0021-972X/84/5802-0327$02.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 58, No. 2
Printed in U.S.A.

Differential Effect of Single High Dose and Divided Small Dose Administration of Human Chorionic Gonadotropin on Leydig Cell Steroidogenic Desensitization*

A. G. H. SMALS, G. F. F. M. PIETERS, G. H. J. BOERS, J. M. M. RAEMAKERS, A. R. M. M. HERMUS, Th. J. BENRAAD, and P. W. C. KLOPPENBORG

Division of Endocrinology, Department of Medicine, and Division of Experimental and Chemical Endocrinology (T.J.B.), University of Nijmegen, Nijmegen, The Netherlands

ABSTRACT. This study compared the effect of a single high dose of hCG (1500 IU) with that of the same dose administered in multiple small doses (300 IU, once daily for 5 days) on Leydig cell steroidogenesis. Administration of a single high dose of hCG to seven healthy men raised the mean plasma testosterone (T) level to peak levels 2.1 ± 0.2 (SEM) × the baseline value at 48 h. Thereafter plasma T decreased to below normal (0.7 ± 0.1 × baseline) 7 days after the injection. The mean 17-hydroxyprogesterone (17-OHP) level peaked at 24 h (2.5 ± 0.2 × baseline) and then also fell to a nadir value of 0.6 ± 0.2 × baseline on day 7. Reflecting the early accumulation of 17-OHP over T, the 17 OHP/T ratio reached its maximum (1.6 ± 0.1 × baseline) at 24 h at the same time when plasma estradiol [(E_2) 4.4 ± 0.6 × baseline] and the ratio E_2/T (2.7 ± 0.3 × baseline) achieved their maximal values. Administration of 1500 IU hCG in five divided doses of 300 IU daily increased the mean plasma T levels to peak value of 2.1 ± 0.2 × baseline at 5 days and the levels remained elevated thereafter. The response of T as reflected by the area under the curve was almost twice as great as in the single dose study (2844 ± 360 *vs.* 1647 ± 214). In contrast to the single high dose experiment, mean plasma 17-OHP levels in the divided dose protocol did not peak at 24 h but only gradually increased. As the increase of T exceeded the 17-OHP increase at almost all time intervals, no accumulation of 17-OHP over T occurred as in the single dose experiment. Instead the 17-OHP/T ratio fell to a nadir value of 0.6± 0.1 × baseline on day 7. The initial E_2 peak was absent in the divided dose protocol and the E_2/T ratio only marginally increased. Considering both experiments together a close relation was found between the hCG-induced increases in E_2 and 17-OHP ($r = +0.88$, $P < 0.001$), as well as the ratio 17 OHP/T ($r = +0.64$, $P < 0.02$). Multiple small dose hCG administration in contrast to a single high dose does not desensitize but rather enhances Leydig cell steroidogenesis, probably by preventing the early accumulation of E_2 and thereby the steroidogenic enzyme suppression which occurs after massive doses of hCG. (*J Clin Endocrinol Metab* **58:** 327, 1984)

DIRECT and indirect evidence of Leydig cell steroidogenic desensitization after administration of massive doses of gonadotropins has been well documented both in laboratory animals and man (1–17). This desensitization has been associated with decrease in the activities of the major microsomal enzymes 17α-hydroxylase and 17,20-lyase leading to accumulation of intermediates of androgen biosynthesis such as progesterone and 17-hydroxyprogesterone (17-OHP) (3–5, 7, 11, 14–16, 18). The decrease in these key enzymes after a single injection of hCG has been attributed to hCG-induced aromatase stimulation and thereby estradiol (E_2) increase, with subsequent E_2-induced inhibition of enzyme activity, mediated through a specific estrogen receptor in Leydig cells (4, 8, 9, 19, 20, 20a).

