CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS

D.J. KWEKKEBOOM

CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS

(KLINISCH NIET-FUNCTIONERENDE EN GONADOTROFE HYPOFYSEADENOMEN)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. C.J. RIJNVOS
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
DONDERDAG 2 NOVEMBER 1989 OM 13.30 UUR.

DOOR

DIRK JAN KWEKKEBOOM

GEBOREN TE GOES

PROMOTIECOMMISSIE

Promotor: Prof. Dr. S.W.J. Lamberts

Overige leden: Prof. Dr. J.C. Birkenhäger
Prof. Dr. G.P. van Rees
Prof. Dr. J.J. van der Werff ten Bosch

Co-promotor: Dr. F.H. de Jong

CONTENTS

1. INTRODUCTION

1.1 CYTOLOGY OF THE ANTERIOR PITUITARY LOBE AND PHYSIOLOGY OF GONADOTROPIN SECRETION.

1.1.1 Function and cytology of the anterior pituitary lobe.

The pituitary gland, surrounded by the spenoid bone and covered with the sellar diaphragm, lies in the sella turcica, near the hypothalamus and optic chiasm. The anterior pituitary lobe, which constitutes the major part of the pituitary gland, produces various hormones: growth hormone (GH), prolactin (PRL), adenocorticotroph hormone (ACTH), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Based on light microscopy, the glandular cells of the adenohypophysis can be divided in three types: chromophobe, acidophilic and basophilic cells, which represent, approximately, 50, 40 and 10% of the total number of cells. The chromophilic, i.e. acidophilic and basophilic, cells contain secretory granules which have great affinity for specific dyes, while the chromophobe cells do not contain visible secretory granules when investigated by light microscopy. On electron microscopic examination, however, the majority of chromophobe cells can be shown to have small secretory granules, and therefore these cells should also be regarded as active glandular cells (1).

Immunocytochemistry allows a more sophisticated classification of secretory cells. Hormone-specific antibodies that bind intracellularly to the hormone to be studied are applied to the pituitary tissue. Thereafter, the tissue is incubated with a second antibody coupled to peroxidase, and finally the tissue is incubated in 3,3'-diaminoazobenzidine which forms a brown precipitate in the sites that contain the hormone. Thus, it is possible to classify cells according to the hormones they contain. The frequency distribution of secretory cells according to immunocytochemistry is given in figure 1. It is presumed that each cell-type contains only one hormone, with the exception of

cells that contain the gonadotropins LH and FSH. Though some of these cells may contain either of the gonadotropins (3,4), the current view is that almost all gonadotroph cells contain both LH and FSH (2,5). It should be noticed, however, that the presence of more than one hormone is not limited to gonadotroph cells, as cells that contain both GH and PRL have been demonstrated recently (6). Therefore, the percentages given in figure 1 should be regarded as rough estimates.

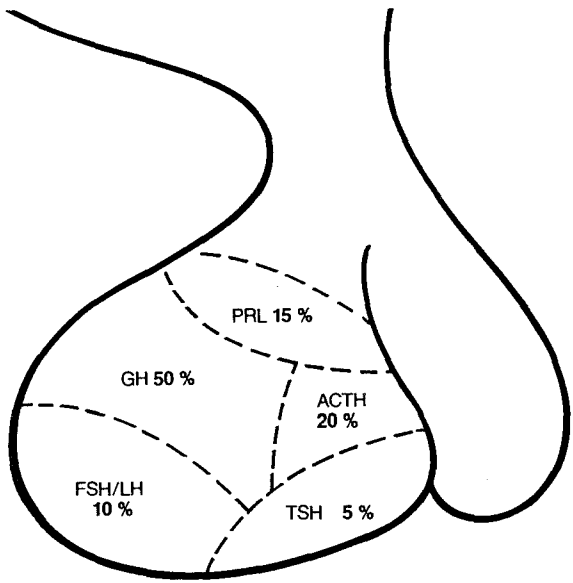

Figure 1: Frequency distribution of secretory cells in the anterior pituitary according to immuncytochemistry. (From: Kovacs K et al.: Anatomy and histology of the normal and abnormal pituitary gland (2))

1.1.2 Structure and physiology of the gonadotropins.

The gonadotropins LH and FSH have a molecular weight of approximately 30.000 and are composed of an α- and a ß-subunit, just like TSH and the placental hormone chorionic gonadotropin

(CG). The α-subunits of these glycoprotein hormones are identical, while the ß-subunits are hormone-specific (7,8). The interspecies similarity in a number of animals is greatest for the ß-subunits, while the α-subunits may differ widely (9).

The hypothalamic hormone gonadotropin releasing hormone (GnRH) or luteinizing hormone releasing hormone (LHRH) reaches the pituitary by route of hypothalamic hypophyseal portal system. It stimulates the production and release of the gonadotropins. The release of GnRH is influenced by opioids and mono-amines, whereas steroids can influence the release of these two substances (10).

In both sexes, the release of gonadotropins is influenced by the circulating levels of the steroids of which they promote the production. In men testosterone inhibits the release of LH (11), while in women estrogens may exert both positive and negative feedback on LH release, depending on the serum concentrations of these steroids during the menstrual cycle. Testosterone in men and estradiol in women also suppress FSH release, but the gonadal hormone inhibin is thought to be more important in regulating FSH levels (12). Apart from a direct effect on the pituitary gonadotroph, estradiol and testosteron are thought to affect the opioid control of GnRH release (10,13).

In both sexes the gonadotropins stimulate the release of steroids by their target organs. In men LH stimulates the production of testosterone in the testicular interstitial cells, while in women LH stimulates the ovarian producion of progestins, androgens and estrogens. In men FSH, through its effect on the Sertoli cells, has a role in the control of spermatogenesis (14). In women FSH, through its action on the ovarian granulosa cells promotes follicle growth and stimulates the synthesis of estradiol from androgens (15).

1.1.3 Dynamic tests of gonadotropin release in normal subjects.

Administration of GnRH results in a rise in serum gonadotropins and free α-subunit, both in men and in women (16,17).

Administration of TRH to normal men has been reported to cause

slight increases in LH or FSH levels. The reported results, however, are contradictory, as some groups report an increase in LH but not FSH levels (18,19), while others report the opposite (20,21).

The opiate antagonist naloxone causes a rise in serum gonadotropin concentrations in men and premenopausal women. This is due to the antagonizing effect of this drug on the inhibitory action of opioids on GnRH release (10,13,22).

Administration of estradiol in women and of testosterone in men causes a decrease in serum gonadotropin and α-subunit levels (11,23-26).

1.2 PITUITARY NONFUNCTIONING AND GONADOTROPH ADENOMAS.

1.2.1 Prevalence and presenting symptoms.

Pituitary adenomas are benign neoplasms arising in the adenohypophyseal cells. Pituitary adenomas can be classified according to several characteristics: according to the clinical presentation (1), to the hormones hypersecreted **in vivo** (2), to the chromophilia of the tumor tissue (3), or to the immunocytochemical properties of the tumor tissue (4). The second and fourth classification are the ones most commonly used, the classification based on elevated serum hormone concentrations being pre-operative, the classification based on tumor immunocytochemistry being post-operative.

In figure 2 the prevalence of various types of pituitary adenomas in large series of surgically removed pituitary tumors is shown. The adenomas are classified according to the immunocytochemical properties of the tissue.

When one compares figure 2 with figure 1, which lists the frequencies of secretory cells according to immunocytochemistry in the normal pituitary, a marked infrequency of adenomas that contain one of the glycoprotein hormones, LH, FSH or TSH, can be noticed. Like PRL secreting cells, glycoprotein hormone secreting cells account for about 15% of the secretory cells of the anterior pituitary (figure 1). However, PRL secreting adenomas represent 25-30% and glycoprotein hormone secreting adenomas less than 5% of all operated adenomas.

Based on elevated serum gonadotropin concentrations, the gonadotroph cell adenomas are rarely encountered: they account for 1.7 to 3.3% of the total of surgically removed pituitary adenomas (29,30). Almost all patients who are clinically recognized as having a gonadotroph adenoma are men (29-56; for reviews see 40,57). In virtually all patients serum FSH levels are elevated and frequently serum α-subunit concentrations are also high. Hypersecretion of LH is seldomly described and may in a substantial number of reported cases be ascribed to elevated levels of uncombined gonadotropin subunits which crossreact in

the LH radioimmunoassay (RIA) (40).

Pure α-subunit secreting pituitary adenomas have also been reported predominantly in men (51,55,58-62). Though it is questionable whether these adenomas can be regarded gonadotroph adenomas, some of these tumors have been shown to secrete gonadotropins when cultured (63) (see Chapter 1.2.5).

Clinically nonfunctioning adenomas do not cause any signs or symptoms related to the hypersecretion of a pituitary hormone. They are characterized by the absence of hypersecretion of any pituitary hormone **in vivo**.

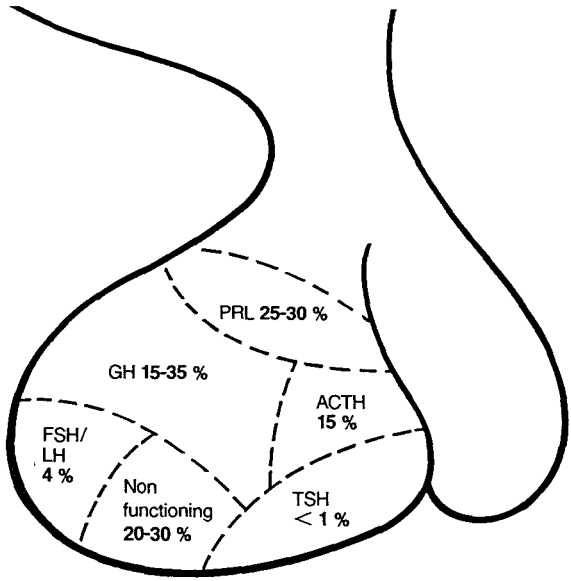

Figure 2: Prevalence of pituitary adenomas classified according to immunocytochemistry in large series of surgically removed tumors. In the group of nonfunctioning adenomas no or very few hormone positive cells could be detected. Except for adenomas that contain both gonadotropins, plurihormonal adenomas have not been listed. (From: Kovacs et al. (27) and Wilson et al. (28))

Patients with a gonadotroph, α-subunit secreting or clinically nonfunctioning pituitary adenoma have several clinical characteristics in common:

-They are elderly people. From the literature, the mean age of the patients can be estimated to be 50 to 60 years in all three patient groups (30-34,36,37,39,41-56,58-61,64-66).

-Unlike most patients with a GH or PRL producing adenoma, these patients have no symptoms caused by overproduction of a hormone.

-Almost all patients have macroadenomas with suprasellar extension. Symptoms caused by local pressure of the tumor tissue, i.e. visual field defects, loss of visual acuity and headache, are present in the majority of cases. Loss of libido and impotence in men and sudden amenorrhoea in women are also frequent. Hypopituitarism may be present (28,29,31-37,40,43,44, 57,58,60,67-69).

1.2.2 Etiology.

The cause of gonadotroph, α-subunit secreting and clinically nonfunctioning pituitary adenomas is unknown. There are, however, indications that support the hypothesis that primary hypogonadism might play a role in the development of these tumors:

-A high incidence of pituitary gonadotroph cell like adenomas can be found 15 months or more after gonadectomy in both male and female rats (70).

-In some patients with a gonadotroph adenoma, castration or ovarian ablation had been performed decades before the pituitary tumor was diagnosed (52,71,72).

-Gonadotroph, α-subunit secreting, and clinically nonfunctioning pituitary adenomas occur in elderly patients. Apart from adenomas that cause symptoms, subclinical pituitary adenomas which do not give rise to any endocrinological abnormalities or complaints have been found in 13% of the cases at unselected autopsies of men and women over 80 years of age. About 50% of these tumors have no immunocytochemical activity (73). In both normal men and women, sex steroids are low in aging as compared

to young subjects. In men there is an age-dependent decrease in serum free testosterone concentrations and a moderate increase in serum gonadotropin levels (74), while in elderly, postmenopausal, women estrogen concentrations are low and serum gonadotropin levels are high.

On the other hand, the majority of patients with a gonadotroph, clinically nonfunctioning or α-subunit secreting adenoma have a history of normal gonadal function and have children. In most men with such pituitary adenomas, a significant rise in serum testosterone in response to CG is noted (75), while in premenopausal patients ovarian estradiol production may be normal or become normal after therapy (54), indicating that primary defects in the gonads do not play a role. Also, in aging men a moderate age-associated decrease in serum free testosterone concentrations occurs (74), while in postmenopausal women serum estrogen concentrations are dramatically low in comparison with those in premenopausal women. If hypogonadism were an etiological factor in the development of gonadotroph, α-subunit secreting and clinically non-functioning pituitary adenomas, the incidence of these tumors should be higher in women than in men. However, no sex-related difference in the incidence of these tumors exists.

Lastly, a hypothalamic cause of these tumors, in particular changes in GnRH pulse frequency or amplitude, should be considered. In ovariectomized monkeys bearing hypothalamic lesions a GnRH pulse given every hour restores the release of LH and FSH. Higher frequencies reduce the release of gonadotropins whereas lower frequencies lead to a rise in FSH levels only (76). In men with idiopathic hypogonadotropic hypogonadism, a reduction in the pulse frequency of administered GnRH leads to increased serum FSH concentrations (77). In the majority of patients with a gonadotroph pituitary adenoma serum FSH concentrations are high whereas LH levels are not elevated. In patients with a clinically nonfunctioning pituitary adenoma serum gonadotropin concentrations may be low (see Chapter 1.2.4). If serum gonadotropin concentrations in these patients were caused by abnormalities in the GnRH pulse frequency, GnRH pulse frequency might be expected to be low in patients with a gonadotroph pituitary adenoma, and

high in patients with a clinically nonfunctioning pituitary tumor.

In conclusion, whether gonadotroph, α-subunit secreting and clinically nonfunctioning adenomas are caused by mild or overt hypogonadism, remains unclear. Also, it is uncertain whether these tumors are caused by primary pituitary or primary hypothalamic abnormalities (40).

1.2.3 Morphology.

Most gonadotroph adenomas are chromophobic on light microscopic examination, and some may in part be basophilic. The cells are arranged in a sinusoidal pattern (47).

Immunocytochemistry reveals in most of the tumors cells that are positive for FSH, α-subunit or, less frequently, LH. In women, tumors that have the ultrastructural features of gonadotroph adenomas, do not immunostain in about 50% of the cases (47, see below).

On electron microscopic examination, most gonadotroph adenomas can be shown to consist of elongated cells with small nuclei. The Rough Endoplasmatic Reticulum (RER) can be moderately or well developed, and may form cisternae. Numerous free ribosomes may be observed. Small secretory granules measuring 50-250 nm vary in number from cell to cell and from tumor to tumor. A striking feature of the cytoplasm is the abundance of microtubules (30,47). Oncocytic transformation of the cells, i.e. an abundance of mitochondria, may be observed in a number of cases (30). Horvath and Kovacs (47) noted a difference in the fine structure of gonadotroph cell adenomas from women compared to those from men: a honey-comb like Golgi apparatus could be observed in the adenomas from women. The functional relevance of this observation is not clear. It should also be noticed that only 1 of 15 women with a gonadotroph adenoma described by these authors had documented elevated serum gonadotropin concentrations, and that their definition of these adenomas depends largely on the immunocytochemical or ultrastructural features of the cells.

Morphological data on pure α-subunit secreting adenomas are

scarce. Tumor material from patients with elevated serum α-subunit concentrations immunostains for α-subunit, but not for the gonadotropins (58). Ultrastructurally, this group of patients has not been described as a separate entity.

Clinically nonfunctioning adenomas appear chromophobic on light microscopic examination. Their cells may be arranged in a sinusoidal or diffuse pattern. By immunocytochemistry, these adenomas are either negative for all pituitary hormones or they contain only a few scattered cells staining for the glycoprotein hormones. Alternatively, they may consist of cells reacting with antibodies to α-subunit (78,79). Electron microscopy reveals tumors consisting of small cells with a poorly developed RER, clusters of free ribosomes and a variable number of small, rod-shaped mitochondria. Secretory granules, measuring up to 250 nm, are scarce. Oncocytic transformation may be noticed (80).

1.2.4 In vivo hormone data and dynamic tests on gonadotropin release.

Virtually all patients with a pituitary gonadotroph adenoma, as defined by elevated serum gonadotropin concentrations, are men (see 40,57 for reviews). Serum FSH concentrations are supranormal in the majority of cases, and can be accompanied by high levels of FSHß or α-subunit. Elevated serum LH concentrations are reported infrequently and can often be ascribed to crossreaction of α-subunit in the LH immunoassay (30,39,40,43,46). In some cases, however, reported LH levels are too high to be caused by α-subunit crossreactivity (54) or are accompanied by high testosterone levels (36,37,50).

Pure α-subunit secreting pituitary adenomas, characterized by elevated serum concentrations of only α-subunit, have been described also chiefly in men (51,55,58-62). The infrequency of pure α-subunit secreting adenomas and gonadotroph adenomas in women may be due to the fact that most female patients are postmenopausal; the high normal values of the gonadotropins and α-subunit after the menopause will mask the secretion of these hormones by the tumor.

Clinically nonfunctioning pituitary adenomas are characterized by the absence of hypersecretion of any pituitary hormone **in vivo**. Serum LH, FSH and α-subunit concentrations may be within the normal range or may be low (45,55,64,69).

Administration of GnRH to patients with a gonadotroph, α-subunit secreting or clinically nonfunctioning adenoma may result in either a supranormal, normal or low response in serum gonadotropin and subunit concentrations (38,58-60,75, see Chapter 5).

Administration of TRH to patients with a gonadotroph adenoma results in exaggerated responses in serum LH and FSH levels compared to normal subjects (18, see Chapter 5).

Hormone responses to other substances will be discussed in Chapter 1.2.6.

1.2.5 In vitro hormone data.

Surgically removed pituitary tumor cells can be dispersed and cultured. Adenomas which cause elevated serum gonadotropin concentrations will release these glycoprotein hormones **in vitro**, while virtually no other hormones can be detected. **In vivo** and **in vitro** hormone responses to drugs and hormones are well correlated (36,40,63,81).

The release of gonadotropins and their subunits from cultured gonadotroph adenoma cells can be stimulated by TRH and GnRH (39,43,53,71). The dopamine agonist bromocriptine can suppress the release of FSH, LH and their subunits to a variable extent (43).

Pituitary adenomas which cause elevated serum levels of only α-subunit, may release both α-subunit and FSH **in vitro** (63).

In recent years, it has been reported that clinically nonfunctioning adenomas may release gonadotropins and their subunits **in vitro** (45,53,63,64,71,82). Compared to gonadotroph adenomas, clinically nonfunctioning adenomas release only small amounts of these hormones and subunits (63). Expression of one or more glycoprotein hormone genes (α-subunit, LHß, FSHß and TSHß) may be observed, while GH and PRL gene expression is absent (55).

1.2.6 Experimental treatments and therapy.

Transsphenoidal surgery is the accepted treatment for gonadotroph, α-subunit secreting and clinically nonfunctioning pituitary adenomas. This may be followed by radiotherapy (28,35,58). Improvement of visual field defects after surgery is observed in the majority of cases (28,35,83), and peripheral concentrations of hormones or subunits that were hypersecreted preoperatively return to the normal range (35,58). During the 6-week postoperative period, mortality in a large series was 0.4%, while major morbidity like cerebrospinal fluid leak and bacterial meningitis occurred in about 10% of the cases (28).

