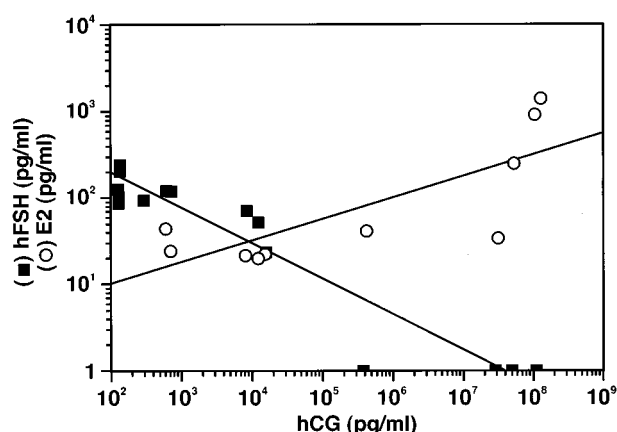


Figure 3 Correlation of 17β-oestradiol (E2, ○) and hFSH (■) concentrations with hCG serum concentrations. Data for one representative patient (F.R.) are given. hFSH concentrations were negatively correlated to hCG (*P*<0.01; *r*=-0.96) as determined by linear regression analysis, whereas E2 concentrations were positively correlated (*P*<0.01; *r*=0.78). hCG concentrations decreased during polychemotherapy by a factor 10⁶.

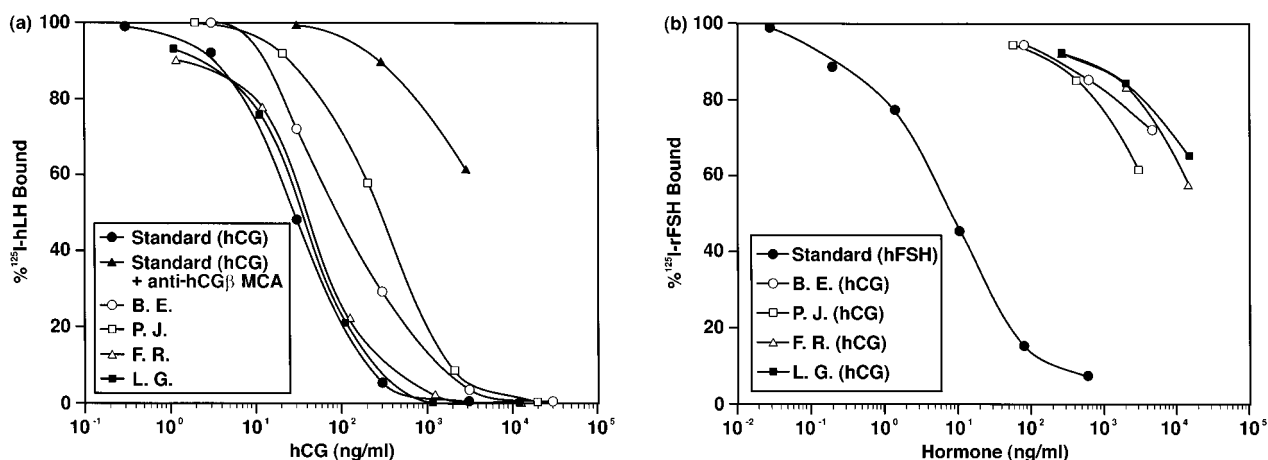


Figure 4 (a) LH and (b) FSH binding capacity of hCG in the sera of four patients with testicular cancer. (a) The hCG standard and the four patients exhibited parallel displacement curves. Immunoabsorption of the hCG standard with a MCA specific for hCG β (INN-hCG-2; standard+MCA) caused a 99.5% decrease in signal, demonstrating that hCG was almost completely neutralised. (b) The ability of tumour-derived hCG in the dialysed sera of four patients with testicular cancer to block binding of [¹²⁵I]FSH to its receptor was found to be approximately 0.01–0.1% in terms of the hFSH standard.