In man repeated administration of hCG 24 or 48 h after a massive dose was ineffective in further raising plasma testosterone (T) (11, 13, 14). In contrast plasma E_2 and 17-OHP levels further increased, which is compatible with estrogen-mediated augmentation of the 17,20-lyase block (11, 14) induced by the first injection. In this study we compared the effect of a single high dose of hCG (1500 IU) with that of the same dose in multiple small doses (300 IU daily for 5 days) on circulating T, 17-OHP, and E_2 levels in normal men in order to determine the role of estrogens in the process of hCG-induced Leydig cell desensitization. The data indicate that repeated small doses of hCG in contrast to one large dose do not desensitize but rather enhance Leydig cell steroidogenesis.

Subjects and Methods

Seven normal men (laboratory personnel, with proven fertility and normal testicular size without a history of gynaecomas-

Received May 19, 1983.
Address requests for reprints to: Dr. A. G. H. Smals, Division of Endocrinology, University of Nijmegen, Nijmegen, The Netherlands.
* Presented in part at the 65th Annual Meeting of The Endocrine Society, San Antonio, TX, June 8–10, 1983.

tia, cryptorchidism, or prenatal exposure to diethyl-stilbestrol), mean age, 31.2 ± 10.1 (SD); range, 18–48 yr, volunteered for the study and gave informed consent. In Exp I the men received a single im injection of 1500 IU hCG (Pregnyl, Organon, Oss, The Netherlands) at 0800 h on day 0. Blood for measurement of T, 17-OHP, and E_2 was collected before and 1, 2, 3, 4, 5, and 7 days after the injection. In Exp II, subjects were given 300 IU hCG daily for 5 days starting at 0800 h on day 0. Blood for hormone determinations was collected at 0800 h immediately before the first injection and 1, 2, 3, 4, 5, and 7 days thereafter. On days 1, 2, 3, and 4 blood was sampled immediately before the subsequent hCG injection. In four men Exp II was preceded by Exp I; in 3 men the order was reversed. There was always an interval of at least 2 weeks. Plasma 17-OHP, T, and E_2 levels were measured by RIA. After incubation of 2 ml plasma with 8000 dpm [^{3}H]T, 17-OHP, and E_2, used as internal standards to correct for procedural losses, the plasma samples were extracted with 15 ml diethylether and the extracts dried under a purified stream of air and redissolved in diethylether. The extraction residues were chromatographed for 3.5 h in a modified Bush A solvent system [petroleum-methanol-water (450:350:200)] using Whatman no I paper. The areas of E_2 (1–4 cm), 17-OHP (9–13 cm), and T (14–18 cm) were located by radioscanning and eluted with 1, 2, and 8 ml water-ethyleneglycol (0.2%), respectively. ^{3}H-Recovery was measured by counting 15% of the eluate. The eluates of T and 17-OHP were radioimmunoassayed as previously described (21, 22). Paper eluate fractions (0.1, 0.2, and 0.3 ml) were incubated overnight at 4 C with 7000 dpm [^{3}H]E_2 (New England Nuclear, Boston, MA, NET 317) and 1:300,000 diluted antiserum, raised in rabbits against E_2-6 carboxy methyl oxime-BSA (Organon, Oss R5-658-TO). Dextran-coated charcoal was used for separation of free and antiserum bound E_2. The sensitivity of the RIA was about 2 pg/tube, the lowest limit of detection of 6 pg/ml plasma. The intraassay replicate variation was 3%. To avoid interassay variation all samples from a single subject were measured in the same assay. Statistical analyses were performed using Wilcoxon's paired rank test (P, values denoted by P), Friedman's nonparametric analysis of variance (P^x), and Spearman's rank correlation test (P^{xx}). The mean values ± 1 SEM are given, unless otherwise stated.