The administration of bromocriptine can lower serum gonadotropin and α-subunit concentrations in patients with a gonadotroph or α-subunit secreting tumor (31,33,41,43,50,62). Improvement of visual field defects in a patient with a gonadotroph adenoma after 1 week of bromocriptine treatment (7.5 mg daily) has been reported, though after 1 year of treatment no change in tumor size could be demonstrated by Computed Tomographic (CT) scan (41). In another patient with a gonadotroph adenoma 2 months of therapy with increasing doses of bromocriptine (from 7.5 to 20 mg daily) resulted in a slight reduction in tumor mass by CT scan (50). Recently, Klibanski et al. (62) reported a decrease in tumor size in 2 of 4 patients with an α-subunit secreting pituitary tumor who were treated with bromocriptine up to 10 mg daily for 6 weeks.

The administration of bromocriptine to patients with clinically nonfunctioning adenomas by several researchers has led to conflicting results: Johnston et al. (66) reported a decrease in tumor size in a patient with a clinically nonfunctioning adenoma after 25 months of bromocriptine treatment (20 mg daily), while after 3 months no change in tumor size by CT scan could be shown. Wass et al. (84) also reported a reduction in tumor size in a patient treated with 7.5 mg bromocriptine daily for 4 months. On the other hand, Barrow et al. (85) did not note any reduction in tumor volume in 7 patients with a clinically nonfunctioning adenoma treated with 7.5 mg bromocriptine daily for 6 weeks,

while Grossman et al. (86) observed the same in 12 patients treated for 3 to 36 months (median: 6.5 months) with 3 different dopamine agonists. Lastly, Zarate et al. (87) found no amelioration of vision or tumor volume reduction in 7 patients treated for 2 to 23 weeks with bromocriptine in dosages of 15-22.5 mg daily.

The administration of testosterone in men and estradiol in women with a gonadotroph adenoma lowers serum gonadotropin and α-subunit concentrations, but to a lesser extent than in normal subjects (34,54). No data on tumor volume after long-term treatment with steroids are available.

In a patient with a gonadotroph adenoma, treatment with a GnRH analog for 3 weeks resulted in an increased secretion of LH and α-subunit (37). Recently, 2 groups reported on the effects of treatment for 8 to 9 weeks with these analogs in patients with a gonadotroph adenoma. The effects on serum LH and FSH concentrations were variable, while serum α-subunit levels were higher than pretreatment values in all patients. No change in tumor volume after GnRH analog treatment could be demonstrated by CT scan in any patient (51,56).

1.3 REFERENCES.

1. Junqueira LC, Carneiro J, Contopoulos AN. Basic histology. Lange Medical Publishers, Los Altos 1975;pp 375-6.
2. Kovacs K, Horvath E, Ezrin C. Anatomy and histology of the normal and abnormal pituitary gland. In: DeGroot L et al., eds. Endocrinology. WB Saunders Company, Philadelphia 1989;pp 266-7.
3. Pelletier G, Leclerc R, Labrie F. Identification of gonadotropic cells in the human pituitary by immunoperoxidase technique. Mol Cell Endocrinol 1976;6:123-36.
4. Childs GV, Hyde C, Naor Z, Catt K. Heterogeneous luteinizing hormone and follicle stimulating hormone storage patterns in subtypes of gonadotropes separated by centrifugal elutiration. Endocrinology 1983;113:2120-28.
5. Pelletier G, Robert F, Hardy J. Identification of human anterior pituitary cells by immunoelectron microscopy. J Clin Endocrinol Metab 1978;46:534-42.
6. Lloyd RV, Anagnostou D, Cano M, Barkan AL, Chandler WF. Analysis of mammosomatotropic cells in normal and neoplastic human pituitary tissues by the reverse hemolytic plaque assay and immunocytochemistry. J Clin Endocrinol Metab 1988; 66:1103-10.
7. Hagen C, McNeilly AS. Identification of human luteinizing hormone, follicle-stimulating hormone, luteinizing hormone ß-subunit and gonadotrophin α-subunit in foetal and adult pituitary glands. J Endocr 1975;67:49-57.
8. Pierce JG. The subunits of pituitary thyrotropin - their relationship to other glycoprotein hormones. Endocrinology 1971;89:1331-44.
9. Vaitukaitis JL, Ross GT, Reichert LE Jr., Ward DN. Immunologic basis for within and between species cross-reactivity of luteinizing hormone. Endocrinology 1972;91:1337-42.
10. Rasmussen DD. New concepts in the regulation of hypothalamic gonadotropin releasing hormone (GnRH) secretion. J Endocrinol Invest 1986;9:427-37.
11. Marynick SP, Loriaux DL, Sherins RJ, Pita JC, Lipsett MB. Evidence that testosterone can suppress pituitary gonadotropin secretion independently of peripheral aromatization. J Clin Endocrinol Metab 1979;49:396-8.
12. De Jong FH. Inhibin. Physiol Rev 1988;68:555-607.
13. Bicknell RJ. Endogenous opioid peptides and hypothalamic neuroendocrine neurones. J Endocr 1985;107:437-46.
14. Martin CR. Endocrine physiology. Oxford University Press, New York, 1985;pp 600-1.
15. Chappel SC, Ulloa-Aguirre A, Coutifaris C. Biosynthesis and secretion of follicle-stimulating hormone. Endocrine Rev 1983;4:179-211.
16. Dufau ML, Beitins IZ, Mc Arthur JW, Catt KJ. Effects of luteinizing hormone releasing hormone (LHRH) upon bioactive and immunoreactive serum LH levels in normal subjects. J Clin Endocrinol Metab 1976;43:658-67.
17. Rosemberg E, Bulat G. Immunoreactive α and ß subunits of follicle stimulating and luteinizing hormones in peripheral blood throughout the menstrual cycle and following stimula-

tion with synthetic gonadotropin releasing hormone (GnRH). J Endocrinol Invest 1979;2:233-9.
18. Snyder PJ, Muzyka R, Johnson J, Utiger RD. Thyrotropin releasing hormone provokes abnormal follicle-stimulating hormone (FSH) and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 1980;51:744-8.
19. Anderson MS, Bowers CY, Kastin AJ, Schalch DS, Schally AV, Snyder PJ, Utiger RD, Wilber JF, Wise AJ. Synthetic thyrotropin releasing hormone. N Eng J Med 1971;285:1279-83.
20. Mortimer CH, Besser GM, McNeilly AS, Turnbridge AS, Gomez Pan A, Hall R. Interaction between secretion of gonadotropins, prolactin, growth hormone, thyrotrophin, and corticosteroids in man: the effect of LH/FSH-RH, TRH and hypoglycemia alone and in combination. Clin Endocrinol (Oxf) 1973;2:317-26.
21. Bremner WJ, De Kretser DM, Burger HG. Increases in serum concentrations of follicle stimulating hormone (FSH) during thyrotrophin-releasing hormone (TRH) infusions in normal men. Clin Endocrinol (Oxf) 1977;7:399-404.
22. Vermeulen A, Deslypere JP, Kaufman JM. Influence of antiopioids on luteinizing hormone pulsatility in aging men. J Clin Endocrinol Metab 1989;68:68-72.
23. Veldhuis JD, Evans WS, Rogol AD, Kolp L, Thorner MO, Stumpf P. Pituitary self-priming actions of gonadotropin-releasing hormone. J Clin Invest 1986;77:1849-56.
24. Deslypere JP, Kaufman JM, Vermeulen T, Vogelaers D, Vandalem JL, Vermeulen A. Influence of age on pulsatile luteinizing hormone release and responsiveness of the gonadotrophs to sex hormone feedback in men. J Clin Endocrinol Metab 1987; 64:68-73.
25. Veldhuis JD, Samojlik E, Evans WS, Rogol AD, Ridgeway EC, Crowley WF, Kolp S, Checinska E, Kirschner MA, Thorner MO, Stumpf P. Endocrine impact of pure estradiol replacement in postmenopausal women: alterations in anterior pituitary hormone release and circulating sex steroid hormone concentrations. Am J Obstet Gynecol 1986;155:334-9.
26. Andreasson B, Bostofte E. Influence of 2 mg estradiol-17ß on circulating FSH, LH, total and unconjugated estradiol levels in post-menopausal women. Acta Obstet Gynecol Scand 1981;60: 555-8.
27. See Ref 2;pp 271-81.
28. Wilson CB, Dempsey LC. Transsphenoidal microsurgical removal of 250 pituitary adenomas. J Neurosurg 1978;48:13-22.
29. Beckers A, Stevenaert A, Mashiter K, Hennen G. Follicle-stimulating hormone-secreting pituitary adenomas. J Clin Endocrinol Metab 1985;61:525-8.
30. Trouillas J, Girod C, Sassolas G, Claustrat B, Lheritier M, Dubois MP, Goutelle A. Human pituitary gonadotropic adenoma; histological, immunocytochemical, and ultrastructural and hormonal studies in eight cases. J Pathology 1981;135:315-36.
31. Berezin M, Olchovsky D, Pines A, Tadmor R, Lunenfeld B. Reduction of follicle-stimulating hormone (FSH) secretion in FSH-producing pituitary adenoma by bromocriptine. J Clin Endocrinol Metab 1984;59:1220-3.
32. Borges JLC, Ridgway EC, Kovacs K, Rogol AD, Thorner MO.

Follicle-stimulating hormone-secreting pituitary tumor with concomitant elevation of serum α-subunit levels. J Clin Endocrinol Metab 1984;58:937-41.
33. Chapman AJ, Macfarlaine IA, Shalet SM, Beardwell CG, Dutton J, Sutton ML. Discordant serum α-subunit and FSH concentrations in a woman with a pituitary tumour. Clin Endocrinol 1984;21:123-9.
34. Friend JN, Judge DM, Sherman BM, Santen RJ. FSH-secreting pituitary adenomas: stimulation and suppression studies in two patients. J Clin Endocrinol Metab 1976;43:650-7.
35. Harris RI, Schatz NJ, Gennarelli T, Savino PJ, Cobbs WH, Snyder PJ. Follicle-stimulating hormone-secreting pituitary adenomas: correlation of reduction of adenoma size with reduction of hormonal hypersecretion after transsphenoidal surgery. J Clin Endocrinol Metab 1983;56:1288-93.
36. Peterson RE, Kourides IA, Horwith M, Vaughan Jr. ED, Saxena BB, Fraser RAR. Luteinizing hormone- and α-subunit-secreting pituitary tumor: positive feedback of estrogen. J Clin Endocrinol 1981;52:692-8.
37. Roman SH, Goldstein M, Kourides IA, Comite F, Bardin CW, Krieger DT. The luteinizing hormone-releasing hormone (LHRH) agonist [D-Trp^{6}-Pro^{9}-Net] LHRH increased rather than lowered LH and α-subunit levels in a patient with an LH-secreting pituitary tumor. J Clin Endocrinol Metab 1984;58:313-9.
38. Snyder PJ, Johnson J, Muzyka R. Abnormal secretion of glycoprotein α-subunit and follicle-stimulating hormone (FSH) ß-subunit in men with pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 1980;51:579-84.
39. Snyder PJ, Bashey HM, Kim SU, Chappel SC. Secretion of uncombined subunits of luteinizing hormone by gonadotroph cell adenomas. J Clin Endocrinol Metab 1984;59:1169-75.
40. Snyder PJ. Gonadotroph cell adenomas of the pituitary. Endocrine Rev 1985;6:552-63.
41. Vance ML, Ridgway EC, Thorner MO. Follicle-stimulating hormone- and α-subunit-secreting pituitary tumor treated with bromocriptine. J Clin Endocrinol Metab 1985;61:580-4.
42. Whitaker MD, Prior JC, Scheithauer B, Dolman L, Durity F, Pudek MR. Gonadotrophin-secreting pituitary tumour: report and review. Clin Endocrinol 1985;22:43-8.
43. Lamberts SWJ, Verleun T, Oosterom R, Hofland L, van Ginkel LA, Loeber JG, van Vroonhoven CCJ, Stefanko SZ, de Jong FH. The effects of bromocriptine, thyrotropin-releasing hormone, and gonadotropin-releasing hormone on hormone secretion by gonadotropin-secreting pituitary adenomas in vivo and in vitro. J Clin Endocrinol Metab 1987;64:524-30.
44. Wide L, Lundberg PO. Hypersecretion of an abnormal form of follicle stimulating hormone associated with suppressed luteinizing hormone secretion in a woman with a pituitary adenoma. J Clin Endocrinol Metab 1981;53:923-30.
45. Asa SL, Gerrie BM, Singer W, Horvath E, Kovacs K, Smyth HS. Gonadotropin secretion in vitro by human pituitary null cell adenomas and oncocytomas. J Clin Endocrinol Metab 1986; 62:1011-9.
46. Demura R, Jibiki K, Kubo O, Odagiri E, Demura H, Kitamura K, Shizume K. The significance of α-subunit as a tumor marker for gonadotropin-producing pituitary adenomas. J Clin

Endocrinol Metab 1986;63:564-9.
47. Horvath E, Kovacs K. Gonadotroph adenomas of the human pituitary: sex-related fine-structural dichotomy. Am J Pathol 1984;117:429-40.
48. Korsic M, Belas-Bahun N, Surdonja P, Besenski N, Horvat S, Plavsic V. Infarction of FSH-secreting pituitary adenoma. Acta Endocrinol 1984;107:149-54.
49. Koide Y, Kugai N, Kimura S, Fujita T, Kameya T, Azukizawa M, Ogata E, Tomono Y, Yamashita K. A case of pituitary adenoma with possible simultaneous secretion of thyrotropin and follicle-stimulating hormone. J Clin Endocrinol Metab 1982;54:397-403.
50. Klibanski A, Deutsch PJ, Jameson JL, Ridgway EC, Crowley WF, Hsu DW, Habener JF, Black PMcL. Luteinizing hormone-secreting pituitary tumor: biosynthetic characterization and clinical studies. J Clin Endocrinol Metab 1987;64:536-42.
51. Klibanski A, Jameson JL, Biller BMK, Crowley Jr. WF, Zervas NT, Rivier J, Vale WW, Bikkal H. Gonadotropin and α-subunit responses to chronic gonadotropin-releasing hormone analog administration in patients with glycoprotein hormone-secreting pituitary tumors. J Clin Endocrinol Metab 1989; 68:81-6.
52. Nicolis G, Shimshi M, Allen C, Halmi NS, Kourides IA. Gonadotropin-producing pituitary adenoma in a man with long-standing primary hypogonadism. J Clin Endocrinol Metab 1988;66:237-41.
53. Daniels M, Newland P, Dunn J, Kendall-Taylor P, White MC. Long-term effects of a gonadotrophin-releasing hormone agonist ([D-Ser(But)^6]GnRH(1-9)nonapeptide-ethylamide) on gonadotrophin secretion from human pituitary gonadotroph cell adenomas in vitro. J Endocr 1988;118:491-96.
54. Cook DM, Watkins S, Snyder PJ. Gonadotrophin-secreting pituitary adenomas masquerading as primary ovarian failure. Clin Endocrinol 1986;25:729-38.
55. Jameson JL, Klibanski A, Black PMcL, Zervas NT, Lindell CM, Hsu DW, Ridgway EC, Habener JF. Glycoprotein hormone genes are expressed in clinically nonfunctioning pituitary adenomas. J Clin Invest 1987;80:1472-8.
56. Sassolas G, Lejeune H, Trouillas J, Forest MG, Claustrat B, Lahlou N, Loras B. Gonadotropin-releasing hormone agonists are unsuccesful in reducing tumoral gonadotropin secretion in two patients with gonadotropin-secreting pituitary adenomas. J Clin Endocrinol Metab 1988;67:180-5.
57. Ridgway EC. Glycoprotein hormone production by pituitary tumors. In: Black PMcL et al., eds. Progress in endocrine research and therapy (vol 1). Raven Press, New York, 1984;pp 343-63.
58. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 1983;59:585-9.
59. Kourides IA, Weintraub BD, Rosen SW, Ridgway EC, Kliman B, Maloof F. Secretion of alpha subunit of glycoprotein hormones by pituitary adenomas. J Clin Endocrinol Metab 1976; 43:97-106.
60. Ridgway EC, Klibanski A, Ladenson PW, Clemmons D, Beitins IZ, McArthur JW, Martorana MA, Zervas NT. Pure alpha-secreting pituitary adenomas. N Eng J Med 1981;304: 1254-9.

61. Ishibashi M, Yamaji T, Takaku F, Teramoto A, Fukushima T. Secretion of glycoprotein hormone α-subunit by pituitary tumors. J Clin Endocrinol Metab 1987;64:1187-93.
62. Klibanski A, Shupnik MA, Bikkal HA, Black PMcL, Kliman B, Zervas NT. Dopaminergic regulation of α-subunit secretion and messenger ribonucleic acid levels in α-secreting pituitary tumors. J Clin Endocrinol Metab 1988;65:96-102.
63. Snyder PJ, Bashey HM, Phillips JL, Gennarelli TA. Comparison of hormonal secretory behavior of gonadotroph cell adenomas in vivo and in culture. J Clin Endocrinol Metab 1985;61: 1061-5.
64. Yamada S, Asa SL, Kovacs K, Muller P, Smyth HS. Analysis of hormone secretion by clinically nonfunctioning human pituitary adenomas using the reverse hemolytic plaque assay. J Clin Endocrinol Metab 1989;68:73-80.
65. Eseri MM, Bevan JS, Burke CW, Adams CBT. Effect of bromocriptine treatment on the fibrous tissue content of prolactin-secreting and nonfunctioning macroadenomas of the pituitary gland. J Clin Endocrinol Metab 1986;63:383-8.
66. Johnston DG, Hall K, McGregor A, Ross WM, Kendall-Taylor P, Hall R. Bromocriptine therapy for "nonfunctioning" pituitary tumors. Am J Med 1981;71:1059-61.
67. Arafah BM. Reversible hypopituitarism in patients with large nonfunctioning pituitary adenomas. J Clin Endocrinol Metab 1986;62:1173-9.
68. Sassolas G, Trouillas J, Lejeune H, Claustrat B, Girod C. A clinical and biological study of ten cases of pituitary gonadotropic adenomas, revealed by immunocytochemistry and intratumorous assay. In: Lamberts SWJ et al., eds. Trends in diagnosis and treatment of pituitary adenomas. Free University Press, Amsterdam, 1984;pp 267-72.
69. Mukai K. Pituitary adenomas. Immunocytochemical study of 150 tumors with clinicopathologic correlation. Cancer 1983; 52:648-53.
70. Griesbach WE, Purves HD. Basophil adenomata in the rat hypophysis after gonadectomy. Br J Cancer 1960;14:49-59.
71. Surmont DWA, Winslow CLJ, Loizou M, White MC, Adams EF, Mashiter K. Gonadotrophin and alpha subunit secretion by human 'functionless' pituitary adenomas in cell culture: long term effects of luteinizing hormone releasing hormone and thyrotrophin releasing hormone. Clin Endocrinol 1983; 19:325-36.
72. Kovacs K, Horvath E, Rewcastle NB, Ezrin C. Gonadotroph cell adenoma of the pituitary in a woman with longstanding hypogonadism. Arch Gynecol 1980;229:57-65.
73. Kovacs K, Ryan N, Horvath E, Singer W, Ezrin C. Pituitary adenomas in old age. J Gerontol 1980;35:16-22.
74. Deslypere JP, Vermeulen A. Leydig cell function in normal men: effect of age, lifestyle residence, diet, and activity. J Clin Endocrinol Metab 1984;59:955-62.
75. Snyder PJ, Brigdeli H, Gardner DF, Mihailovic V, Rudenstein RS, Sterling FH, Utiger RD. Gonadal function in fifty men with untreated pituitary adenomas. J Clin Endocrinol Metab 1979;48:309-14.
76. Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PR, Knobil E. Frequency and amplitude of gonadotro-

pin-releasing hormone stimulation and gonadotropin secretion in the Rhesus monkey. Endocrinology 1981;109:376-85.
77. Gross KM, Matsutomo AM, Bremner WJ. Differential control of luteinizing hormone and follicle-stimulating hormone secretion by luteinizing hormone-releasing hormone pulse frequency in man. J Clin Endocrinol Metab 1987;64:675-80.
78. Kovacs K, Horvath E, Ryan N, Ezrin C. Null cell adenoma of the human pituitary. Virchows Arch [Pathol Anat] 1980; 387:165-74.
79. Landolt AM, Heitz PU. Alpha-subunit-producing pituitary adenomas. Virchows Arch [Pathol Anat] 1986;409:417-31.
80. Landolt AM, Oswald UW. Histology and ultrastructure of an oncocytic adenoma of the human pituitary. Cancer 1973; 31:1099-1105.
81. Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SWJ. Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. J Endocrinol Invest 1984;7:307-11.
82. Mashiter K, Adams E, Van Noorden S. Secretion of LH, FSH and PRL shown by cell culture and immunocytochemistry of human functionless pituitary adenomas. Clin Endocrinol 1981; 15:103-12.
83. Blaauw G, Braakman R, Cuhadar M, Hoeve LJ, Lamberts SWJ, Poublon RML, Singh R, Wijngaarde R. Influence of transsphenoidal hypophysectomy on visual deficit due to a pituitary tumour. Acta Neurochir (Wien) 1986;83:79-82.
84. Wass JAH, Williams J, Charlesworth M, Kingsley DPE, Halliday AM, Doniach I, Rees LH, McDonald WI, Besser GM. Bromocriptine in management of large pituitary tumours. Br Med J 1982; 284:1908-11.
85. Barrow DL, Tindall GT, Kovacs K, Thorner MO, Horvath E, Hoffman Jr. JC. Clinical and pathological effects of bromocriptine on prolactin-secreting and other pituitary tumors. J Neurosurg 1984;60:1-7.
86. Grossman A, Ross R, Charlesworth M, Adams CBT; Wass JAH, Doniach I, Besser GM. The effect of dopamine agonist therapy on large functionless pituitary tumours. Clin Endocrinol 1985;22:679-86.
87. Zarate A, Moran C, Kleriga E, Loyo M, Gonzalez-Angulo A, Aquilar-Parada E. Bromocriptine therapy as pre-operative adjunct of non-functional pituitary macro-adenomas. Acta Endocrinol 1985;108:445-50.