These data have been the subject of debate because of high hCG cross-reactivity in the respective hLH assays (Berthelsen *et al.* 1983, Morrish *et al.* 1990). Morrish *et al.* (1990), used an hLH assay with hCG cross-reactivity of 12%. These methodological limitations confirm that true gonadotrophin concentrations can be assessed only with highly specific MCA-based immunoassays. In our studies of 12 patients with excessively high hCG values at diagnosis, none showed the dramatically increased hLH

serum concentrations reported by others. In contrast, complete suppression of hLH (<2 pg/ml) was demonstrable in five of 12 individuals, and similar data were reported in a study of 54 patients with testicular cancer (Joos *et al.* 1993). In patients with increased hCG concentrations (mean 193.5 U/ml), LH was significantly lower (0.77 mIU/ml) than in those with hCG-negative tumours; however, full suppression comparable to that observed in our studies was not reported (Joos *et al.* 1993).

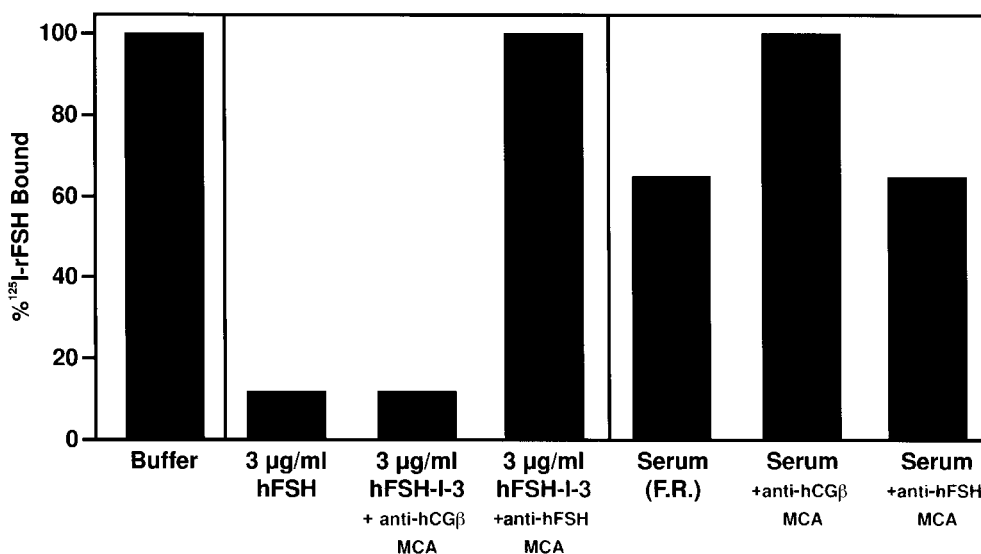


Figure 5 Effective block of FSA in the serum of patient F.R. by an MCA directed against hCG β (INN-hCG-2). The FSA of tumour-derived hCG was about 0.1% in terms of FSH-I-3 on a w/w basis. The FSA in the serum of patient F.R. (1.5 × 10⁷ pg hCG/ml) was totally blocked by the addition of an anti-hCG β MCA (INN-hCG-2), whereas addition of an anti-hFSH MCA (INN-hCG-117) had no effect. The displacement capacity of the hFSH standard (hFSH-I-3, 3 µg/ml) was entirely blocked by the anti-hFSH MCA (INN-hCG-117) which does not recognise [¹²⁵I]rFSH, but an anti-hCG MCA (INN-hCG-2) had no effect. Mean values of duplicates are given.

Concerning hFSH, the majority of studies have demonstrated suppression in patients with increased hCG concentrations (Nijman *et al.* 1987, Joos *et al.* 1993). Complete suppression of hFSH secretion, with undetectable serum concentrations, has not been observed to date despite the application of a highly sensitive immunoassay as seen in 11 of the 12 patients in our study. This finding, in parallel with the situation for hLH, is attributable to the superiority of the hFSH immunoassay used, which lacks hCG cross-reactivity, in addition to the fact that our patients were selected on the basis of excessively increased hCG concentrations at diagnosis (Berger *et al.* 1988, Madersbacher *et al.* 1993b).