Results

After administration of a single dose of hCG (1500 IU) plasma T levels increased significantly ($P^x < 0.0005$) from a mean of 565 ± 38 (SE) ng/100 ml before to a maximum of 1178 ± 78 ng/100 ml (2.1 ± 0.2 × baseline, $P < 0.02$ *vs.* t = 0) 48 h after the injection (Fig. 1). Thereafter the mean level decreased to the pretreatment value on day 5, and was below baseline 7 days after the injection (411 ± 39 ng/100 ml, $P < 0.05$ *vs.* t = 0). Small divided doses of hCG significantly ($P^x < 0.0005$) increased the mean plasma T level from 573 ± 31 ng/100 ml at t = 0 ($P > 0.10$ *vs.* Exp. I) to a peak of 1135 ± 91 ng/100 ml (2.1 ± 0.2 × baseline, $P > 0.10$ *vs.* Exp. I) on day 5 after the first injection. Despite a slight decrease to 1.7 ± 0.2 × baseline on day 7 ($P < 0.02$ *vs.* day 5), the

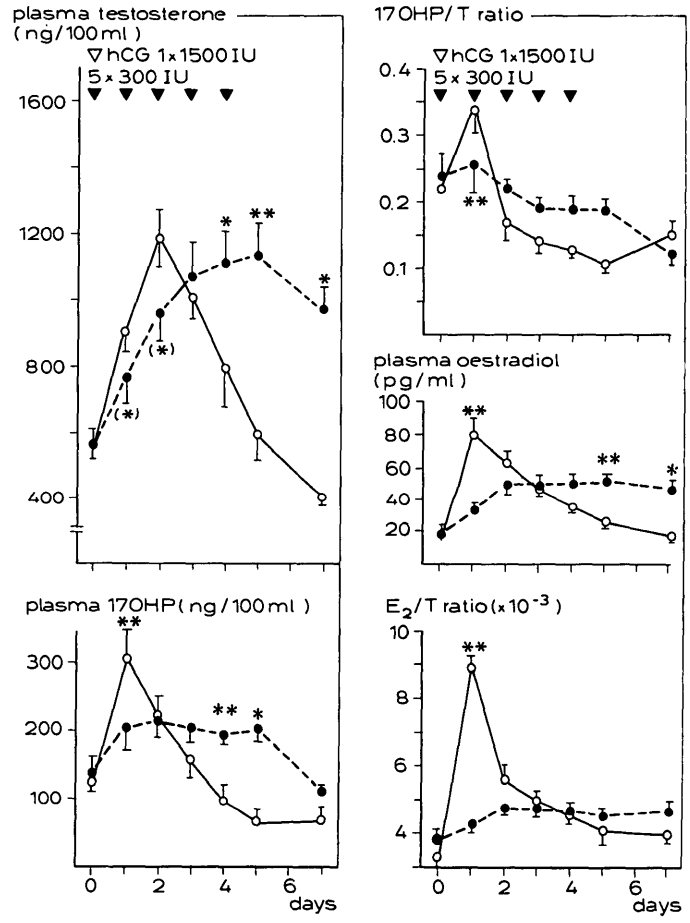

FIG. 1. The mean absolute responses of plasma T, 17-OHP, Ei2 and their ratios, 17 OHP/T and E_2/T to a single high dose injection of hCG (Pregnyl, 1500 IU im) (O——O) and repeated small doses (300 IU daily for 5 days) (●- - -●) in seven normal men. The *asterisks* indicate statistically significant differences between the single and multiple dose experiments. (*), $P < 0.10$; *, $P < 0.05$; **, $P < 0.02$.

mean plasma T value (975 ± 58 ng/100 ml) remained significantly elevated for a much longer period than in the single high dose experiment ($P < 0.05$ *vs.* Exp. I). Comparing the plasma T responses in both experiments by calculating the areas under the 0- to 7-day T curves, the overall response in the divided dose experiment was almost two-fold greater than in the single dose experiment (2844 ± 360 *vs.* 1647 ± 214 area U, $P < 0.05$), six of seven men having an increase of 69 to 113% (mean 92 ± 17%). Only in one man was the area similar in both protocols (1881 and 1797 area U).