2. AIMS AND SCOPE OF THE THESIS.

This thesis deals with clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. From the literature discussed in the preceding chapter several questions arise. Below these questions are listed with a reference to where the subject concerned was mentioned or discussed in the preceding chapter, as well as with a reference to the chapter where these questions will be discussed in detail.

1. (1.2.5) Do all clinically nonfunctioning pituitary adenomas release gonadotropins and their subunits **in vitro**? (4,5,6)

2. (1.2.5) Can the hormone release from clinically nonfunctioning adenomas **in vitro** be suppressed and stimulated with hormones and drugs? (5,6)

3. (1.2.3,1.2.5) Is the secretory activity of clinically nonfunctioning, α-subunit secreting, and gonadotroph adenomas better reflected in results from cell culture than in results from immunocytochemistry? (6)

4. (1.2.1,1.2.5) Are clinically nonfunctioning, α-subunit secreting, and gonadotroph pituitary adenomas to be regarded as one group? (5,6)

5. (1.2.4) Is the exaggerated response in serum gonadotropin levels to TRH limited to patients with a gonadotroph adenoma? (5)

6. (1.2.6) Can bromocriptine significantly suppress serum gonadotropin and α-subunit concentrations in patients with a clinically nonfunctioning adenoma and will these patients benefit from prolonged treatment with bromocriptine? (5,8)

7. (1.1.2,1.2.2) By analogy with the findings in aging men, do serum estradiol concentrations decrease and gonadotropin levels increase in aging postmenopausal women? (7)

8. (1.2.6) Can GnRH analogs be beneficial in patients with gonadotroph pituitary adenomas? (8)

9. Which drugs are promising in the future treatment of gonadotroph, α-subunit secreting, and clinically nonfunctioning pituitary adenomas? (8)

The major subjects discussed in the various chapters are listed below:

An introduction to the literature on clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas was given in **Chapter 1.**

The presenting symptoms of these adenomas and some **in vivo** data are discussed in **Chapter 3.**

Confounding factors in the in vitro research of these adenomas are analyzed in **Chapter 4.**

The correlations between **in vivo** and **in vitro** hormone data, effects of TRH and bromocriptine, and similarities between gonadotroph and clinically nonfunctioning adenomas are discussed in **Chapter 5.**

Additional **in vitro** data are presented in **Chapter 6.**

The etiology of clinically nonfunctioning and gonadotroph adenomas is discussed in the light of the age-dependent changes in gonadotropin and sex steroid levels in normal subjects in **Chapter 7.**

The role of 2 drugs that might be useful in the therapy of clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas is discussed in **Chapter 8.**

A discussion of the major conclusions of this thesis is presented in **Chapter 9.**

3. THE CLINICAL PRESENTATION OF "NONFUNCTIONING" PITUITARY ADENOMAS.

Adapted from: Kwekkeboom DJ, Leunisse M, van der Zwan L, de Jong FH, Lamberts SWJ. "Nonfunctioning" pituitary adenomas in vivo and in vitro. Advances in the Biosciences Vol. 69; Pergamon Press, Oxford 1988;pp 417-20.

ABSTRACT

Thirtytwo patients with the clinical diagnosis "nonfunctioning pituitary adenoma" were treated by transsphenoidal surgery. These were 20 men and 12 women; the mean age was 58 years. Deteriorating vision was the most frequent initial complaint (n=20;63% of the patients). Circulating FSH and LH levels were low in 9 and normal in 2 women, while 16 men had high or normal and 2 had low values. However, 9 men had low testosterone (T) levels. High subunit levels may have accounted for the discrepancy between LH and T levels.

INTRODUCTION

Nonfunctioning adenomas of the pituitary are being called nonfunctioning because they do not cause any symptoms by overproduction of a hormone. Generally, patients present with loss of visual acuity and/or visual field defects (1,2), while hypersecretion of pituitary hormones seems absent. In recent years, however, hypersecretion of gonadotropins and gonadotropin subunits by nonfunctioning adenomas has been reported (for reviews see 3,4). Moreover, many clinically nonfunctioning adenomas have been reported to secrete LH, FSH or gonadotropin subunits **in vitro** (3-5).

PATIENTS AND METHODS

Patients. To evaluate the **in vivo** presentation of nonfunctioning adenomas, we studied a group of 32 patients, who were clinically diagnosed as having a "nonfunctioning" adenoma of the pituitary and who were treated by transsphenoidal surgery between 1980 and 1986. There were 20 men and 12 women. The mean age was 58 years.

Primary complaints, i.e. complaints which, in retrospect, were noticed first, of patients were looked up in patient files, and, if necessary, additional information was obtained in retrospective interviews.

Morphologic techniques applied to identify the removed tissue consisted of trichrome and PAS-stains as well as immunocytochemistry with specific antibodies as previously described (5).

Radioimmunoassays for GH, PRL, and ACTH were performed using commercial kits. Gonadotropins in serum were measured using polyclonal antibodies with 20-50% (U/U) crossreactivity for α-subunit and LHß. Standards used in these assays were: for LH MRC 68/40, and for FSH MRC 69/104.

RESULTS

In 32 patients who were clinically diagnosed as having a nonfunctioning adenoma of the pituitary, and in whom the diagnosis was confirmed by histologic examination of the removed tissue, sufficient data were available to evaluate the nature and duration of primary complaints. Deteriorating vision was the most frequent primary complaint; it was present in 20 patients (63%). Impotence or loss of libido was present in 9, headache in 7. As to the duration of the complaints, it is noteworthy that most of the patients (17/20) who sought advice because of worsening vision were treated within 2 years after the onset of their complaints, while in the group that had sexual complaints many (6/9) were treated only after 4 to 15 years. Most of the patients who had these latter complaints were men (8/9).

Serum levels of LH and FSH were estimated in 29 patients.

Nine women had lowered values for both hormones, while in two levels were in the normal range. In the remaining 18 men the situation was strikingly different: 2 had high levels of LH and FSH, 14 were within the normal range, while only 2 had lowered values. However, 9 men had low testosterone levels.

DISCUSSION

Deteriorating vision is the most frequent initial complaint in patients with a nonfunctioning adenoma of the pituitary. However, especially in men impotence or loss of libido are noticed quite often, though in many of these patients it takes a long time before the diagnosis of a pituitary adenoma is established. The reason for this delay may be that patients do not seek medical attention for this type of complaint or that the physician does not consider the possibility of a pituitary tumor.

In 9 out of 11 women with a nonfunctioning adenoma we observed low immuno-active LH and FSH levels, while in 16 out of 18 men immuno-active gonadotropin levels were normal or high. However, in 8 men a discrepancy between LH and testosterone levels existed. Since gonadotropin levels were assessed using immuno-assays with high crossreactivity of α-subunit and LHß, seemingly normal LH levels in men with low testosterone may have been caused by crossreactivity of circulating subunits.

REFERENCES

1. Mukai K. Pituitary adenomas.Immunocytochemical study of 150 tumors with clinicopathologic correlation. Cancer 1983;52: 648-53.
2. Wilson CB, Dempsey LC. Transspenoidal microsurgical removal of 250 pituitary adenomas. J Neurosurg 1978;48:13-22.
3. Ridgway EC. Glycoprotein hormone production by pituitary tumors. In: Black PMcL, Ed. Progress in endocrine research and therapy (Vol 1), Raven Press, New York, 1984:pp 343-63.
4. Snyder PJ. Gonadotroph cell adenomas of the pituitary. Endocrine Rev 1985;6:552-63.
5. Lamberts SWJ, Verleun T, Oosterom R, Hofland L, van Ginkel LA, Loeber JG, van Dongen KJ, de Jong FH. Hormone secretion by gonadotroph pituitary tumors in vivo and in vitro: the effects of TRH and bromocriptine. J Clin Endocrinol Metab

1987;64:524-30.

4. CONFOUNDING FACTORS IN THE INTERPRETATION OF GONADOTROPIN AND GONADOTROPIN-SUBUNIT RELEASE FROM CULTURED HUMAN PITUITARY ADENOMAS.

D.J. Kwekkeboom, F.H. De Jong and S.W.J. Lamberts.

Department of Medicine , University Hospital Dijkzigt, Rotterdam, The Netherlands.

Journal of Steroid Biochemistry, in press.

ABSTRACT

Culture data of 31 human pituitary nonfunctioning adenomas and effects of crossreactivity and **in vitro** culturing conditions on immunoreactivity of gonadotropins and subunits were investigated.

Using immunoradiometric assays for FSH and LH and radioimmunoassays for α-subunit and LHß-subunit cross-reactivities were reduced to a minimum.

Repeated freezing and thawing had no effect on immunoreactivity of hormones and subunits tested.

Incubation at 37°C did not affect the immunoreactivity of purified subunit preparations and no recombination of α-subunit and LHß into intact LH could be demonstrated after coincubation of the subunits. FSH immunoreactivity in culture media from 3 pituitary tumors was not affected by incubation at 37°C. LH from a purified preparation and LH in culture media from 3 pituitary adenomas showed a rapid decrease of LH immunoreactivity when left at 37°C.

Concomitant with decreasing LH levels at 37°C, a rise in the

concentration of α-subunit occurred. A direct correlation between gain in α-subunit and loss of LH was found. LHβ levels remained stable while LH decreased. This observation may be attributed to an increase in LHβ levels which is compensated by the loss of LH, which has a relatively high crossreactivity in the LHβ immunoassay.

LH, FSH, α-subunit, LHß or a combination of these glycoproteins could be demonstrated in 26 out of 31 cultured tumors from patients operated upon because of a clinically nonfunctioning adenoma. In none of the media of 15 adenomas in which both α-subunit and LH were detected, could α-subunit levels have been caused by dissociation of LH at 37°C. In two cases, measured LH levels could have been caused by crossreactivity of α-subunit and FSH.

It was concluded that: 1. in research of nonfunctioning pituitary adenomas data on gonadotropin and gonadotropin-subunit secretion may suffer from bias caused by crossreactivity; 2. that dissociation of LH into subunits at 37°C is relatively unimportant in **in vitro** research of nonfunctioning adenomas; 3. that virtually all nonfunctioning pituitary adenomas contain or release gonadotropins and/or subunits.

INTRODUCTION

Secretion of LH, FSH, and the subunits of these hormones, α-subunit, LHß and FSHß by human "nonfunctioning" pituitary adenomas has been reported by several authors over the past few years (1-23, for reviews see 11,16). **In vivo** hypersecretion of gonadotropins or α-subunit can be estimated to be relatively rare (4-17 % in larger series) (8,11,16,21). However, many, if not virtually all nonfunctioning adenomas can be shown to produce gonadotropins or gonadotropin subunits **in vitro** (15,16,20,23-26).

Several causes might contribute to this discrepancy between **in vivo** and **in vitro** data:

1. The majority of nonfunctioning adenomas appear to secrete gonadotropins and/or their subunits at a low rate: levels of

their secretory products **in vivo** may therefore well fall into the normal or even low range, while **in vitro** secretion of these products can be easily detected.

2. A relatively high secretion of LH, FSH or one of the gonadotropin-subunits **in vitro** may cause appreciable immunoreactive levels of one of the other hormones because of mutual crossreactivity.

3. The **in vitro** cell culture model may have features that are lacking under **in vivo** conditions.

The aim of the present study was to investigate the effects of crossreactivity and **in vitro** culturing conditions on immunoreactivity of gonadotropins and gonadotropin-subunits in an attempt to evaluate **in vivo** and **in vitro** data in this type of tumors in a more satisfying way.

MATERIALS AND METHODS

In vitro investigations

In vitro cell culture data were obtained as described before (27). In short, surgically removed tissue of nonfunctioning pituitary adenomas was washed several times, incubated with dispase, and transformed into a cell suspension using a Dounce-type homogenizer. Centrifugation in a discontinuous Ficoll-Isopaque gradient was applied to separate pituitary cells from blood elements. Finally, cells were suspended in Eagle's Minimum Essential Medium (MEM), containing 10% fetal calf serum (FCS). Thereafter, cells were cultured at 37°C in Costar multiwell plates, usually at a concentration of 200.000 cells per well. Incubations, with or without addition of drugs or hormones were performed in fourfold and generally lasted for 24 to 92 hours.

To evaluate the effect of exposure of LH, FSH, α-subunit and LHß-subunit to the conditions of cell culture and immunoassay, we investigated the changes of immunoreactive gonadotropin and subunit levels after incubation at elevated temperature. Two different purified LH and FSH preparations, purified α-subunit and LHß preparations and a combination of these two subunit preparations, were diluted in MEM containing 10% FCS. Of each

preparation, two separate dilutions were left at 37°C and at 20°C for 0, 4, 24, and 48 hours respectively. Each incubation was performed in triplicate. Preparations used were for LH: MRC 68/40 and a preparation from KABI, Stockholm, Sweden; for FSH: a preparation from IRE-Medgenix, Brussels, Belgium, and a preparation from KABI; for α-subunit MRC 78/554, and for LHß-subunit MRC 78/556. Culture media of three pituitary adenomas that contained LH, FSH, α-subunit and LHß-subunit were also incubated at 37°C for 0, 4, 24 and 48 hours.

To evaluate the effect of repeated freezing and thawing, a procedure frequently used to lyse cells after culture in order to be able to estimate hormone contents of cells, on LH, FSH and gonadotropin-subunit levels 10 patient sera were frozen and thawed 1 to 5 times, followed by estimation of hormone levels.

The relation between testosterone (T) and LH values was evaluated in 14 men with a nonfunctioning pituitary adenoma. A comparison was made between LH levels as measured using double antibody immunoassays (antibodies from KABI and UCB) and LH levels as measured using the radioimmunometric assay mentioned below.

Immunoassays

Immunoassays applied were: for LH and FSH immunoradiometric assays (IRMAs) supplied by IRE-Medgenix, Brussels, Belgium. Incubation time of these assays was 2 hours. Intra- and inter-assay coefficients of variation (CVs) of these assays were for LH <5% and <15%, for FSH <3% and <8%, respectively. Sensitivities (as defined by two standard deviations of the blank) were for the LH IRMA 0.05 ng/ml (0.3 mIU/ml) and for the FSH IRMA 0.03 ng/ml (0.3 mIU/ml).

For the estimation of α-subunit and LHß double antibody RIAs were applied using antibodies purchased from UCB, Brussels, Belgium. Incubation in these assays was overnight. Intra- and interassay CVs of these assays were: for α-subunit <6% and <11%, for LHß <7% and <13%, respectively. Sensitivities (as defined by a 10 % decrease of the initial binding) were for the α-subunit assay 0.2 ng/ml and for the LHß assay 0.5 ng/ml. Standards used

were for LH: MRC 68/40 (potency: 6.6 mIU/ng), for FSH: a preparation from KABI, Stockholm, Sweden (potency: 8.7 mIU/ng), for α-subunit: MRC 78/554 (potency: 1 mIU/ng) and for LHß: MRC 78/556 (potency: 1 mIU/ng).

Crossreactivities in the immunoassays were determined using the above mentioned standard preparations.

Statistics

For comparison of slopes of regression lines Student's t-test was used. Other tests used are mentioned in the text.

RESULTS

Crossreactivities of gonadotropins and subunits in the various immunoassays have been summarized in Table 1.

Table 1. Crossactivity of hormones and subunits in immunoassays

Assays	LH assay	FSH assay	α-subunit assay	LHβ assay
Hormone				
LH	100	0.06 (0.08)	3.9 (0.6)	53.4 (8.1)
FSH	0.5 (0.4)	100	20.0 (2.3)	1.1 (0.1)
α-subunit	0.4 (2.7)	0.06 (0.5)	100	1.8 (1.8)
LHβ-subunit	< 0.5 (<3.2)	<0.01 (<0.1)	0.2 (0.2)	100

Crossreactivity of hormones and subunits in gonadotropin- and subunit immunoassays, in ng/ng and, between brackets, in mIU/mIU; values are percentages.

The effect of repeated freezing and thawing on immunoreactivity of LH, FSH, α-subunit and LHß is summarized in Table

2. Applying Spearman's Rank Correlation Test on individual serum levels, no significant changes of hormone concentrations could be shown.

Table 2. Effects of repeated freezing and thawing on glycoprotein hormone immunoreactivity in patient serum samples. Individual serum sample levels after thawing once have been taken as 100%. Values listed are mean percentages, and, between brackets, standard deviations, of 10 samples.

	Frequency of thawing				
	1x	2x	3x	4x	5x
Hormone levels					
LH	100	98 (3.1)	99 (6.3)	95 (6.6)	96 (5.5)
FSH	100	103 (5.0)	104 (2.5)	101 (6.6)	103 (3.6)
α-subunit	100	100 (7.4)	98 (11.1)	98 (11.3)	105(11.7)
LHß	100	104 (7.6)	100 (8.1)	100 (9.9)	103(13.8)

The relation between peripheral levels of testosterone and LH in 14 men with a nonfunctioning pituitary adenoma is summarized in Table 3. In 3 of these 14 men α-subunit levels and in one LHß levels were elevated. Using a radioimmunoassay with polyclonal antibodies, a discrepancy between LH and T levels was observed in 8 men. Using the immunoradiometric assay, a discrepancy between LH and T levels was noticed in only 3 patients. None of these three had elevated subunit levels.