What are the mechanisms responsible for these observations? First, an assay artefact caused by blocking of the respective capture MCA had to be excluded. hCG in the respective sera was present in an 10^8 molar excess over hFSH. In the two-step IEMA for hFSH, blocking the capture MCA by high concentrations of hCG can be excluded, as neither anti-hFSH MCA used in the hFSH-IEMA cross-reacts with hCG (Berger *et al.* 1988), spiking the hFSH standard with 5 µg hCG/ml did not alter the signal of the hFSH IEMA, and no bioactive hFSH was detected in these sera, as demonstrated by RRA.

In parallel with the immunoassays, the RRA was carefully designed: in the RRA for hCG, the MCA used for hCG neutralisation (INN-hCG-2) interfered with neither the [125 I]hLH tracer (even in a 20 000-fold molar excess) nor hFSH, as shown in the RRA for FSH. The MCA used in the FSH RRA (INN-hFSH-117) was both hormone- and species-specific and, therefore, recognised neither [125 I]rFSH nor hCG but, rather, neutralised the hFSH standard (Berger *et al.* 1988). As artefacts can be excluded, the potential pathomechanism involved must be considered. One possible pathway might be the induction of inhibin production by Sertoli cells, potent downregulators of pituitary hFSH secretion (De Jong 1988, Morris *et al.* 1988). It has been shown that FSH and LH independently stimulate inhibin secretion (De Jong 1988). We have shown herein that tumour-derived hCG in the sera of patients with testicular cancer competes at the level of the FSH receptor. As estimated by results of the RRA, the intrinsic FSA of hCG is 0.01–0.1% as compared with hFSH, comparable to that of highly purified hCG (Siris *et al.* 1978). Recalculating the respective pretreatment hCG concentrations (10^8 pg/ml) in terms of FSA, these sera have an apparent FSH activity of approximately 10^4 – 10^5 pg/ml and, therefore, far exceed physiological values. The calculated FSA of hCG in these sera would, at least *in vitro*, effectively stimulate the release of inhibin from Sertoli cells (Morris *et al.* 1988).

The second, more likely mechanism is mediated by increased steroid hormone production (Morrish *et al.* 1990, Joos *et al.* 1993). In fact, increased oestrogen and testosterone concentrations have frequently been observed in patients with testicular cancer. Joos *et al.* (1993) reported

greater testosterone (mean: 9.6 ng/ml) and E2 (mean 113.7 pg/ml) concentrations in hCG-positive patients with testicular cancer compared with those without increased hCG (testosterone 5.5 ng/ml, E2 31.7 pg/ml). Similarly, Cochran *et al.* (1975) observed greater E2 concentrations (76 pg/ml) in hCG-positive patients with testicular cancer than in hCG-negative individuals. We determined oestrogen concentrations in selected serum samples and also noted significantly increased concentrations (10- to 20-fold compared with physiological male values). It has been suggested that these increased steroid hormone concentrations are caused by hCG-induced stimulation of testosterone secretion by Leydig cells, and subsequent testosterone conversion via peripheral aromatisation to oestrogens. Oestrogen and testosterone are important downregulators of pituitary gonadotrophin secretion. The pathomechanism leading to endocrine disturbances in patients with testicular cancer has been studied in detail by incubating normal testicular tissue and germ cell tumour tissue with hCG in the range of 100 ng/ml corresponding to approximately 1000 mIU/ml (Morrish *et al.* 1990). It is noteworthy that pretreatment hCG concentrations in all our patients exceeded this value. Morrish *et al.* (1990) reported that normal testicular tissue responded to hCG by increased steroid hormone production, particularly of oestrogen. This increased production was not demonstrable in testicular tumour tissue, presumably as a result of the blocking of multiple enzymes necessary for steroidogenesis (Morrish *et al.* 1990).

In conclusion, we have demonstrated that secretion of pituitary gonadotrophins, particularly of hFSH, is fully suppressed in the majority of those patients with testicular cancer who have significantly increased serum concentrations of hCG. As estimated by the results of RRA, the intrinsic FSA of tumour-derived hCG is 0.01–0.1% compared with FSH. Recalculating the respective hCG serum concentrations at diagnosis ($\approx 10^8$ pg/ml) in terms of FSA, these sera have an apparent FSH-activity of $\approx 10^4$ – 10^5 pg/ml. These endocrine disturbances are most probably caused by the increased steroid hormone concentrations seen in these patients, which can be attributed at least in part to the FSH-like activity of hCG.

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