Single high dose hCG administration increased the mean plasma 17-OHP to maximum levels 2.5 ± 0.2 × baseline at 24 h ($P < 0.02$ *vs.* t = 0). Thereafter 17-OHP levels significantly decreased ($P^x < 0.0005$) to baseline on day 3 and to nadir values (0.6 ± 0.1 × baseline, $P < 0.05$ *vs.* t = 0) on days 5 and 7 ($0.10 < P > 0.05$ *vs.* t = 0). In contrast to Exp. I, in the divided dose protocol the mean 17-OHP levels did not peak at 24 h but only

gradually increased ($P^x < 0.005$) and plateaued from day 2 until day 5 at a level 1.6–1.7 × baseline. Thereafter the 17-OHP levels fell to pretreatment values. In Exp I a transient accumulation of 17-OHP over T, as reflected by an elevated 17-OHP/T ratio (1.6 ± 0.1 × baseline, $P < 0.02$ *vs.* t = 0), was found at 24 h. Thereafter the ratio fell significantly ($P^x < 0.01$) to a nadir value of 0.6 ± 0.1 × baseline at day 5 ($P < 0.05$ *vs.* t = 0). In contrast to Exp I, in the divided dose protocol the ratio 17-OHP/T did not peak at 24 h, but rather gradually fell throughout the experiment ($P^x < 0.0005$) to a nadir value of 0.6 ± 0.1 × baseline ($P < 0.05$ *vs.* t = 0) on day 7 after the first injection. At no time point beyond 24 h did the 2 curves differed significantly.

Parallel with plasma 17-OHP levels and the ratio 17-OHP/T, E_2 levels in Exp I dramatically increased from a mean basal value of 19 ± 2 pg/ml to a peak level 4.4 ± 0.6 × baseline ($P < 0.02$ *vs.* t = 0) at 24 h and then significantly decreased ($P^x < 0.001$) to a nadir value 20% below the pretreatment level on day 7. As the increase in plasma E_2 exceeded the rise in plasma T the ratio E_2/T also steeply increased, achieving its maximum (2.7 ± 0.3 × baseline, $P < 0.02$ *vs.* t = 0) at 24 h. Thereafter the ratio rapidly fell reaching pretreatment values on day 7. In contrast to the single high dose experiment plasma E_2 levels in Exp II rather gradually increased from a basal level of 20 ± 2 pg/ml to a plateau 2.3–2.5 × baseline between days 2 and 7. As the increase in plasma E_2 almost paralleled the rise in T, the E_2/T ratio in the divided dose protocol did not peak at 24 h but instead marginally increased to highest levels 1.3 to 1.4 × baseline between days 2 and 7.

Considering Exps I and II together, a close direct correlation was found between the 24-h increases of E_2 and 17-OHP ($r = 0.88$, $P^{xx} < 0.001$) and to a lesser extent between the increases of E_2 and the ratio 17-OHP/T ($r = 0.64$, $P = 0.02$). The correlation between the increase of E_2 at 24 h and the response of T as reflected by the area under the curve ($r = -0.46$, $0.10 > P^{xx} > 0.05$) lacked statistical significance.