Table 3. LH and Testosterone levels in 14 men with a pituitary nonfunctioning adenoma. Levels are stated in reference to normal values for the immunoassay.

	LH by RIA			LH by IRMA			Total
	high	normal	low	high	normal	low	
Testosterone							
normal	2	4	-	-	6	-	6
low	-	6	2	-	3	5	8

In figure 1 the effect of prolonged exposure to 37°C on LH immunoreactivity of two LH preparations is depicted. The MRC reference preparation 68/40 showed a significantly slower decay of LH immunoreactivity than the KABI preparation ($P<0.05$). Regression lines of LH decay in culture media of three nonfunctioning pituitary adenomas were parallel to that of the KABI preparation, but differed significantly from that of MRC 68/40.

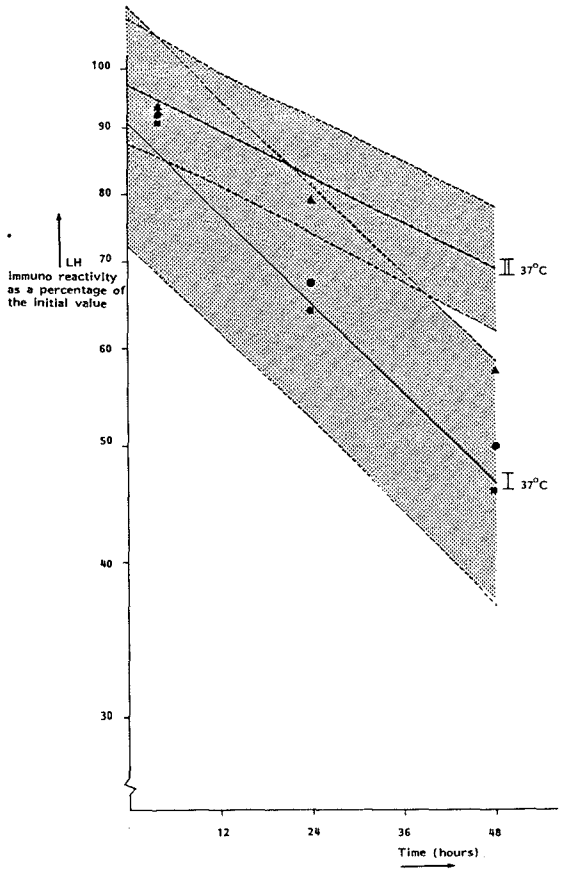

Figure 1. Effect of prolonged exposure to 37°C on LH immunoreactivity. Regression lines and (shaded area) 95% confidence limits for the prediction of individual values. I : KABI preparation; II: MRC 68/40; ● ▲ ■ : LH in media of cultured cells from pituitary adenomas.

At a temperature of 20°C LH immunoreactivity showed a significant, though slow decrease (data not shown). The slope of the regression line at 20°C was significantly lower than that of the same preparation at 37°C (0.0008/h. versus 0.006).

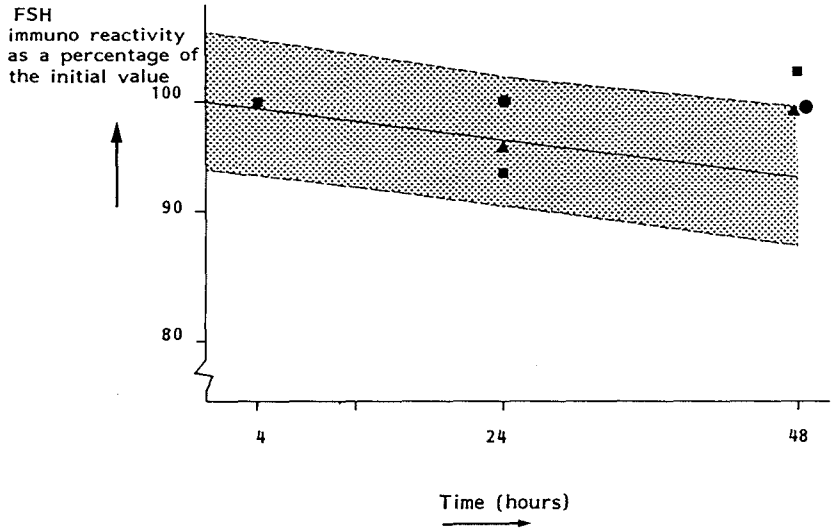

Figure 2. Effect of prolonged exposure to 37°C on FSH immunoreactivity. Regression line and (shaded area) 95% confidence limits for the prediction of individual values. ● ▲ ■ : FSH in media of cultured cells from pituitary adenomas.

In figure 2 the effect of exposure to 37°C on FSH immunoreactivity in the KABI and Medgenix preparations is shown. As the slopes of the lines for the two tested preparations did not differ significantly, data were combined. Data on FSH decay in individual tumorous cell cultures did not show a significant regression, however. Exposure to 20°C did not have any effect on FSH levels of purified preparations (data not shown).

Exposure to a temperature of 37°C of α-subunit and LHß-subunit did not affect immunoreactivity of the subunits (data not shown). Neither did we notice any effect on α-subunit or LHß immunoreactivity when these purified preparations were combined and left at 37°C for 48 hours. Moreover, no change in LH immunoreactivity

of the combined α-subunit and LHß preparation was observed after 48 hours of exposure to 37°C.

A rise in α-subunit levels concomitant with the decrease in LH levels in LH preparations was noticed, after incubation at 20°C and 37°C. The gain in α-subunit levels was directly correlated to the loss of LH immunoreactivity, irrespective of temperature or preparation tested. This relationship is depicted in fig 3. The gain in α-subunit (ng) could be estimated to be 41 ± 4% (slope (b) ± standard error of the slope (sb)) of the loss in LH immunoreactivity, expressed in ng of the MRC 68/40 standard. Data on the relationship between loss of immunoreactive LH and gain of α-subunit in pituitary cell culture media were in concordance with this regression line.

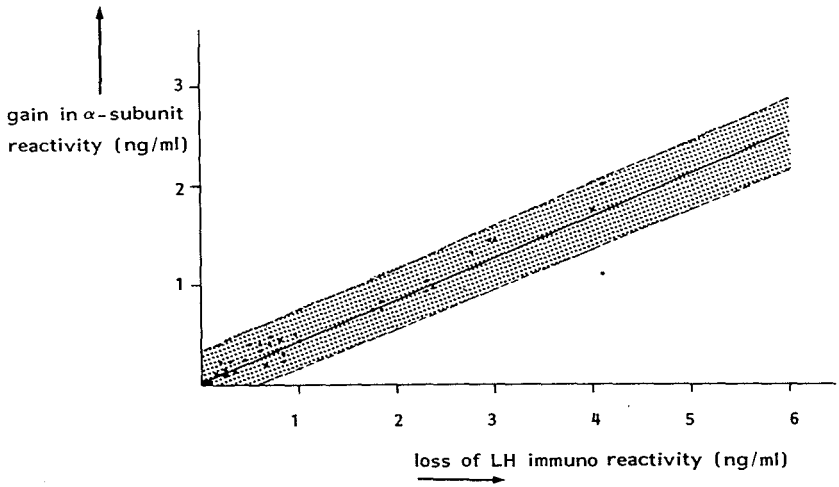

Figure 3. Gain in α-subunit immunoreactivity (ng/ml) versus loss of LH immunoreactivity (ng/ml) in LH preparations. Regression line and (shaded area) 95% confidence limits for the prediction of individual values.

No significant difference in LHß immunoreactivity in the LH preparations tested could be shown, neither after exposure to 37°C nor to 20°C.

Culture data of pituitary tumor cells from 31 patients who were operated upon because of a clinically nonfunctioning adenoma and in whom the existence of a pituitary tumor was confirmed by histologic examination of the removed tissue, are listed in Table 4. In media in which FSH was demonstrated, values per 24 hours of culturing and 10^6 cells ranged from 0.2 to 367.8 ng/ml; for α-subunit values ranged from 0.3 to 320.0 ng/ml, for LHß from 2.4 to 82.1 ng/ml and for LH from 0.4 to 6.7 ng/ml. In two culture media, measured LH and LH and LHß levels, respectively, could have been caused by crossreactivity of α-subunit and FSH. In none of the media in which α-subunit was detected could levels of this subunit have been caused by dissociation of LH at 37°C.

Table 4. Frequency distribution of hormones and subunits in cell cultures of 31 nonfunctioning pituitary adenomas.

Hormones and subunits detected	♂	♀	Total
LH, FSH, LHβ, α-subunit	10	3	13
α-subunit	1	3	4
LHβ	2	1	3
FSH	1	0	1
FSH, α-subunit	1	1	2
FSH, LHβ	0	1	1
LH, LHβ,α-subunit	2	0	2
ACTH	1	2	3
None	0	2	2
Total	18	13	31

DISCUSSION

Several authors have reported secretion of gonadotropins and their subunits by pituitary nonfunctioning adenomas **in vivo** and **in vitro** (for reviews see 11,16). However, the results presented were often obtained using radioimmunoassays in which separate subunits or intact hormones show considerable mutual crossreactivity. The immunoassays for FSH, LH and α-subunit used in this study, show low crossreactivity for the other gonadotropins or subunits. An example of the importance of low crossreactivity in immunoassays is given in Table 3: LH levels as measured using radioimmunoassays showed a discrepancy with testosterone levels in 8 out of 14 patients with a pituitary nonfunctioning adenoma. Using the immunoradiometric assay, a discrepancy between LH and T levels was noticed in only 3 patients. In these patients hypogonadism or circulating biologically inactive forms of LH may have been present.

Other factors which might confound the interpretation of data are the influence of repeated freezing and thawing of samples and the spontaneous dissociation of gonadotropins or the reassociation of gonadotropin-subunits during culture.

The results obtained after repeated freezing and thawing show that there are no significant changes in the levels of immunoreactive LH, FSH, α-subunit or LHß. This is in concordance with the findings of other investigators (28).

Loeber et al. (29), reported that LH in solution dissociates at elevated temperatures into subunits, regardless of the presence or absence of enzyme inhibitors. Similar studies have not been reported for gonadotropins in the medium of cultured gonadotropin producing pituitary tumors. After incubation of two different purified LH preparations for various periods at 37°C and at 20°C a clear decay of LH levels was found, accompanied by a rise in α-subunit levels, while LHß immunoreactivity did not change. Significantly different slopes for the decay of LH preparations at 37°C were obtained. This difference might be due to a different method of purification of the preparations or to a difference in constituting LH components (30). Cell culture

media of gonadotropin producing pituitary adenomas showed decreases of immunoassayable LH levels that were comparable with the decay of the KABI LH preparation.

After log transformation of the data, straight regression lines of time of exposure versus LH levels as a percentage of the level before exposure to 37°C could be obtained. This means that a constant percentage of the LH available dissociated. Assuming a constant secretion rate of LH by LH producing pituitary tumors, and therefore an average exposure time of LH to 37°C of 24 hours in cell cultures lasting 48 hours, this means that 28% of the LH released may dissociate.

The loss of initially present immunoreactive LH after exposure to 20°C or 37°C was directly correlated to an increase in α-subunit levels. In cell cultures of gonadotropin secreting pituitary adenomas, in which both LH and α-subunit are found, α-subunit levels will have to exceed not only the amount of immunoreactivity which may be due to crossreactivity of LH present, but also the amount due to dissociation of LH initially present, to prove that α-subunit secretion by the adenomatous cells actually took place. In none of the 15 cultures of pituitary adenomas listed in Table 4 in which both α-subunit and LH were detected, could α-subunit levels have been caused by dissociation of secreted LH. This is due to the fact that amounts of LH released by these nonfunctioning adenomas **in vitro** are low in comparison with released amounts of subunits.

Dissociation of LH at 20°C was noticed. As the LH IRMA only takes 2 hours of incubation, and the slope of the decay line at 20°C is rather flat, an accurate estimation of the LH level can be obtained. In the α-subunit assay, however, samples are incubated for 24 hours. In these samples, 2.2 % of the initial LH, if present, will have dissociated, giving rise to 0.41 x 2.2% = 0.9% α-subunit immunoreactivity in relation to LH levels. This is far less than LH crossreactivity in the α-subunit assay (being 3.9%), but presumably forms a part of it.

Rising α-subunit levels accompanying decreasing LH levels at 37°C and 20°C suggest the dissociation of LH into α-subunit and LHß-subunit. However, we did not find a significant trend

indicating an increase in LHß concomitant to LH decrease. This observation can be explained by a rise in "true" LHß levels that is about equal to the decrease of LH crossreacting in the LHß assay. So, we assume that the gain in LHß accompanying LH dissociation is about equal to the the crossreaction of LH in the LHß assay, i.e. 53% (ng/ng). In the evaluation of LHß concentrations, this means that LHß immunoreactivity has to exceed 53% of the LH levels to allow any conclusion as to what hormone is actually being measured.

A decrease in FSH immunoreactivity of purified FSH preparations during exposure to 37°C was found. In comparison to LH, however, the rate of FSH decay was low. FSH produced by gonadotropin secreting pituitary tumours did not show a significant trend of decay at all, just as purified FSH at 20°C could not be shown to decrease in immunoreactivity. Therefore, dissociation of FSH under **in vitro** conditions is not likely to affect the interpretation of the results.

Apart from dissociation of LH at various temperatures, we also investigated the possibility of association of subunits into LH at 37°C as reported by various authors (31,32). We did not find decreasing subunit levels or increasing LH levels. The reason for this may be that we studied combinations of subunits in relatively low concentrations (up to 100 ng/ml), while it has been pointed out (33) that high concentrations (>1 mg/ml) are needed to favour association.

Summarizing, it is concluded that: 1. in research of nonfunctioning pituitary adenomas data on gonadotropin and gonadotropin-subunit secretion may suffer from bias caused by crossreactivity of these hormones and subunits in their respective immunoassays; 2. that dissociation of LH into subunits at 37°C occurs, but is relatively unimportant in **in vitro** research of pituitary nonfunctioning adenomas, because amounts of LH released by this type of tumor are low in comparison with released amounts of α-subunit; 3. that virtually all human pituitary nonfunctioning adenomas contain or release gonadotropins and/or their subunits.

REFERENCES

1. Beckers A, Stevenaert A, Mashiter K, Hennen G. Follicle-stimulating hormone-secreting pituitary adenomas. J Clin Endocrinol Metab 1985;61:525-8.
2. Berezin M, Olchovsky D, Pines A, Tadmor R, Lunenfeld B. Reduction of follicle-stimulating hormone (FSH) secretion in FSH-producing pituitary adenoma by bromocriptine. J Clin Endocrinol Metab 1984;59:1220-3.
3. Borges JLC, Ridgway EC, Kovacs K, Rogol AD, Thorner MO. Follicle-stimulating hormone-secreting pituitary tumor with concomitant elevation of serum α-subunit levels. J Clin Endocrinol Metab 1984;58:937-41.
4. Chapman AJ, Macfarlaine IA, Shalet SM, Beardwell CG, Dutton J, Sutton ML. Discordant serum α-subunit and FSH concentrations in a woman with a pituitary tumour. Clin Endocrinol 1984; 21:123-29.
5. Friend JN, Judge DM, Sherman BM, Santen RJ. FSH-secreting pituitary adenomas: stimulation and suppression studies in two patients. J Clin Endocrinol Metab 1976;43:650-7.
6. Harris RI, Schatz NJ, Gennarelli T, Savino PJ, Cobbs WH, Snyder PJ. Follicle-stimulating hormone-secreting pituitary adenomas: correlation of reduction of adenoma size with reduction of hormonal hypersecretion after transsphenoidal surgery. J Clin Endocrinol Metab 1983;56:1288-93.
7. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 1983;59:585-9.
8. Kourides IA, Weintraub BD, Rosen SW, Ridgway EC, Kliman B, Maloof F. Secretion of alpha subunit of glycoprotein hormones by pituitary adenomas. J Clin Endocrinol Metab 1976;43: 97-106.
9. Peterson RE, Kourides IA, Horwith M, Vaughan ED Jr., Saxena BB, Fraser RAR. Luteinizing hormone- and α-subunit-secreting pituitary tumor: positive feedback of estrogen. J Clin Endocrinol 1981;52:692-8.
10. Ridgway EC, Klibanski A, Ladenson PW, Clemmons D, Beitins IZ, Mcarthur JW, Martorana MA, Zervas NT. Pure alpha-secreting pituitary adenomas. N Eng J Med 1981;304:1254-9.
11. Ridgway EC. Glycoprotein hormone production by pituitary tumors. In: Progress in endocrine research and therapy, Vol 1. Black PMCL et al., eds. Raven Press, New York 1984;pp 343-63.
12. Roman SH, Goldstein M, Kourides IA, Comite F, Bardin CW, Krieger DT. The luteinizing hormone-releasing hormone (LHRH) agonist [D-Trp^{6}-Pro^{9}-Net] LHRH increased rather than lowered LH and α-subunit levels in a patient with an LH-secreting pituitary tumor. J Clin Endocrinol Metab 1984;58:313-9.
13. Snyder PJ, Johnson J, Muzyka R. Abnormal secretion of glycoprotein α-subunit and follicle-stimulating hormone (FSH) ß-subunit in men with pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 1980;51:579-84.
14. Snyder PJ, Muzyka R, Johnson J, Utiger RD. Thyrotropin releasing hormone provokes abnormal follicle-stimulating hormone (FSH) and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. J Clin

Endocrinol Metab 1980;51:744-8.
15. Snyder PJ, Bashey HM, Kim SU, Chappel SC. Secretion of uncombined subunits of luteinizing hormone by gonadotroph cell adenomas. J Clin Endocrinol Metab 1984;59:1169-75.
16. Snyder PJ. Gonadotroph cell adenomas of the pituitary. Endocrine Rev 1985;6:552-63.
17. Trouillas J, Girod C, Sassolas G, Claustrat B, Lheritier M, Dubois MP, Goutelle A. Human pituitary gonadotropic adenoma; histological, immunocytochemical, and ultrastructural and hormonal studies in eight cases. J Pathology 1981;135:315-36.
18. Vance ML, Ridgway EC, Thorner MO. Follicle-stimulating hormone- and α-subunit-secreting pituitary tumor treated with bromocriptine. J Clin Endocrinol metab 1985;61:580-4.
19. Whitaker MD, Prior JC, Scheithauer B, Dolman L, Durity F, Pudek MR. Gonadotrophin-secreting pituitary tumour: report and review. Clin Endocrinol 1985;22:43-8.
20. Lamberts SWJ, Verleun T, Oosterom R, Hofland L, van Ginkel LA, Loeber JG, van Vroonhoven CCJ, Stefanko SZ, de Jong FH. The effects of bromocriptine, thyrotropin-releasing hormone, and gonadotropin-releasing hormone on hormone secretion by gonadotropin-secreting pituitary adenomas in vivo and in vitro. J Clin Endocrinol Metab 1987;64:524-30.
21. Mukai K. Pituitary adenomas. Immunocytochemical study of 150 tumors with clinicopathologic correlation. Cancer 1983;52: 648-53.
22. Wide L, Lundberg PO. Hypersecretion of an abnormal form of follicle stimulating hormone associated with suppressed luteinizing hormone secretion in a woman with a pituitary adenoma. J Clin Endocrinol Metab 1981;53:923-30.
23. Asa SL, Gerrie BM, Singer W, Horvath E, Kovacs K, Smyth HS. Gonadotropin secretion in vitro by human pituitary null cell adenomas and oncocytomas. J Clin Endocrinol Metab 1986;62: 1011-9.
24. Snyder PJ, Bashey HM, Phillips JL, Gennarelli TA. Comparison of hormonal secretory behavior of gonadotroph cell adenomas in vivo and in culture. J Clin Endocrinol Metab 1985;61: 1061-5.
25. Surmont DWA, Winslow CLJ, Loizou M, White MC, Adams EF, Mashiter K. Gonadotrophin and α subunit secretion by human 'functionless' pituitary adenomas in cell culture: long term effects of luteinizing hormone releasing hormone and thyrotrophin releasing hormone. Clin Endocrinol 1983;19: 325-36.
26. Mashiter K, Adams E, van Noorden S. Secretion of LH, FSH and PRL shown by cell culture and immunocytochemistry of human functionless pituitary adenomas. Clin Endocrinol 1981;15: 103-12.
27. Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SWJ. Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. J Endocrinol Invest 1984;7:307-11.
28. Lambert A, Tsatsoulis A, Shalet S, Frost J, Robertson WR. Bioactive and immunoactive luteinising hormone, stability in vitro in human blood and plasma. J Endocrinol 1986;111 suppl:59.
29. Loeber JG, Nabben-Fleuren JWGM, Elvers LH, Segers MFG, Lequin

RM. Spontaneous dissociation of human pituitary luteinizing hormone in solution. Endocrinology 1978; 103:2240-6.
30. Van Ginkel LA, Loeber JG. Heterogeneity of human lutropin. Detection and identification of α- and ß-subunits. Acta Endocrinol 1985;110:182-92.
31. Weintraub BD, Stannard BS, Rosen SW. Combination of ectopic and standard human glycoprotein hormone alpha with beta subunits: discordance of immunologic and receptor-binding activity. Endocrinology 1977;101:225-35.
32. Reichert LE, Midgley AR, Niswender GD, Ward DN. Formation of a hybrid molecule from subunits of human and bovine luteinizing hormone. Endocrinology 1970;87:534-41.
33. De la Llosa P, Justisz M. Reversible dissociation into subunits and biological activity of ovine luteinizing hormone. Biochim Biophys Acta 1969;181:426-36.

5. GONADOTROPIN RELEASE BY CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS IN VIVO AND IN VITRO: RELATION TO SEX AND EFFECTS OF TRH, GnRH AND BROMOCRIPTINE.

D.J. Kwekkeboom, F.H. de Jong and S.W.J. Lamberts.

Department of Medicine, University Hospital Dijkzigt, The Netherlands.

Journal of Clinical Endocrinology and Metabolism 1989;68:1128-35.

ABSTRACT

We studied the **in vivo** hormonal levels and **in vitro** hormone and subunit release in a group of 22 patients who were operated upon because of a clinically nonfunctioning or gonadotroph pituitary adenoma. **In vivo**, 5 of the 22 patients, all of whom were men, had hypersecretion of FSH, LHß or α-subunit. An elevated ratio of serum α-subunit to LH and FSH was found in 6 of 8 women **in vivo**, although in all 6 women serum LH, FSH and α-subunit levels were low. LH, FSH, α-subunit, LHß or a combination of these glycoprotein hormones could be demonstrated in 19 of 22 cultured adenomas.

We conclude that: 1. virtually all clinically nonfunctioning adenomas contain or release gonadotropins or their subunits **in vitro**; 2. **in vivo** hypersecretion of these hormones and subunits occurs infrequently and in this series only in men; 3. an elevated ratio of α-subunit to LH and FSH is frequently found in women and may prove a useful diagnostic tool; 4. responses to TRH and bromocriptine do not depend on baseline gonadotropin levels, neither **in vivo** nor **in vitro**, implying that the distinction between gonadotroph adenomas and adenomas without hypersecretion

of gonadotropins **in vivo** is absent where hormone dynamics are concerned.

INTRODUCTION

Production, both **in vivo** and **in vitro**, of LH, FSH and their subunits, α-subunit, LHß and FSHß by human pituitary adenomas has been reported by several authors (1-30, for reviews see 11,16). Based on the presence or absence of elevated serum gonadotropin or subunit concentrations, it is possible to distinguish between gonadotroph and clinically nonfunctioning pituitary adenomas. In most of the clinically nonfunctioning adenomas, immunostaining for the anterior pituitary hormones is negative (21), but some may immunostain for gonadotropins or subunits (23). Moreover, many clinically nonfunctioning adenomas produce gonadotropins or gonadotropin subunits **in vitro** (15,16, 20,23-26). Therefore, it may be questioned whether gonadotroph and clinically nonfunctioning tumors represent two different entities. Most reports on **in vivo** and **in vitro** gonadotropin production by clinically nonfunctioning adenomas and gonadotroph adenomas deal with small groups of patients, making general conclusions difficult. We investigated the **in vivo** hormonal levels, and the **in vitro** hormone- and subunit release in a group of 22 patients who were operated upon because of a clinically nonfunctioning or gonadotroph pituitary adenoma, and evaluated the influence of several parameters on hormone and subunit release **in vivo** and **in vitro**.

PATIENTS AND METHODS

Patients

We studied 22 patients who were treated by transsphenoidal surgery because of a clinically nonfunctioning or gonadotroph pituitary adenoma between 1980 and 1987. Serum gonadotropin and subunit concentrations had been measured in all patients, and in each the presence of a pituitary tumor was verified by histologic examination of the removed tissue. The protocol was approved by the hospital's ethical committee and each patient gave informed

consent for the studies.

In vivo investigations

The responses to 200 μg TRH and 100 μg GnRH were studied on separate days. Blood samples were collected before and 10, 20, 30, 60 and 120 minutes after iv TRH or GnRH injection. Bromocriptine (2.5 mg) or a placebo was given orally and blood samples were collected hourly for 12 hours on other days.

In vitro investigations

In vitro cell culture data were obtained as described previously (31). Surgically removed pituitary tumor tissue was washed several times, incubated with dispase, and the cells dispersed using a Dounce type homogenizer. The tumor cells were seperated from blood cells by discontinuous Ficoll-Isopaque gradient centrifugation, and then were suspended in Eagle's Minimum Essential Medium (MEM) containing 100 g/L fetal calf serum (FCS) and cultured at 37°C in Costar multiwell plates, generally at a concentration of 200.000 cells per well. After renewal of the media on day 4, the cells were incubated with or without TRH (100 nmol/L; Hoechst, Frankfurt am Main, FRG), GnRH (100 nmol/L; Hoechst) or bromocriptine (10 nmol/L; Sandoz, Basel, Switzerland) in quadruplicate for 24 to 72 hours. Thereafter, the media were stored at -20°C for hormone assay. In most cultures, cells obtained both before and after these incubations were lysed in distilled water containing 1 g/L bovine serum albumin by repeated freezing and thawing and the hormone concentrations in these lysates were measured.

Immunoassays

Prolactin, GH and ACTH were measured using radioimmunoassay (RIA) kits obtained, respectively, from IRE-Medgenix, Brussels, Belgium, Sorin, Milano, Italy, and The Radiochemical Center, Amersham, UK (20,31). TSH was measured using a kit obtained from Behring, Marburg, FRG. The sensitivity of the assay was 0.1 mU/L and the crossreactivities of subunits and gonadotropins were <1% and <0.01%, respectively.

FSH and LH were measured using immunoradiometric assay kits supplied by IRE-Medgenix, Brussels, Belgium. The sensitivity of these assays was 0.5 IU/L. α-subunit and LHß were measured by RIA using antibodies purchased from UCB, Brussels, Belgium. The sensitivity of the α-subunit assay was 0.3 µg/L and that of the LHß assay was 1.0 µg/L. LH was expressed in terms of the MRC 68/40 reference preparation, FSH in terms of the MRC 78/549 reference preparation, and α-subunit and LHß in terms of MRC 78/554 and 78/556, respectively.

The crossreactivities on a weight/weight basis were: LH assay -FSH 0.5%, α-subunit 0.4%, LHß <0.5%; FSH assay -LH and α-subunit 0.06 %, LHß <0.01%; α-subunit assay -LH 3.9%, FSH 20.0%, LHß 0.2%; LHß assay -LH 53.4%, FSH 1.1%, and α-subunit 1.8%. The intra- and interassay coefficients of variation of the assays were, respectively, <5% and <15% for LH, <3% and <8% for FSH, <6% and <11% for α-subunit, and <7% and <13% for LHß.

Statistics

In vitro hormone release was evaluated using one way analysis of variance (ANOVA). Log transformation of data was used to stabilise variance. For the comparison of treatment means the Newman Keuls method was applied (32). P values <0.05 were considered significant.

RESULTS

In vivo investigations

The mean age of the 14 men was 54.3 ± 11.7 (SD) years (range 32-69) and that of the 8 women was 60.6 ± 10.3 (range 40-71) (P>0.05 by Student's t test). Eighteen patients (10 men and 8 women) had visual symptoms, 9 men had impotence and decreased libido, 1 woman had decreased libido, and 5 men and 2 women had headaches or drowsiness.

Signs of hypopituitarism, manifested by low serum T4 levels and/or a decreased response to metyrapone, were present in 5 of 21 (24%) and 13 of 16 (81%) patients, respectively, and 1 patient who had autoimmune thyroiditis had an elevated serum TSH and a

low T4 level. Thirteen patients had slightly elevated serum prolactin values (range: 12.3-38.1 μg/L; normal values: <12 μg/L in men and <15 μg/L in women). No patient had an elevated plasma GH or ACTH concentration. The presence or absence of abnormal levels of the above mentioned hormones did not differ significantly between sexes (P>0.05 by Fisher exact tests). Serum testosterone levels were low in 8 men.

Table 1. Serum gonadotropin and subunit concentrations in 22 patients with a clinically nonfunctioning or gonadotroph pituitary adenoma. Concentrations are means of 2 or more values.

	Serum					
	LH (IU/L)	FSH (IU/L)	α-subunit μg/L	LHβ μg/L	α-subunit/ gonadotropin ratio (%)	cell-culture number
Men	3.1	24.1*	1.2*	< 1.0	4.4	
	3.4	46.2*	2.7*	< 1.0	5.4	2
	2.2	12.8*	0.7	< 1.0	4.7	1
	2.7	4.9	0.8	< 1.0	10.5	
	2.5	4.6	0.6	< 1.0	8.5	
	2.8	3.9	0.9	< 1.0	13.4	5
	5.5	4.7	1.0	< 1.0	9.8	
	3.2	3.9	0.8	< 1.0	11.3	
	1.6*	4.0	0.9	< 1.0	16.1*	4
	1.0*	2.5	2.6*	< 1.0	74.3*	8
	1.0*	4.4	0.6	1.1*	11.1	10
	1.5*	3.2	0.3*	< 1.0	6.4	
	1.8*	4.2	< 0.3*	< 1.0	< 5.0	6
	2.1	3.8	0.5	< 1.0	8.5	a
Women						
premenopausal	3.6	12.5	0.5	< 1.0	3.1	a
menopausal						
	6.3*	14.3*	0.5*	< 1.0*	2.4	7
	1.6*	4.6*	0.7*	< 1.0*	11.3*	
	0.9*	6.2*	0.6*	< 1.0*	8.5*	
	1.2*	8.2*	0.7*	< 1.0*	7.5*	3
	< 0.5*	4.0*	0.8*	< 1.0*	> 17.8*	
	< 0.5*	4.9*	0.6*	<1.0*	> 11.1*	
	0.6*	1.8*	0.8*	< 1.0*	33.3*	9
Normal values						
Men	1.9-9.2	1.6-11.1	0.4-1.1	< 1.0	3.8-16.0	
premenopausal women	1.1-49.5	1.8-46.0	0.3-2.3	< 1.0-3.8	2.4-16.7	
menopausal women	17.5-86.6	26.2-107.7	1.3-4.0	1.4-5.4	1.4-3.3	

***: abnormal concentration. The numbers in the cell culture column correspond to the patient numbers in Figure 1 and Table 3. a: Cell culture in which ACTH was detected.**

The serum gonadotropin and subunit levels in the individual patients are shown in Table 1. The α-subunit/gonadotropin ratio listed in the table was computed by dividing the α-subunit level by the sum of the LH and FSH levels and multiplying the result by 100. Three of the 22 patients (14%), all of whom were men, had gonadotroph adenomas, defined by supranormal serum LH or FSH concentrations. In comparison to normal values, 6 of the 14 men had elevated serum FSH, α-subunit, LHß or an increased α-subunit/gonadotropin ratio. Low serum gonadotropin and α-subunit levels were found in 7 of the 8 women, and the α-subunit/gonadotropin ratio was high in 6 women. Compared to their respective normal values, low serum LH, FSH, and α-subunit levels and an increased α-subunit/gonadotropin ratio were found significantly more frequently in women than in men (chi-square tests; $P<0.01$ in all instances).

The mean absolute serum LH, FSH, and α-subunit levels and the α-subunit/gonadotropin ratio were similar in the men and women.

Light microscopy revealed a basophilic adenoma in one patient, and a chromophobe adenoma in 21 patients.

In vitro investigations

No hormones were detected in 1 patient's cells or media, while LH, FSH, α-subunit, LHß or a combination were found in the media and/or cells from 19 tumors (Table 2). TSH was found in one culture. No GH or prolactin was found in any culture. ACTH alone was found in 2 tumors, although neither patient had any biochemical findings of Cushing's disease; the tumor tissue of one of these patients was basophilic.

The absence or presence of LH, FSH, α-subunit and LHß did not differ significantly between men and women ($P>0.05$; Fisher exact tests), nor was there a significant difference in the simultaneous presence of all four measured gonadotropins and subunits between the sexes.

Table 2. Frequency distribution of hormones and subunits in media and/or cells of clinically nonfunctioning pituitary adenomas or gonadotroph pituitary adenomas from 22 patients.

Hormones and subunits detected	♂	♀	Total
LH, FSH, LHβ,α-subunit	7	1	8
α-subunit	1	2	3
LHβ	2	0	2
FSH	1	0	1
FSH, α-subunit	1	1	2
FSH, LHβ	0	1	1
LH, LHβ,α-subunit	1	0	1
LH, FSH, LHβ,α-subunit, TSH	0	1	1
ACTH	1	1	2
None	0	1	1
Total	14	8	22

The presence of gonadotropins and subunits in culture media could be evaluated in 20 of the 22 tumors, since in 2 tumors only cell hormone analyses were done. A discrepancy between media and cell gonadotropin and subunit content was found in the cultures of 6 patients: LH, LHß or FSH were present in the cells, but were not detected in the culture media from 2, 3 and 3 tumors, respectively. In the media of 2 tumors the measured LH or LH and LHß levels, respectively, could have been caused by crossreactivity of α-subunit or FSH.

The frequency of release of LH, FSH, α-subunit and LHß did not differ significantly between sexes, although the LH release was detected in 6 of 12 cultures from men, and in only 1 of 8 cultures from women. The release of LH, FSH, LHß and α-subunit per 24 hours and 10^6 cells did not differ significantly between sexes ($P>0.05$; Mann-Whitney U-tests).

In media in which FSH was detected, the values per 24 hours and 10^6 cells ranged from 1.3 to 3200.0 IU/L, for α-subunit from 0.3 to 320.0 μg/L, for LH from 2.7 to 29.5 IU/L and for LHß from 2.4 to 82.1 μg/L.

Comparison of in vitro and in vivo hormone levels

In 20 patients in whom **in vitro** release of hormones was measured, the amounts of LH and FSH released **in vitro** per 10^6 cells and 24 hours and the serum hormone values were not correlated. For α-subunit, however, a significant correlation was found (Spearman Rank correlation test ; r_s=0.574, P<0.01). As LHß was detected **in vivo** in only one patient, no correlation test was performed.

In vivo effects of TRH, GnRH and bromocriptine

In 6 patients the effects of placebo administration were studied for 12 hours. Their serum LH, FSH and α-subunit levels varied by up to 38%, 32% and 25% of the respective baseline levels. Therefore, responses over 40% of the baseline level for LH, over 35% of the baseline level for FSH and over 30% of the baseline level for α-subunit were considered significant.

The response to TRH was evaluated in 18 patients (11 men and 7 women). A significant increment of LH, FSH and α-subunit occurred in 1, of LH and α-subunit in 3, of FSH and α-subunit in 2, of FSH only in 1 , of LH only in 2, and of α-subunit only in 4. Thus, 13 of 18 patients (72%) had a rise in serum gonadotropin or α-subunit levels. The percentage increase was independent of the baseline hormone levels (Spearman Rank correlation tests), and was not related to sex (Mann-Whitney U-tests). The number of significant responses also did not differ significantly between men and women (chi-square tests).

Bromocriptine administration in 3 patients resulted in a significantly decreased serum α-subunit level in 1 patient. In 2 of these 3 patients, a TRH test had been performed. Both had an increase in one or more glycoprotein hormone levels after TRH administration.

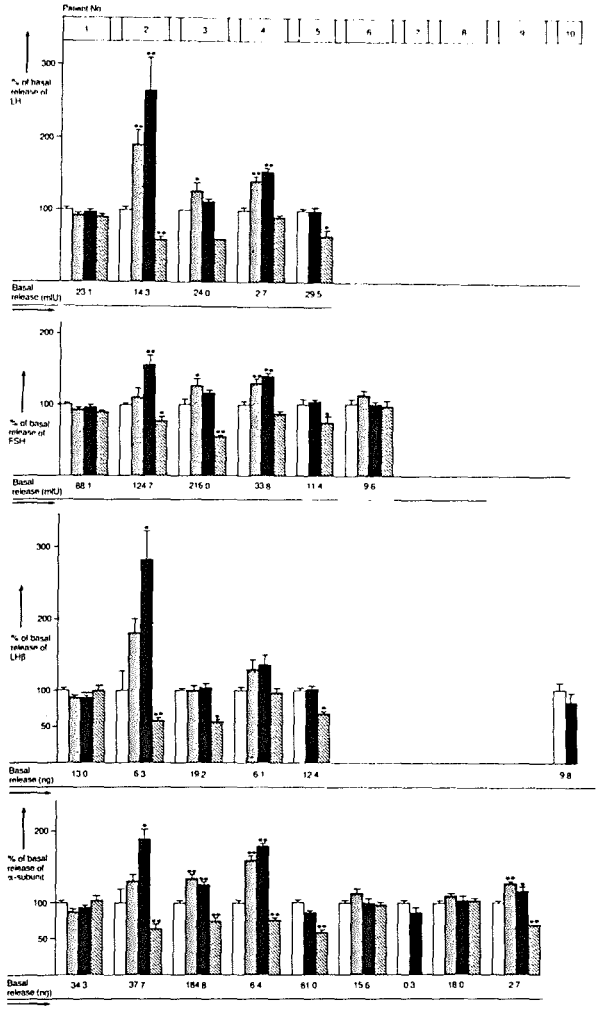

Fig. 1 Effects of TRH, GnRH and bromocriptine on mean (± SE) glycoprotein hormone release by 10 human nonfunctioning adenomas in vitro. Release is shown per 10^6 cells/mL and per 24 hours. (In patient 3 LH values during incubation with bromocriptine were undetectable). □ control; ▨ TRH 100 nmol/L; ■ GnRH 100 nmol/L; ▧ bromocriptine 10 nmol/L. *: P<0.05; ** P<0.01 by Newman Keuls method for comparison of means in ANOVA.

GnRH tests were performed in 4 patients. Serum LH levels increased by 81 to 238% and α-subunit levels increased by 33 to 125% in these 4 patients. Serum FSH levels increased by 39 to 200% in 3 patients.