Discussion

The present study clearly demonstrated that the effect of hCG on Leydig cell steroidogenesis differs depending on the mode of its administration; hCG in small divided doses elicited an almost 2-fold greater increase in T than the same dose given by a single injection despite similar average serum hCG levels over the 7-day period. Furthermore, a single high dose of hCG gave rise to temporary accumulation of 17-OHP over T at 24 h after the injection, compatible with the occurrence of a steroidogenic block localized at the 17,20-lyase step. Such a steroidogenic block did not occur after administration of the same dose of hCG in small divided doses. The enhancement of T production by small doses of hCG as compared to a single high dose and the occurrence of 17,20-lyase block 24 h after high dose administration but not after the repeated lower doses suggest that the Leydig cells are desensitized only by massive doses of hCG. The 20–30% fall in plasma T, 17-OHP, and E_2 7 days after single high dose hCG administration may reflect overall attenuation of Leydig cell steroidogenesis early in the biosynthetic pathway before 17-OHP (18). In rodents desensitization of Leydig cell steroidogenesis generally has been associated with a decrease of the cytochrome P 450 dependent key enzymes 17α-hydroxylase and 17,20-lyase (2–4, 6–9). If accumulation of 17-OHP over T indicates that in man high doses of hCG induce a steroidogenic enzymatic block, then the fall in 17 OHP/T after repeated small doses of hCG can be interpreted to mean enhancement of 17,20-lyase activity by repeated hCG administration, facilitating Leydig cell steroidogenesis. In fact Payne and co-workers (23, 24) very recently demonstrated in rats that repeated low dose hCG administration for 6 days increased activities of both 17α-hydroxylase and 17,20-lyase and thereby T production by the Leydig cell despite substantial LH receptor loss. The same dose in a single injection decreased the activities of both enzymes as evidenced by desensitization of the Leydig cell, lowered T production, and accumulation of 17-OHP over T. The mechanism by which hCG increased these enzyme activities (*de novo* synthesis, inhibition of enzyme decay, or removal of an inhibitor) is not clear (25). The data in the present study indirectly suggest that in man a similar mechanism might be operative: repeated low doses of hCG enhance Leydig cell steroidogenesis, whereas the same total dose given as single injection down-regulates steroid biosynthesis by inducing an, albeit transient, enzymatic 17,20-lyase block. The decrease in 17-OHP accumulation relative to testosterone after the peak might be due to relief of the enzymatic block later and/or tropic effects on other enzymes involved in testicular steroidogenesis which may facilitate T production (24, 26).

In rodents the decrease in 17α-hydroxylase and 17,20-lyase after high dose hCG administration has been attributed to hCG mediated increases of E_2 by some authors (4, 8, 9, 19, 20, 20a, 27, 28), but denied by others (29–33). Our data in man demonstrate that there is a close temporal relationship between the response patterns of E_2 and 17-OHP in the high dose experiment and also between the hCG-induced E_2, 17-OHP, and 17 OHP/T increments after 24 h when considering both experiments together. Accumulation of 17-OHP over T only occurred when there was a concomitant steep rise in E_2 relative to T levels as in the high dose experiment. After small divided dose hCG administration no 24-h E_2 peak

occurred and there was no accumulation of 17-OHP over T, but rather a decrease in the 17 OHP/T ratio. In this experiment plasma E_2 levels paralleled the rise in T until 24 h although from 48 h on there was a slight preponderance of E_2 over T, which may be due to hCG-induced stimulation of aromatase (34–36). Together these results provide indirect evidence for a causal relation between the hCG-induced initial E_2 release and the occurrence of the 17,20-lyase block.

Summarizing, the data demonstrate that small divided dose hCG administration in contrast to a single high dose enhanced testicular T production probably by preventing early E_2 accumulation and thereby suppression of steroidogenic enzymes which occurs after massive doses of hCG.

Acknowledgments

The authors would like to acknowledge the technical assistance of Mrs. W. van de Velden-van Leeuwen, Mr. E. Wilms, Mr. H. Hofman, and the secretarial assistance of Mrs. W. Straten.