In vitro effects of TRH, GnRH and bromocriptine

The effects of bromocriptine (10 nmol/L), TRH (100 nmol/L) and GnRH (100 nmol/L) were studied **in vitro** in 8, 7 and 10 tumors, respectively. Bromocriptine inhibited hormone release significantly in 5 of the 8 tumors. TRH and GnRH significantly stimulated hormone release in 4 of 7 and 4 of 10 tumors, respectively (Fig. 1). The number of cultures in which significant responses to one or more agents were found did not differ significantly between those cultured for 24 hours and those cultured longer (1 of 4 and 4 of 6 cultures, respectively; P=0.26, Fisher exact test).

No relationship between the type of hormones or subunits released and susceptibility to suppression or stimulation was found (Fisher exact tests; P>0.05). Neither was there a relationship between the amount of glycoprotein hormones released and the effectiveness of GnRH, bromocriptine and TRH (Mann-Whitney U-tests; P>0.05).

In the 7 cultures in which bromocriptine, GnRH and TRH were tested, the release of one or more gonadotropins and subunits changed significantly in response to all three substances in 4, while 3 responded to none (P<0.05; Fisher exact test).

Comparison of in vitro and in vivo responses to TRH, GnRH, and bromocriptine

In 8 patients the **in vivo** responses to TRH, GnRH or bromocriptine (Table 3) could be compared with **in vitro** results (Fig 1). Nine of the 10 significant changes of serum LH, FSH,and α-subunit concentrations in response to TRH, GnRH and bromocriptine administration **in vivo** were mimicked **in vitro**. Serum gonadotropin and α-subunit concentrations did not change significantly after TRH, GnRH or bromocriptine administration on 15 occasions **in vivo**, but **in vitro** the levels of the corresponding hormone or

subunit responded to these substances with a significant change on 8 occasions. In the 8 patients 10 **in vivo** tests were performed. The tumors of these 8 patients responded **in vitro** to 7 tests with a significant change of the LH, FSH or α-subunit concentration; this response was mimicked for at least one hormone **in vivo** in 6 tests. In the remaining 3 tests, no significant changes occurred in any hormone, either **in vivo** or **in vitro** ($P<0.05$; Fisher exact test).

Table 3. Effects of TRH (200 μg), GnRH (100 μg) and bromocriptine (2.5 mg) on hormone- and subunit levels in 8 patients

	TRH test			Bromocriptine test		
Patient	LH (IU/L) basal-max	FSH (IU/L) basal-max	α-subunit (μg/L) basal-max	LH (IU/L) basal-min	FSH (IU/L) basal-min	α-subunit (μg/L) basal-min
1				2.2-2.2	12.8-13.6	0.7-0.6
2	3.4-5.5*	46.2-51.3	2.7-4.4*			
3	1.3-2.1*	9.7-12.0	0.7-0.8	1.2-1.2	8.2- 7.6	0.7-0.6
4	1.6-2.1	4.0- 5.7*	0.9-1.2*			
5				2.8-1.8	3.9- 2.7	0.9-0.5*
6	1.8-1.7[a]	4.2- 4.5	<0.3-<0.3			
8	1.0-1.7*[a]	2.5- 3.1[a]	2.6-3.3			
	GnRH test					
4	1.6-5.4*	4.0- 8.4*	0.9-1.2*			
9	0.6-1.2*[a]	1.8- 5.4*[a]	0.8-1.2*			

Patient numbers refer to figure 1. *: significant response, as defined in the text. a: hormone not detected in vitro.

DISCUSSION

The absence of a specific clinical syndrome caused by overproduction of a hormone is characteristic, but not specific,

for patients who have a clinically nonfunctioning pituitary adenoma or a gonadotroph adenoma. Complaints caused by local tumor growth, i.e. visual complaints, drowsiness and headache, were predominant in this series, being present in 20 of the 22 patients (91%).

In vivo hypersecretion of FSH, α-subunit or LHß was found in 5 of the 22 patients (23%). Other groups have reported elevated serum gonadotropin and subunit concentrations in 4-17% of such patients (8,11,16,21). This relatively low prevalence of gonadotroph adenomas in comparison to clinically nonfunctioning adenomas contrasts with the reports of **in vitro** release of gonadotropins and subunits by clinically nonfunctioning adenomas (15,16,20,23-26). It appears that most, if not all, such nonfunctioning adenomas contain or release LH, FSH, α-subunit and LHß (23,25,26). Our finding that 19 of 22 ademonas contained or released gonadotropins and/or subunits **in vitro** provides further evidence that the term nonfunctioning might at best be limited to the clinical presentation.

Two adenomas in this group contained or released ACTH **in vitro**, but neither patient had Cushing's disease. In both patients the 0800 h. plasma cortisol concentrations were within the normal range and one patient had a normal decrease of plasma cortisol in response to 1 mg dexamethasone and a normal cortisol production rate. Therefore, these two tumors can be regarded as silent corticotroph adenomas (33,34).

In one cultured adenoma no hormones or subunits could be demonstrated; it is unclear whether this tumor actually produced no glycoprotein hormones at all, or whether a substance not tested for, i.e. FSHß, was secreted. In the cultured tumor from one patient TSH was released in combination with LH, FSH, LHß and α-subunit, but the patient's serum glycoprotein hormone and subunit concentrations were not elevated, and the serum T4 concentration was normal (91 nmol/L). Asa et. al. (23) also have reported tumors that released all three glycoprotein hormones and α-subunit. They suggested that the tumor cells might derive from a precursor cell of the glycoprotein cell line.

A significant correlation between hormone release per 24 hours

and per 10^6 cells **in vitro** and **in vivo** hormonal levels was found for α-subunit, but not for LH and FSH. These findings may be due to the fact that in 14 of the 20 cultures in which a correlation could be computed α-subunit was released, whereas LH and FSH were released in only 7 and 9 cultures. Moreover, the quantity of normal pituitary tissue which might be present **in vivo** and the tumor volume might bias the correlation between **in vitro** and **in vivo** data.

In this series, all 5 patients with elevated serum gonadotropin and/or subunit concentrations were men. This observation correlates with the fact that the majority of pituitary adenomas with hypersecretion of gonadotropins or subunits so far described have been found in men (1-3,5,6,9,11-16,18-20,24,27,29,30,35,36).

However, most women who have a clinically nonfunctioning pituitary adenoma are menopausal, and the elevated serum gonadotropin and subunit values that occur normally in menopausal women may mask the secretion of gonadotropins and subunits by these tumors (11,16). Serum LH, FSH, and α-subunit levels were low in 7 of the 8 women in this series. However, 6 of these 7 women had an increased α-subunit/gonadotropin ratio, indicating that their LH and FSH levels were more profoundly decreased than were α-subunit levels, either as a consequence of hypopituitarism or as a consequence of α-subunit secretion by the pituitary tumor. The latter possibility is favoured, but not proven, by the fact that in 4 of 5 women with an elevated serum α-subunit/gonadotropin ratio **in vivo**, α-subunit release **in vitro** was found.

Elevated serum gonadotropin and subunit concentrations can be used as a tool in the diagnosis of clinically nonfunctioning adenomas. In the absence of hypersecretion of other hormones, or in the presence of anterior pituitary insufficiency, an elevated α-subunit/gonadotropin ratio may prove an additional diagnostic tool. In this series, inclusion of an elevated α-subunit/gonadotropin ratio as a sign of secretion of α-subunit by a pituitary tumor increased the percentage of patients with elevated hormone values of some type from 23% to 55%.

Stimulation of gonadotropin and subunit secretion from nonfunctioning and gonadotroph pituitary tumors by TRH and GnRH

and their suppression by bromocriptine, **in vivo** as well as **in vitro**, have been reported before (7-16,18,20,23,29,37). We found significant responses to TRH in 4 of 7 cultures (57%) **in vitro** and in 13 of 18 patients (72%) **in vivo**. No relation was found between the height of the response to TRH and baseline gonadotropin levels, either **in vivo** or **in vitro**. This fact implies that the distinction between gonadotroph and nonfunctioning adenomas, based on immunocytochemical staining of the tissue or on the presence of elevated serum gonadotropin levels **in vivo**, is absent as far as hormone dynamics are concerned.

Susceptibility to suppression by bromocriptine and stimulation by TRH and GnRH were found to be linked **in vitro**, suggesting that the presence of TRH receptors in adenomatous cells (as reported by Peillon et al., 38) is accompanied by the presence of GnRH- and dopamine-receptors (as reported by Bevan and Burke, 39). **In vivo**, however, this correlation was not confirmed. Moreover, 8 of 15 hormone and subunit levels that did not respond to TRH or bromocriptine **in vivo** responded with significant changes to these substances **in vitro**. This discrepancy may result from one or more of the following: 1. Most **in vitro** incubations lasted for 48 to 72 hours, whereas **in vivo** a single dose of an agent was administered, resulting in a relatively short exposure to the effective dose. 2. Not every patient received a placebo, but individual reactions to placebo administration varied, so significant individual changes may have been missed. In 8 patients 10 tests were performed. Significant responses of at least one hormone or subunit **in vitro** were mimicked **in vivo** in 6 of 7 tests. In the remaining 3 tests, no significant changes occurred in any hormone, either **in vitro** or **in vivo**. As hormone release **in vitro** could be significantly lowered by bromocriptine in 5 out of 8 cultures, this correlation of **in vitro** and **in vivo** hormone responses may indicate that in more than 50% of the patients with a clinically nonfunctioning or gonadotroph pituitary adenoma suppression of gonadotropin or subunit levels with bromocriptine may be accomplished, especially if administration of this drug is repeated.

We conclude that: 1. virtually all clinically nonfunctioning

adenomas contain or release gonadotropins or their subunits **in vitro**; 2. **in vivo** hypersecretion of these hormones and subunits occurs infrequently and in this series only in men; 3. an elevated ratio of α-subunit to LH and FSH is frequently found in women and may prove a useful diagnostic tool; 4. responses to TRH and bromocriptine do not depend on baseline gonadotropin levels, either **in vivo** or **in vitro**, implying that the distinction between gonadotroph adenomas and adenomas without hypersecretion of gonadotropins **in vivo** is absent in so far as hormone dynamics are concerned.

REFERENCES

1. Beckers A, Stevenaert A, Mashiter K, Hennen G. Follicle-stimulating hormone-secreting pituitary adenomas. J Clin Endocrinol Metab 1985;61:525-8.
2. Berezin M, Olchovsky D, Pines A, Tadmor R, Lunenfeld B. Reduction of follicle-stimulating hormone (FSH) secretion in FSH-producing pituitary adenoma by bromocriptine. J Clin Endocrinol Metab 1984;59:1220-3.
3. Borges JLC, Ridgway EC, Kovacs K, Rogol AD, Thorner MO. Follicle-stimulating hormone-secreting pituitary tumor with concomitant elevation of serum α-subunit levels. J Clin Endocrinol Metab 1984;58:937-41.
4. Chapman AJ, Macfarlaine IA, Shalet SM, Beardwell CG, Dutton J, Sutton ML. Discordant serum α-subunit and FSH concentrations in a woman with a pituitary tumour. Clin Endocrinol 1984;21:123-9.
5. Friend JN, Judge DM, Sherman BM, Santen RJ. FSH-secreting pituitary adenomas: stimulation and suppression studies in two patients. J Clin Endocrinol Metab 1976;43:650-7.
6. Harris RI, Schatz NJ, Gennarelli T, Savino PJ, Cobbs WH, Snyder PJ. Follicle-stimulating hormone-secreting pituitary adenomas: correlation of reduction of adenoma size with reduction of hormonal hypersecretion after transsphenoidal surgery. J Clin Endocrinol Metab 1983;56:1288-93.
7. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 1983;59:585-9.
8. Kourides IA, Weintraub BD, Rosen SW, Ridgway EC, Kliman B, Maloof F. Secretion of alpha subunit of glycoprotein hormones by pituitary adenomas. J Clin Endocrinol Metab 1976;43:97-106.
9. Peterson RE, Kourides IA, Horwith M, Vaughan ED Jr., Saxena BB, Fraser RAR. Luteinizing hormone- and α-subunit-secreting pituitary tumor: positive feedback of estrogen. J Clin Endocrinol 1981;52:692-8.
10. Ridgway EC, Klibanski A, Ladenson PW, et al. Pure alpha-secreting pituitary adenomas. N Eng J Med 1981;304:1254-9.
11. Ridgway EC. Glycoprotein hormone production by pituitary tumors. In: Black PMcL, ed. Secretory tumors of the pituitary

gland. New York, Raven Press, 1984:343-63.
12. Roman SH, Goldstein M, Kourides IA, Comite F, Bardin CW, Krieger DT. The luteinizing hormone-releasing hormone (LHRH) agonist [D-Trp^6-Pro^9-NEt] LHRH increased rather than lowered LH and α-subunit levels in a patient with an LH-secreting pituitary tumor. J Clin Endocrinol Metab 1984;58:313-9.
13. Snyder PJ, Johnson J, Muzyka R. Abnormal secretion of glycoprotein α-subunit and follicle-stimulating hormone (FSH) ß-subunit in men with pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 1980;51:579-84.
14. Snyder PJ, Muzyka R, Johnson J, Utiger RD. Thyrotropin releasing hormone provokes abnormal follicle-stimulating hormone (FSH) and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 1980;51:744-8.
15. Snyder PJ, Bashey HM, Kim SU, Chappel SC. Secretion of uncombined subunits of luteinizing hormone by gonadotroph cell adenomas. J Clin Endocrinol Metab 1984;59:1169-75.
16. Snyder PJ. Gonadotroph cell adenomas of the pituitary. Endocrine Rev 1985;6:552-63.
17. Trouillas J, Girod C, Sassolas G, et al. Human pituitary gonadotropic adenoma; histological, immunocytochemical, and ultrastructural and hormonal studies in eight cases. J Pathology 1981;135:315-36.
18. Vance ML, Ridgway EC, Thorner MO. Follicle-stimulating hormone- and α-subunit-secreting pituitary tumor treated with bromocriptine. J Clin Endocrinol Metab 1985;61:580-4.
19. Whitaker MD, Prior JC, Scheithauer B, Dolman L, Durity F, Pudek MR. Gonadotrophin-secreting pituitary tumour: report and review. Clin Endocrinol 1985;22:43-8.
20. Lamberts SWJ, Verleun T, Oosterom R, et al. The effects of bromocriptine, thyrotropin-releasing hormone, and gonadotropin-releasing hormone on hormone secretion by gonadotropin-secreting pituitary adenomas in vivo and in vitro. J Clin Endocrinol Metab 1987;64:524-30.
21. Mukai K. Pituitary adenomas. Immunocytochemical study of 150 tumors with clinicopathologic correlation. Cancer 1983; 52:648-53.
22. Wide L, Lundberg PO. Hypersecretion of an abnormal form of follicle stimulating hormone associated with suppressed luteinizing hormone secretion in a woman with a pituitary adenoma. J Clin Endocrinol Metab 1981;53:923-30.
23. Asa SL, Gerrie BM, Singer W, Horvath E, Kovacs K, Smyth HS. Gonadotropin secretion in vitro by human pituitary null cell adenomas and oncocytomas. J Clin Endocrinol Metab 1986;62: 1011-9.
24. Snyder PJ, Bashey HM, Phillips JL, Gennarelli TA. Comparison of hormonal secretory behavior of gonadotroph cell adenomas in vivo and in culture. J Clin Endocrinol Metab 1985; 61:1061-5.
25. Surmont DWA, Winslow CLJ, Loizou M, White MC, Adams EF, Mashiter K. Gonadotrophin and alpha subunit secretion by human 'functionless' pituitary adenomas in cell culture: long term effects of luteinizing hormone releasing hormone and thyrotrophin releasing hormone. Clin Endocrinol 1983;19: 325-36.

26. Mashiter K, Adams E, van Noorden S. Secretion of LH, FSH and PRL shown by cell culture and immunocytochemistry of human functionless pituitary adenomas. Clin Endocrinol 1981; 15:103-12.
27. Demura R, Jibiki K, Kubo O, et al. The significance of α-subunit as a tumor marker for gonadotropin-producing pituitary adenomas. J Clin Endocrinol Metab 1986;63:564-9.
28. Capella C, Buffa R, Usellini L, et al. Alpha and beta subunits of glycoprotein hormones in argyrophil pituitary tumors with small granule cells. Ultrastructural Pathol 1983;4:35-50.
29. Klibanski A, Deutsch PJ, Jameson JL, et al. Luteinizing hormone-secreting pituitary tumor: biosynthetic characterization and clinical studies. J Clin Endocrinol Metab 1987; 64:536-42.
30. Ishibashi M, Yamaji T, Takaku F, Teramoto A, Fukushima T. Secretion of glycoprotein hormone α-subunit by pituitary tumors. J Clin Endocrinol Metab 1987;64:1187-93.
31. Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SWJ. Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. J Endocrinol Invest 1984;7:307-11.
32. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames, Iowa State University Press, 1980:235.
33. Horvath E, Kovacs K, Killinger DW, Smyth HS, Platts ME, Singer W. Silent corticotropic adenomas of the human pituitary gland. Am J Pathol 1980;98:617-38.
34. Hassoun J, Charpin C, Jaquet P, Lissitzky JC, Grisoli F, Toga M. Corticolipotropin immunoreactivity in silent chromofobe adenomas. Arch Pathol Lab Med 1982;106:25-30.
35. Barrow DL, Tindall GT, Kovacs K, Thorner MO, Horvath E, Hoffman JC Jr.. Clinical and pathological effects of bromocriptine on prolactin-secreting and other pituitary tumors. J Neurosurg 1984;60:1-7.
36. Horvath E, Kovacs K. Gonadotroph adenomas of the human pituitary: sex-related fine-structural dichotomy. Am J Pathol 1984;117:429-40.
37. Macfarlane IA, Beardwell CG, Shalet SM, Ainslie G, Rankin E. Glycoprotein hormone α-subunit secretion in patients with pituitary adenomas: influence of TRH, LRH and bromocriptine. Acta Endocrinol 1982;99:487-92.
38. Peillon F, Bression B, Le Dafniet M, et al. Receptor studies in human pituitary adenomas. Do they contribute to a better understanding of their pathogenesis? In: Landolt AM, Heitz PU, Zapf J, Girard J, Del Pozo E, eds. Advances in pituitary adenoma research. Oxford, Pergamon Press, 1988:67-76.
39. Bevan JS, Burke CW. Non-functioning pituitary adenomas do not regress during bromocriptine therapy but possess membrane-bound dopamine receptors which bind bromocriptine. Clinical Endocrinology 1986;25:561-72.

6. ADDITIONAL DATA ON CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS.

6.1 SIMILARITIES AND DIFFERENCES BETWEEN CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS.

From chapter 5 it can be concluded that virtually all clinically nonfunctioning and gonadotroph pituitary adenomas contain or release gonadotropins or gonadotropin subunits **in vitro**. The distinction between gonadotroph and clinically nonfunctioning adenomas, based on the presumed absence of gonadotropin secretion by clinically nonfunctioning adenomas **in vivo**, is absent under **in vitro** conditions. Apparently, clinically nonfunctioning adenomas produce such small amounts of gonadotropins and gonadotropin subunits, that **in vivo** serum concentrations of these glycoproteins are not elevated. Moreover, adenomatous cells that contain or release gonadotropins and subunits **in vitro** may not be recognized as endocrine active cells by histochemistry or cytochemistry of the tumor tissue.

In Table 1 a comparison is made between the outcomes of histochemistry, cytochemistry of the isolated cells, and cell culture, applied to a number of tumor tissues. TSH was detected by cytochemistry in 8 of 13 tumors, but only in 1 could TSH production be demonstrated **in vitro**. This discrepancy is likely to be due to a lack in specificity of the TSH antibody used in cytochemistry, as in 7 of 8 cases in which TSH production was demonstrated by cytochemistry, α-subunit production was found during cell-culture. In 7 of 11 cases, gonadotropin production by the tumor was demonstrated during cell-culture, but not by histochemistry. Cytochemistry of the tumor cells that were isolated for cell-culture, revealed scattered cells which showed immunopositivity for LH or FSH in 5 of these 7 tumors. From these results, it is clear that isolation of pituitary tumor cells (as described in Chapter 5) and measuring hormone concentrations in the lysates of these cells is a more sensitive method to demonstrate the presence of hormones in the tumor cells than is immunohistochemistry.

Table 1. Results of immunohistochemistry of the removed tumor tissue, immunocytochemistry of isolated tumor cells, and cell culture of these cells from 16 clinically nonfunctioning and gonadotroph pituitary adenomas.

	Immunohistochemistry (tumor tissue)			Immunocytochemistry (isolated cells)				Cell-culture				
Sex	LH	FSH	TSH	LH	FSH	TSH	LH-subunits	LH	FSH	TSH	α-subunit	LHβ
M				-	-			-	-	-	+	-
M				+++	±			+	+	-	+	+
M	-	-		+++	±			+	-	-	+	+
M				+	+++	-		+	+	-	+	+
M	-	-	±	-	±	±		+	+	-	+	+
M	±	-	-	±	-	-	±	-	-	-	-	+
M	-	-	-	-	±	-		-	+	-	+	-
M	±	±	±	-	-	±		+	+	-	+	+
M	-	-	-	-	-	+		+	+	-	+	+
M				-	±	±		-	+	-	-	-
F	-	-	-	-	-	-		-	-	-	+	-
F				-	-	-	-	-	-	-	-	-
F	-	-	-	-	-	±		+	+	-	+	+
F	-	-		-	±	±		+	+	+	+	+
F	-	-	-	±	±	±		-	+	-	+	-
F	-	-	±	-	-	±		-	-	-	+	-
Total	2	1	3	5	8	8	1	8	10	1	13	9
%	18	9	33	31	50	62	50	50	63	6	81	56

Number of cells showing immunopositivity: +++: >50%; ++: 20-50%; +: 10-20%; ±: scattered cells.

The **in vitro** production of gonadotropins and their subunits is a common feature in both clinically nonfunctioning and gonadotroph pituitary adenomas. There are, however, more similarities:

-In both tumortypes hormone or subunit secretion can be stimulated by TRH and can be suppressed by bromocriptine, both **in vivo** and **in vitro**.

-Both tumortypes are diagnosed in elderly patients.

-Immunocytochemistry may or may not reveal cells that are positive for the gonadotropins or their subunits in both gonadotroph and clinically nonfunctioning pituitary adenomas (1-4).

-On electron microscopic examination, there are striking differences between the 2 tumortypes. The RER, moderately to well developed in gonadotroph adenomas, is poorly developed in clinically nonfunctioning adenomas. Secretory granules are more numerous in gonadotroph than in clinically nonfunctioning adenomas. An abundance of microtubules is observed in gonadotroph, but not in clinically nonfunctioning pituitary adenomas. Both, however, may be shown to consist of cells with secretory granules and numerous free ribosomes, while oncocytic transformation may be observed in both (1,5,6).

As the similarities are of greater clinical importance than the differences, clinically nonfunctioning and gonadotroph pituitary adenomas should be regarded as one clinical entity.

6.2 IN VITRO RESPONSES TO HORMONES AND DRUGS.

In Chapter 5 the **in vitro** gonadotropin release from clinically nonfunctiong and gonadotroph pituitary adenomas in response to TRH, GnRH and bromocriptine was discussed. There were no significant differences in intracellular hormone concentrations between cells incubated with or without TRH, GnRH or bromocriptine in 2, 4 and 5 tumors, respectively. In one tumor, the intracellular concentration of α-subunit, but not of LH, FSH or LHß, was significantly elevated after incubation with TRH or GnRH.

6.3 REFERENCES

1. Horvath E, Kovacs K. Gonadotroph adenomas of the human pituitary: sex-related fine-structural dichotomy. Am J Pathol 1984;117:429-40.
2. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 1983;59:585-9.
3. Kovacs K, Horvath E, Ryan N, Ezrin C. Null cell adenoma of the human pituitary. Virchows Arch [Pathol Anat] 1980;387: 165-74.
4. Landolt AM, Heitz PU. Alpha-subunit-producing pituitary adenomas. Virchows Arch [Pathol Anat] 1986;409:417-31.
5. Trouillas J, Girod C, Sassolas G, Claustrat B, Lheritier M, Dubois MP, Goutelle A. Human pituitary gonadotropic adenoma; histological, immunocytochemical, and ultrastructural and hormonal studies in eight cases. J Pathology 1981;135:315-

36.
6. Landolt AM, Oswald UW. Histology and ultrastructure of an oncocytic adenoma of the human pituitary. Cancer 1973;31: 1099-1105.

7. AGE-DEPENDENT CHANGES IN SERUM STEROID AND GONADOTROPIN CONCENTRATIONS.

7.1 CLUES TO THE ETIOLOGY OF CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS.

There are indications supporting the hypothesis that primary hypogonadism might play a role in the development of gonadotroph and clinically nonfunctioning pituitary adenomas:

-A high incidence of pituitary gonadotroph cell like adenomas can be found 15 months or more after gonadectomy in both male and female rats (1).

-In some patients with a gonadotroph adenoma, castration or ovarian ablation had been performed decades before the pituitary tumor was diagnosed (2-4).

-Gonadotroph, α-subunit secreting, and clinically nonfunctioning pituitary adenomas occur in elderly patients. Apart from adenomas that cause symptoms, subclinical pituitary adenomas which do not give rise to any endocrinological abnormalities or complaints have been found in 13% of the cases at unselected autopsies of men and women over 80 years of age. About 50% of these tumors have no immunocytochemical activity (5). In both normal men and women, peripheral levels of "free" sex steroids decrease with aging. In men there is an age-dependent decrease in serum free testosterone concentrations and a moderate increase in serum gonadotropin levels (6), while in postmenopausal women estrogen concentrations are low and serum gonadotropin levels are high.

On the other hand, the majority of patients with a gonadotroph, clinically nonfunctioning or α-subunit secreting adenoma have a history of normal gonadal function and have children. In most men with such pituitary adenomas, a significant rise in serum testosterone in response to CG is noted (7), while in premenopausal patients ovarian estradiol production may be normal or become normal after therapy (8), indicating that primary defects in the gonads do not play a role. Also, in aging men a moderate age-associated decrease in serum free testosterone

concentrations occurs (6), while in postmenopausal women serum estrogen concentrations are dramatically low in comparison with those in premenopausal women. If hypogonadism were an etiological factor in the development of gonadotroph, α-subunit secreting and clinically non-functioning pituitary adenomas, the incidence of these tumors should be higher in women than in men. However, no sex-related difference in the incidence of these tumors exists.

In aging men, an age-associated decrease in plasma free testosterone levels is accompanied by an increase in serum gonadotropin concentrations (6). By contrast, in postmenopausal women circulating sex steroid concentrations do not decrease with age, while serum gonadotropin concentrations do decline with age (Chapter 7.2). Hormone production by the pituitary gonadotroph cells declines in aging postmenopausal women, and increases in aging men. Whether this sex-related difference in gonadotroph cell activity is reflected in the fact that gonadotroph pituitary adenomas are diagnosed for the most part in men is speculative, as high serum gonadotropin concentrations that occur normally in postmenopausal women may mask the secretion of gonadotropins by a gonadotroph pituitary tumor.

In Chapter 7.2 it is discussed that circulating sex steroid concentrations do not influence serum gonadotropin levels in postmenopausal women, while hormone production by the pituitary gonadotroph cell is less responsive to the administration of estradiol and drugs than in premenopausal women. In old men gonadotropin secretion is less influenced by the administration of antiopioids than in young men, possibly implicating a decreased responsiveness of the pituitary gonadotroph cell to GnRH (9). So, compared to young subjects, in both aging men and aging women pituitary gonadotroph cell activity is less affected by drug administration. Whether these relatively autonomously secreting gonadotroph cells which are associated with aging, may develop into clinically nonfunctioning or gonadotroph adenoma cells, is a tempting speculation.

In conclusion, hypogonadism is present in both aging men and aging women and could play a role in the development of gonadotroph and clinically nonfunctioning pituitary adenomas. On the

other hand, relatively autonomously secreting gonadotroph cells which are present in aging subjects might develop into clinically nonfunctioning or gonadotroph pituitary adenomas, independent of circulating sex steroid concentrations. Lastly, a hypothalamic cause of the adenomas should be considered.

REFERENCES

1. Griesbach WE, Purves HD. Basophil adenomata in the rat hypophysis after gonadectomy. Br J Cancer 1960;14:49-59.
2. Nicolis G, Shimshi M, Allen C, Halmi NS, Kourides IA. Gonadotropin-producing pituitary adenoma in a man with long-standing primary hypogonadism. J Clin Endocrinol Metab 1988;66:237-41.
3. Surmont DWA, Winslow CLJ, Loizou M, White MC, Adams EF, Mashiter K. Gonadotrophin and α-subunit secretion by human 'functionless' pituitary adenomas in cell culture: long term effects of luteinizing hormone releasing hormone and thyrotrophin releasing hormone. Clin Endocrinol 1983; 19:325-36.
4. Kovacs K, Horvath E, Rewcastle NB, Ezrin C. Gonadotroph cell adenoma of the pituitary in a woman with longstanding hypogonadism. Arch Gynecol 1980;229:57-65.
5. Kovacs K, Ryan N, Horvath E, Singer W, Ezrin C. Pituitary adenomas in old age. J Gerontol 1980;35:16-22.
6. Deslypere JP, Vermeulen A. Leydig cell function in normal men: effect of age, lifestyle, residence, diet, and activity. J Clin Endocrinol Metab 1984;59:955-62.
7. Snyder PJ, Brigdeli H, Gardner DF, Mihailovic V, Rudenstein RS, Sterling FH, Utiger RD. Gonadal function in fifty men with untreated pituitary adenomas. J Clin Endocrinol Metab 1979;48:309-14.
8. Cook DM, Watkins S, Snyder PJ. Gonadotrophin-secreting pituitary adenomas masquerading as primary ovarian failure. Clin Endocrinol 1986;25:729-38.
9. Vermeulen A, Deslypere JP, De Meirleir K. A new look to the andropause: altered function of the gonadotrophs. J Steroid Biochem 1989;32:163-5.

7.2 SERUM GONADOTROPINS AND THEIR SUBUNITS DECLINE IN AGING NORMAL POSTMENOPAUSAL WOMEN.

D.J. Kwekkeboom[1], F.H. de Jong[1], A.M. van Hemert[2], J.P. Vandenbroucke[3], H.A. Valkenburg[2] and S.W.J. Lamberts[1].

[1] Department of Medicine, and [2] Department of Epidemiology, Erasmus University, Rotterdam, The Netherlands.

[3] Department of Clinical Epidemiology, University Hospital Leiden, The Netherlands.

Submitted.

ABSTRACT

In a group of 680 postmenopausal women participating in a population survey we investigated the relationships between serum gonadotropin, gonadotropin-subunit and prolactin (PRL) concentrations and age, body mass index (BMI) and levels of sex hormone binding globulin (SHBG), estrogens and androstenedione.

Gonadotropin, α-subunit, and LHß levels were negatively correlated with age, while PRL levels did not decrease with age. Predicted serum concentrations in women aged 55 and 75 years, respectively, decreased for LH from 47.1 to 32.4 IU/L, for FSH from 72.1 to 61.6 IU/L, for α-subunit from 2.6 to 1.9 µg/L, and for LHß from 3.2 to 2.4 µg/L. The ratio of α-subunit to LH and FSH decreased and the ratio of LHß to LH increased with age. These changes may either be caused by a direct effect of aging on pituitary gonadotroph cells or by an effect of aging on the hypothalamic regulation of these cells. Serum gonadotropin and subunit concentrations were negatively correlated with the BMI,

but not with circulating estradiol levels.

In addition, we found that estrone and estradiol levels were positively correlated with the BMI, while circulating levels of androstenedione and estrone were more important factors determining estrone and estradiol levels, respectively.

In conclusion: in contrast to what has been reported in normal aging men, serum LH, FSH, α-subunit, and LHß concentrations decrease with age in normal postmenopausal women.

INTRODUCTION

In perimenopausal women an increase in serum LH and FSH concentrations occurs in association with a gradual decline in ovarian estradiol production. It is well known that in postmenopausal women serum LH, FSH and α-subunit concentrations are high in comparison to those in premenopausal women, but whether gonadotropin and subunit levels change during the postmenopause is not known.

In aging men, an age-associated decrease in plasma testosterone levels is accompanied by an increase in serum gonadotropin concentrations (1). As estradiol levels in postmenopausal women have been reported to decrease with age (2), an associated rise in serum gonadotropin concentrations might be expected. On the other hand, the secretion of gonadotropins in postmenopausal women seems to be rather insensitive to changes in serum estradiol concentrations, as the administration of high doses of estradiol does not suppress gonadotropin levels to premenopausal values (3,4).

The aim of the present study was to determine whether aging and endogenous plasma androstenedione and estrogen concentrations influence serum gonadotropin and subunit levels in postmenopausal women. Additionally, we studied the correlations between serum androstenedione, estrogen, SHBG and PRL concentrations.

SUBJECTS AND METHODS

Subjects and parameters

From a large population survey, which took place between 1975 and 1978 and was designed to study the prevalence and determinants of chronic diseases (5), all female participants initially aged 45 to 64 years were selected in 1985 and 1986 for a follow-up study (6). Of the initial selection of 1167 women 71 had died and 87 had moved. Of the remaining 1009 women 855 (85%) participated in the follow-up study.

Age, weight and height were determined, and serum levels of LH, FSH, α-subunit, LHß, estrone, estradiol, androstenedione and SHBG were measured. A complete dataset was available for 697 women, of whom 17 were excluded from the analyses, because they had estradiol levels above 100 pmol/L. The period since menopause in these 680 women was 13.5 ± 7.4 years (range: 1 to 38 years).

The BMI was calculated as the ratio of weight to square height. This is a parameter for the degree of obesity which is independent of height (7).

The α-subunit/gonadotropin ratio was computed by dividing the α-subunit level by the sum of LH and FSH levels and multiplying the result by 100.

The LHß/LH ratio was obtained by multiplying the ratio of LHß to LH by 100.

Immunoassays

Estrone and SHBG levels were measured by radioimmunoassay (RIA) as described elsewhere (8,9). Estradiol and androstenedione levels were measured using RIA kits (Diagnostic Products Corporation, Los Angeles and Eurodiagnostics, Apeldoorn, The Netherlands, respectively). α-Subunit and LHß levels were measured by RIA using antibodies from UCB (Brussels, Belgium). LH, FSH and PRL levels were measured by radioimmunometric assays supplied by IRE-Medgenix (Brussels, Belgium).

Intra- and interassay coefficients of variation were: <14% and <19% for the estrone assay; <12% and <18% for the SHBG assay; <15% and <19% for the estradiol assay; <11% and <17% for the

androstenedione assay; <6% and <11% for the α-subunit assay; <7% and <13% for the LHß assay; <5% and <15% for the LH assay; <3% and <8% for the FSH assay; and <7% and <8% for the PRL assay, respectively.

In the gonadotropin and subunit assays crossreactivities on a weight/weight basis were: LH assay -FSH 0.5%, α-subunit 0.4%, LHß <0.5%; FSH assay -LH and α-subunit 0.06 %, LHß <0.01%; α-subunit assay -LH 3.9%, FSH 20.0%, LHß 0.2%; LHß assay -LH 53.4%, FSH 1.1%, and α-subunit 1.8%. The potencies of the preparations used to determine the crossreactivities were 6.6 IU/μg for LH and 8.7 IU/μg for FSH.

Statistics

Relationships between variables were studied using univariate and multivariate linear regression analysis. Multivariate regression analysis was applied in order to adjust for inter-dependency of the variables. Using the number of years after menopause or years of age as an independent variable yielded virtually the same results. Therefore, in tables and text only age has been mentioned as independent variable.

Multivariate regression analysis was performed including all variables and using step-up- and step-down methods (10). As the latter methods did not greatly affect the significance of outcomes, results obtained using these techniques have not been included in tables or text.

In order to facilitate comparisons between effects of variables, standard regression coefficients were used. These were computed by multiplying the regression coefficient (b) by the SD of the independent variable (x) and dividing the result by the SD of the dependent variable (y) (11). The standard regression coefficient indicates the change in the dependent variable, expressed in terms of the SD of that variable, that is brought about by a change of 1 SD in the independent variable.

RESULTS

Age, BMI, and serum levels of SHBG, gonadotropin-subunits and

hormones in the 680 postmenopausal women studied, have been summarized in Table 1.

Table 1. Descriptive and hormonal characteristics of 680 postmenopausal women.

Variable	Median	Mean	SD	Range
Age (years)	61.4	62.3	5.7	52.9 - 76.2
BMI (kg/h^2)	25.9	26.3	3.9	16.5 - 47.3
LH (IU/L)	39.9	41.8	18.0	3.3 - 162.1
FSH (IU/L)	67.5	68.3	20.8	9.2 - 176.8
α-subunit (µg/L)	2.2	2.3	0.9	0.6 - 16.2
LHβ (µg/L)	2.7	2.9	1.1	0.5 - 9.4
α- subunit/ gonadotropin ratio (%)	2.1	2.2	0.6	1.1 - 6.5
LHβ/LH ratio (%)	7.0	7.5	2.6	1.4 - 46.4
PRL (µg/L)	4.8	6.0	7.3	1.5 - 147.9
Estrone (pmol/L)	130	138	52	25 - 353
Estradiol (pmol/L)	23	27	20	4 - 98
Androstenedione (nmol/L)	3.1	3.4	1.9	0.2 - 16.1
SHBG (nmol/L)	73.0	80.2	51.3	1.6 - 416.6

In Table 2 standard regression coefficients of univariate regression equations have been listed. As has been mentioned under Subjects and Methods, the standard regression coefficient indicates the change in the dependent variable (y), expressed in terms of the SD of that variable, that is brought about by a change of 1 SD in the independent variable (x). For instance, if the variable age changes by one SD, LH levels diminish by 0.23 SD (see Table 2). The most important factors influencing serum gonadotropin and subunit concentrations in postmenopausal women are age and the BMI, while PRL levels are positively correlated with estradiol levels and age. Estradiol and estrone levels are influenced for the most part by estrone and androstenedione levels, respectively.

In Figures 1 to 4 the relationships between age and serum LH, FSH, α-subunit, and LHß concentrations are shown. In figures 5 and 6 the relationships between the BMI and serum FSH concentrations and between estrone and androstenedione levels are represented.

Table 2. Standard regression coefficients of univariate linear regression equations in 680 postmenopausal women. Y denotes the dependent, X the independent variable.