References

1. Haour F, Saez JM 1978 Leydig cell responsiveness to LH-hCG stimulation: mechanism of hCG and steroid induced refractoriness. In: McKerns KW (ed) Structure and function of the gonadotropins. Plenum Press, New York, p 497
2. Cigorrage SB, Dufau ML, Catt KJ 1978 Regulation of luteinizing hormone receptors and steroidogenesis in gonadotropin-desensitized Leydig cells. J Biol Chem 253:4297
3. Dufau ML, Cigorraga S, Baukal AJ, Sorrell S, Bator JM, Neubauer JF, Catt KJ 1979 Androgen biosynthesis in Leydig cells after testicular desensitization by luteinizing hormone releasing hormone and human chorionic gonadotropin. Endocrinology 105:1314
4. Cigorraga SB, Sorrell S, Bator J, Catt KJ, Dufau ML 1980 Estrogen dependence of a gonadotropin-induced steroidogenic lesion in rat testicular Leydig cells. J Clin Invest 65:699
5. Glass AR, Vigersky RA 1980 Resensitization of testosterone production in men after human chorionic gonadotropin induced desensitization. J Clin Endocrinol Metab 51:1395
6. Chasalow F, Marr H, Saez JM 1979 Testicular steroidogenesis after human chorionic gonadotropin desensitization in rats. J Biol Chem 254:5613
7. Nozu K, Deheya A, Zawistowich L, Catt KJ, Dufau ML 1981 Gonadotropin induced receptor regulation and steroidogenic lesions in cultured Leydig cells. J Biol Chem 256:12875
8. Nozu K, Dufau ML, Catt KJ 1981 Estradiol receptor-mediated regulation of steroidogenesis in gonadotropin-desensitized Leydig cells. J Biol Chem 256:1915
9. Nozu K, Matsura S, Catt KJ, Dufau ML 1981 Modulation of Leydig cell androgen biosynthesis and cytochrome P 450 levels during estrogen treatment and human chorionic gonadotropin-induced desensitization. J Biol Chem 256:10012
10. Martikainen H, Huhtaniemie I, Vihko R 1980 Response of peripheral sex steroids and some of their precursors to a single injection of hCG in adult men. Clin Endocrinol (Oxf) 13:157
11. Forest M, Lecoq A, Saez JM 1979 Kinetics of human chorionic gonadotropin induced steroidogenic response of the human testis. II plasma 17α hydroxyprogesterone, Δ^4androstenedione, estrone and 17β estradiol: evidence for the action of human chorionic gonadotropin on intermediate enzymes implicated in steroid biosynthesis. J Clin Endocrinol Metab 49:284
12. Saez JM, Forest M 1979 Kinetics of human chorionic gonadotropin-induced steroidogenic response of the human testis. I plasma testosterone: implications for human chorionic gonadotropin stimulation test. J Clin Endocrinol Metab 49:278
13. Smals AGH, Pieters GFFM, Drayer JIM, Benraad ThJ, Kloppenborg PWC 1979 Leydig cell responsiveness to single and repeated hCG administration. J Clin Endocrinol Metab 49:12
14. Smals AGH, Pieters GFFM, Lozekoot DC, Benraad ThJ, Kloppenborg PWC 1980 Dissociated responses of plasma testosterone and 17 hydroxyprogesterone to repeated human chorionic gonadotropin administration in normal men. J Clin Endocrinol Metab 50:190
15. Smals AGH, Pieters GFFM, Kloppenborg PWC, Lozekoot DC, Benraad TJ 1980 Lack of biphasic steroid response to single human chorionic gonadotropin administration in patients with isolated gonadotropin deficiency. J Clin Endocrinol Metab 50:879
16. Wang C, Rebar RW, Hopper BR, Yen SSC 1980 Functional studies of the luteinizing hormone-Leydig cell androgen axis: exaggerated response in C-18 and C-21 testicular steroids to various modes of luteinizing hormone stimulation. J Clin Endocrinol Metab 51:201
17. Nozu K, Dehejia A, Zawistowich L, Catt KJ, Dufau ML 1982 Gonadotropin-induced desensitization of Leydig cells *in vivo* and *in vitro*: estrogen action on the testis. In: Bardin CW, Sherins RJ (eds) The cell biology of the testis. Ann New York Acad Sci 383:212
18. Smals AGH, Boers GHJ, Roose PHC, Pieters GFFM, Beex LVAM, Kloppenborg PWC 1982 Enhancement of gonadotrophin induced 17,20 lyase suppression one week after human chorionic gonadotrophin priming. Acta Endocrinol (Copehn) 100:308
19. Onoda M, Hall PF 1981 Inhibition of testicular microsomal cytochrome P-450 (17α-hydroxylase/C17,20 lyase) by estrogens. Endocrinology 109:763.
20. Donaldson MD, Saez J, Forest MG 1981 Effects of the aromatase inhibitor 1,4,6,androstatriene 3,17 dione and the antiestrogen tamoxifen on rat testicular function. J Steroid Biochem 14:823

20a. Brinkman AO, Leemborg FG, Roodnat EM, de Jong FH, van der Molen HJ 1980 A specific action of estradiol on enzymes involved in testicular steroidogenesis. Biol Reproduction 23:801

21. Smals AGH, Kloppenborg PWC, Lequin RM, Benraad TJ 1976 The effect of gonadotropin releasing hormone on pituitary gonadal function in Klinefelter's syndrome. Acta Endocrinol (Copenh) 83:829
22. Smals AGH, Kloppenborg PWC, Pieters GFFM, Lozekoot DC, Benraad TJ 1978 Basal and human chorionic gonadotropin stimulated 17 hydroxyprogesterone and testosterone levels in Klinefelter's syndrome. J Clin Endocrinol Metab 47:1144
23. Payne AH, Wong KL, Vega NM 1980 Differential effects of single and repeated administration of gonadotropins on luteinizing hormone receptors and testosterone synthesis in two populations of Leydig cells. J Biol Chem 255:7118
24. Shaughnessy PJ, Payne AH 1982 Differential effects of single and repeated administrations of gonadotropins on testosterone production and steroidogenic enzymes in Leydig cell populations. J Biol Chem 257:11503
25. Chasalow F, Marr H, Detection and charaterization of a hormone-dependent inhibitor of steroidogenesis from rat testes. Program of the 62nd Annual Meeting of the Endocrine Society, Washington, DC, 1980, p 134 (Abstract 239)
26. Hall PF 1970 Gonadotropin regulation of testicular function. In: Nes EK (ed) The androgens of the Testis. Marcel Dekker, New York, p 73
27. Smals AGH, Pieters GFFM, Drayer JIM, Boers GHJ, Benraad TJ, Kloppenborg PWC 1980 Tamoxifen suppresses gonadotropin-induced 17α-hydroxyprogesterone accumulation in normal men. J Clin Endocrinol Metab 51:1026
28. Paltero S, Bartke A, Brodie A, Mayfield D 1983 Effects of testosterone, estradiol, aromatase inhibitor, gonadotropin and prolactin on the response of mouse testes to acute gonadotropin stimulation. J Ster Biochem 18:391
29. Kühn-Velten N, Staib W, Time course of cytoplasmic estradiol receptor and microsomal cytochrome P-450 depletion in rat testis after treatment with hCG and estradiol-17β. 2nd European Workshop on Molecular and Cellular Endocrinology of the Testis. Boekelo, The Netherlands, 1982 (Abstract B12)
30. Brinkman AO, Leemborg FG, van der Molen HM 1981 hCG induced inhibition of testicular steroidogenesis, an estradiol mediated process? Mol Cell Endocrinol 24:65

31. Brinkman A, Leemborg I, Rommerts F, van der Molen H 1982 Translocation of the testicular estradiol receptor is not an obligatory step in the gonadotropin induced inhibition of 17.20 lyase. Endocrinology 110:1834
32. van Bergeyk L, Gooren LJG, de Vries CP, van der Veen EA, Effect of short and long term administration of tamoxifen in adult men. 2nd European Workshop on Molecular and Cellular Endocrinology of the Testis. Boekelo, The Netherlands, 1982 (Abstract B17)
33. Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WY 1983 Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors and sperm production despite high estradiol levels. J Clin Endocrinol Metab 56:720
34. Payne AH, Valladares LE 1980 Regulation of testicular aromatization by luteinizing hormone. In: Steinberger A, Steinberger E (eds) Testicular development, Structure and Function. Raven Press, New York, p 185
35. Pomerantz DK 1981 Human chorionic gonadotropin enhances the ability of gonadotropic hormones to stimulate aromatization in the testis of the rat. Endocrinology 109:1096
36. Valladares LE, Payne AH 1981 Effects of hCG and cyclic AMP on aromatization in purified Leydig cells of immature and mature rats. Biol Reprod 25:752