Y \ X	Age	BMI	Estrone	Estradiol	Androstenedione	SHBG
LH	-0.23***	-0.19***	-0.03	0.01	0.06	0.01
FSH	-0.14***	-0.30***	-0.15***	-0.03	-0.03	0.07
α-subunit	-0.24***	-0.12***	0.08*	0.06	0.08*	-0.01
LHβ	-0.20***	-0.21***	-0.00	-0.02	0.02	0.08
α-subunit/ gonadotropin ratio	-0.08*	0.15***	0.22***	0.10**	0.08*	-0.09*
LHβ/LH ratio	0.16***	0.04	0.06	-0.03	-0.07	0.06
PRL	0.11**	-0.00	0.00	0.12**	0.08*	0.10**
Estradiol	0.04	0.21***	0.38***		0.25***	-0.09*
Estrone	-0.02	0.34***		0.38***	0.49***	-0.12**
Androstenedione	0.02	0.14***				-0.07
SHBG	0.13**	-0.27***	-0.12**	-0.09*	-0.07	
BMI	0.05					

*: P<0.05; **: P<0.01; ***: P<0.001.

To illustrate the effect of age on serum gonadotropin and subunit concentrations, levels of these glycoproteins at 3 age levels have been listed in Table 3. Levels stated are predicted values from univariate regression analyses; predicted values from multivariate regression analyses are virtually the same when mean values are substituted for all other entered variables.

In Table 4 standard regression coefficients of multivariate regression equations have been listed. The most striking difference between the outcomes of univariate and multivariate regression equations is that effects of SHBG levels on several variables which were significant when tested univariately, were no longer significant in multivariate equations. This is probably due to the fact that SHBG levels were very strongly correlated with the BMI. It is also apparent that the standard regression coefficients of age and BMI on all variables were not greatly affected by introducing more variables into the equations. The marginally significant correlations between PRL levels and age, serum androstenedione and SHBG concentrations were no longer significant when 5 high serum PRL concentrations, ranging from 37.5 to 147.9 μg/L, were omitted.

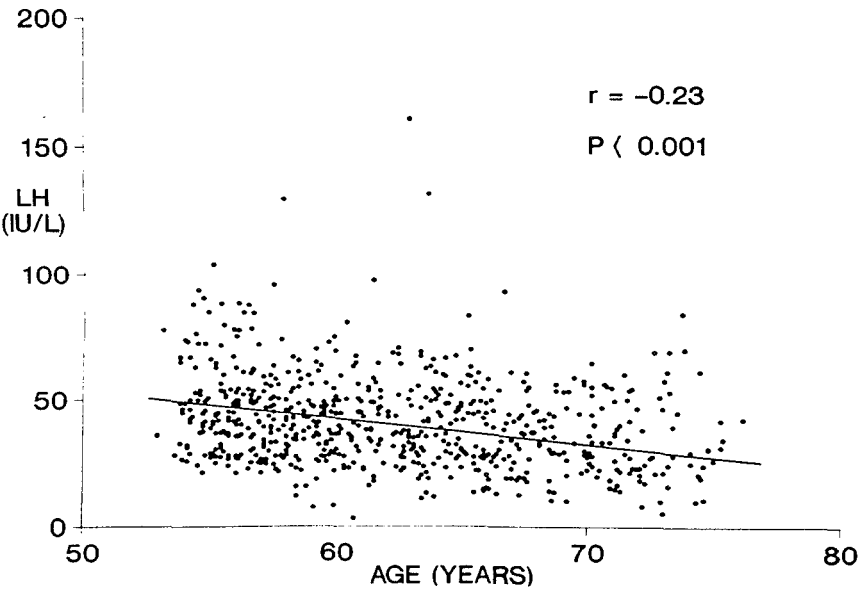

Figure 1. Relationship between LH levels and age in 680 postmenopausal women. The regression line and correlation coefficient refer to univariate regression analysis.

Table 3. Predicted values of gonadotropins and subunits from univariate regression analyses in 680 postmenopausal women.

Age (years)	55	65	75
LH (IU/L)	47.1	39.8	32.4
FSH (IU/L)	72.1	66.8	61.6
α-Subunit(μg/L)	2.6	2.2	1.9
LHß (μg/L)	3.2	2.8	2.4

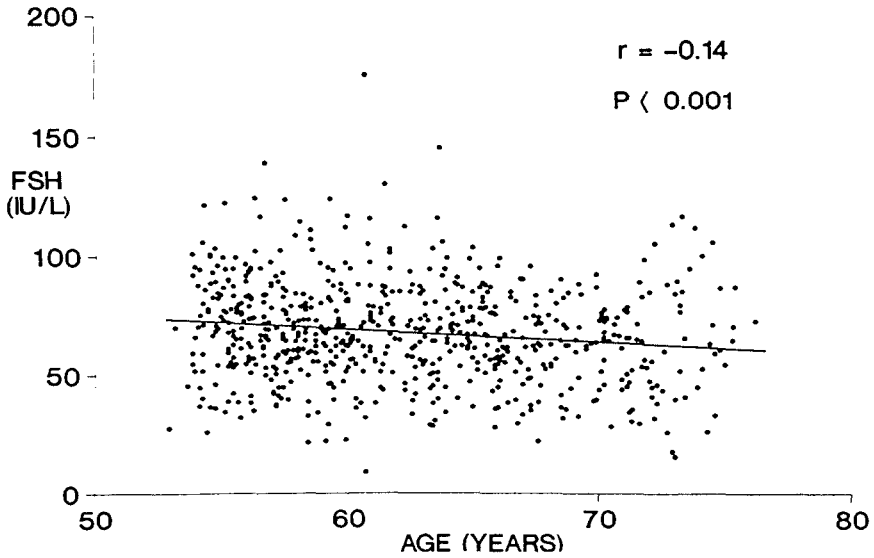

Figure 2. Relationship between FSH levels and age in 680 postmenopausal women. The regression line and correlation coefficient refer to univariate regression analysis.

DISCUSSION

The relationships between estrogens, androstenedione, age and the BMI or "percent ideal weight" in postmenopausal women have been studied by several investigators (2,12-20). In most of these reports, however, only 3 or 4 variables were studied, and the numbers of subjects studied were usually small. In this series, the number of subjects was large and effects of each variable were studied both with and without correction for the influence of other variables. To our knowledge, this is the first report on the influence of age and other variables on gonadotropin and gonadotropin-subunit levels in postmenopausal women. In the next paragraphs the principal relationships will be discussed separately.

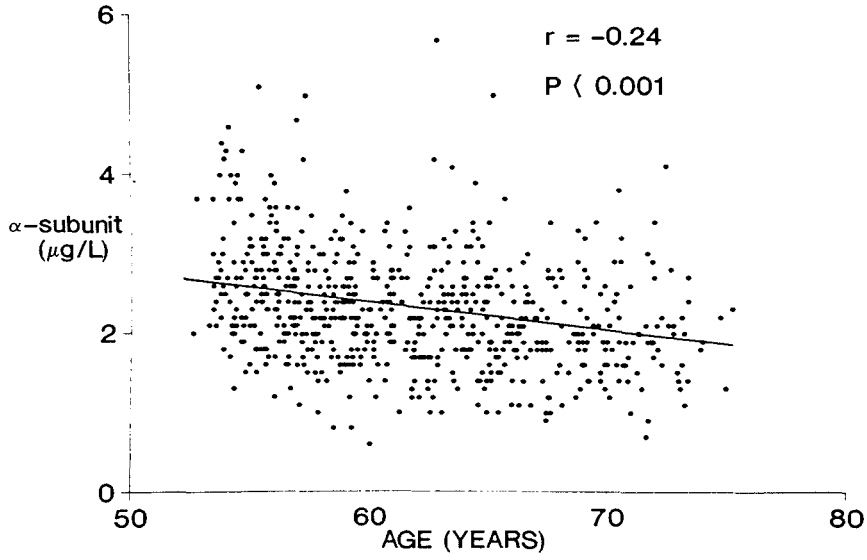

Figure 3. Relationship between α-subunit levels and age in 679 postmenopausal women. The regression line and correlation coefficient refer to univariate regression analysis. One extreme α-subunit level (16.2 μg/L) was omitted; this scarcely changed the slope of the regression line.

LH, FSH, α-subunit, and LHß levels were negatively correlated with age. This effect of age is independent of serum estradiol, estrone, androstenedione or SHBG concentrations, as is clear from the multivariate regression analysis. Aging might affect the secretory activity of the pituitary gland, but this is unlikely as serum PRL concentrations did not decrease with age. Therefore, aging may either affect the secretory activity of the pituitary gonadotroph cells or may influence the hypothalamic control of gonadotropin and subunit secretion, by changing GnRH pulse frequency or amplitude in postmenopausal women. This may either

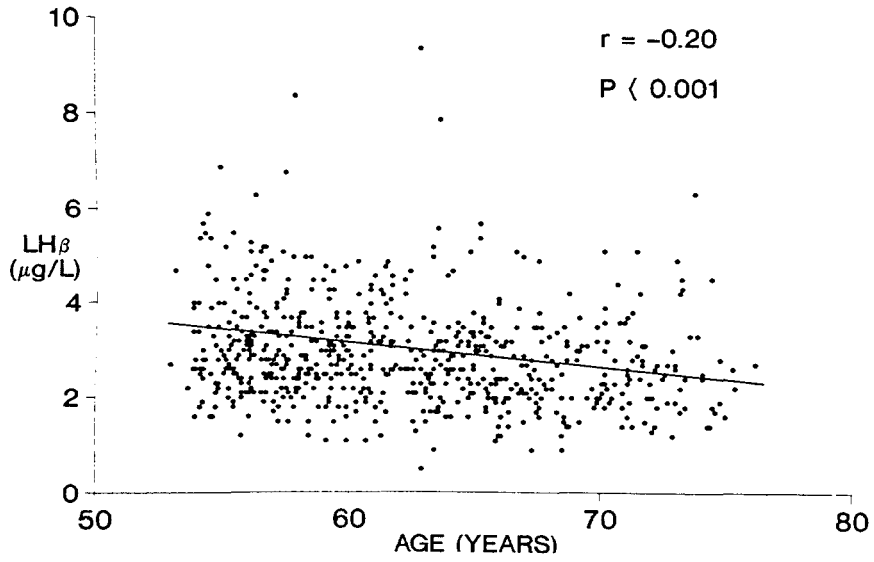

Figure 4. Relationship between LHß levels and age in 680 postmenopausal women. The regression line and correlation coefficient refer to univariate regression analysis.

be a direct effect of aging on GnRH neurones or an effect on factors involved in the regulation of GnRH secretion, such as dopamine or opioids. Hypothalamic dopamine levels might decrease with age, resulting in lower GnRH levels (21,22,23). If this were the case, a positive correlation between PRL levels and age could be expected. In this study, however, PRL levels did not increase significantly with age when the pathologically high serum PRL concentrations of 5 women were omitted, making this explanation less likely. The hypothalamic opioid tonus might increase with age, while ß-endorphin is capable of inhibiting GnRH secretion (23). However, GnRH secretion in postmenopausal women seems to be rather independent of opioid control, as administration of the opiate antagonist naloxone in postmenopausal subjects does not

cause an increase in peripheral LH levels (24). Therefore, it seems most likely that aging affects the secretory activity of either the pituitary gonadotroph cells or of the GnRH neurones.

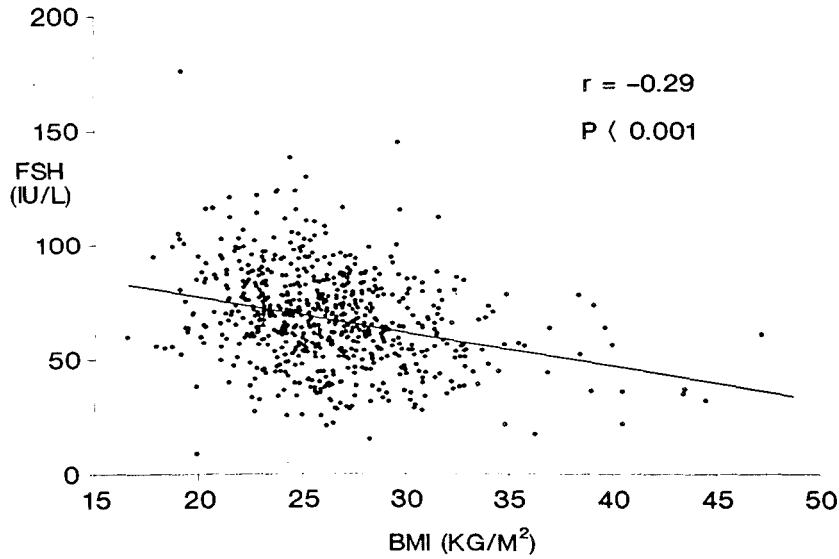

Figure 5. Relationship between FSH levels and the BMI in 680 postmenopausal women. The regression line and correlation coefficient refer to univariate regression analysis.

Gonadotropin and subunit levels were negatively correlated with the BMI. This relationship is independent of alterations in estrogen or SHBG levels correlated with the BMI, as is clear from the multivariate regression analysis. LH levels have been reported to be low as compared to controls in obese premenopausal patients (25,26,27). Increased levels of ß-endorphin in obese women (28), resulting in decreased serum gonadotropin concentrations, might cause this phenomenon, but opioid control of gonadotropin secretion does not appear very important in postmenopausal women, as discussed above.

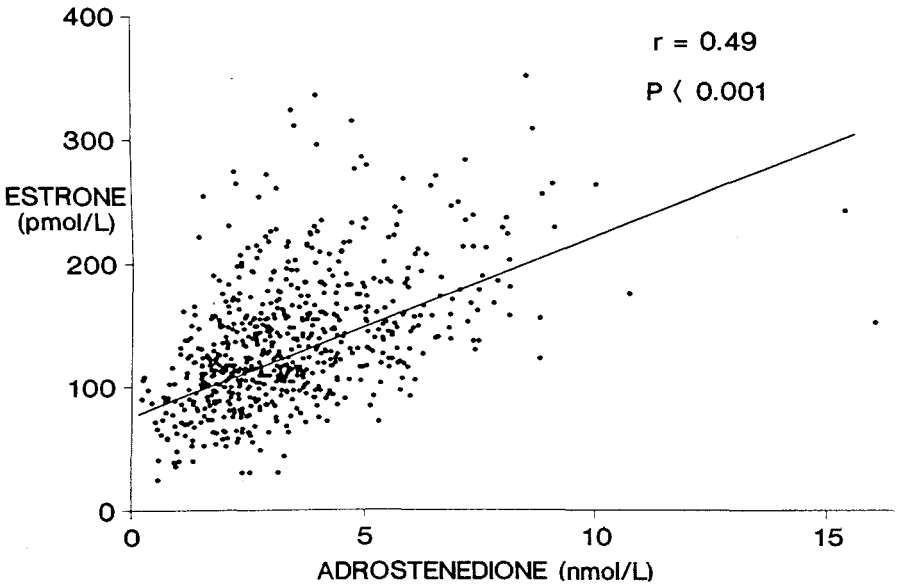

Figure 6. Relationship between estrone levels and androstenedione levels in 680 postmenopausal women. The regression line and correlation coefficient refer to univariate regression analysis.

Androstenedione levels were weakly correlated with LH levels in the multivariate analysis. The reason for this relationship is unclear.

Marginally significant effects of estrone levels on serum FSH concentrations were found. The negative correlation between estrone and FSH levels may be due to the capacity of brain tissue to convert this hormone to estradiol, as has been demonstrated in monkey hypothalami (29). It should be emphasized, however, that the standard regression coefficient of the relationship between serum estrone and FSH concentrations is small, and that therefore this relationship does not seem of great importance.

The α-subunit/gonadotropin ratio was negatively correlated with age. This means that α-subunit levels decrease faster with

age than the gonadotropin levels. In contrast, the BMI affects LH and FSH levels stronger than α-subunit levels.

Table 4. Standard regression coefficients of multivariate linear regression equations in 680 postmenopausal women. Y denotes the dependent, X the independent variable.

Y \ X	Age	BMI	Estrone	Estradiol	Androstenedione	SHBG
LH	-0.23***	-0.19***	-0.03	0.05	0.09*	-0.00
FSH	-0.14***	-0.27***	-0.11*	0.06	0.05	0.01
α-subunit	-0.23***	-0.16***	0.08	0.05	0.05	-0.00
LHβ	-0.20***	-0.21***	0.06	0.01	0.02	0.05
α-subunit/ gonadotropin ratio	-0.07	0.08*	0.19***	0.02	-0.03	-0.03
LHβ/LH ratio	0.16***	0.02	0.15**	-0.06	-0.13**	0.05
PRL	0.09*a	0.01	-0.08	0.13**	0.09*a	0.10*a
Estradiol	0.04	0.08*	0.31***		0.09*	-0.03
Estrone	-0.05	0.24***		0.23***	0.40***	-0.00
Androstenedione	0.02	0.13**				-0.03
SHBG	0.14***	-0.26***	-0.01	-0.03	-0.02	

***: P<0.05; **: P<0.01; ***: P<0.001. a: trend not significant when 5 high serum PRL concentrations (range 37.5 - 147.9 μg/L) were omitted.**

The LHß/LH ratio, on the other hand, showed a positive correlation with age, implying that more free LHß relative to LH is secreted with aging. So, as the years progress, a shift to more free LHß, LH and FSH relative to α-subunit secretion can be shown in postmenopausal women. A positive influence of estrone levels on both the α-subunit/gonadotropin ratio and the LHß/LH ratio was noticed. This again may represent a negative feedback on LH levels of estradiol originating from the conversion of estrone in the hypothalamus (29). The negative correlation of the LHß/LH ratio with androstenedione levels might be ascribed to the positive correlation of LH with this steroid, as discussed above.

Finally, the absence of a significant negative feedback of peripheral estradiol levels on serum gonadotropin and subunit concentrations should be discussed. Several explanations may be offered: firstly, estradiol levels in postmenopausal women may be too low to elicit a response in gonadotropin and subunit levels. Secondly, the secretion of gonadotropins in postmenopausal subjects seems to be hard to influence, as the administration

of high doses of estradiol does not suppress serum gonadotropin concentrations to premenopausal values (3,4), and the administration of naloxone to these women does not result in higher LH levels (24). Lastly, peripheral levels of estradiol may not represent estradiol levels in central nervous tissues: conversion of testosterone, androstenedione, and estrone to estradiol by these tissues has been reported (29,30,31) and may play a more important role in postmenopausal than in premenopausal women, as in postmenopausal women peripheral levels of estradiol are low in comparison with levels of androstenedione and estrone.

SHBG levels were negatively correlated with estrone and estradiol levels, but these relationships appeared to be due to the fact that estrone and estradiol levels were strongly correlated with the BMI ($r=0.34$ and $r=0.21$, respectively), as significance of the correlation between serum SHBG and estrogen concentrations was absent when tested in multivariate regression analysis. SHBG levels were positively correlated with age. This relationship has been reported before (20). Of much more importance, however, is the negative correlation with the BMI, which also has been reported by others (16,17,25,32,33). Abnormalities in hepatic estrogen receptor function have been proposed as a mechanism that might explain the negative correlation between SHBG levels and the BMI (34,35).

Androstenedione levels were shown to increase with the BMI, but not with age. Others have reported levels of androstenedione to increase with body weight (28,33,36,37). As the conversion rates of androstenedione to other steroids also increase with body weight (see below), this fact implies that production rates of androgens become higher as the BMI increases.

Estrone levels were positively correlated with the BMI and androstenedione and estradiol levels, and negatively with SHBG levels. The significant correlation with SHBG, however, was absent when multivariate regression was applied, implying that the effect of SHBG levels was confounded by the correlation of SHBG levels with other variables. The positive effect of body weight on conversion rates of androstenedione to estrone, has been reported by several authors (13,14,38). This effect is due

to the aromatization of androstenedione in fat tissue (39,40,41). The effect of estradiol on estrone levels may represent the peripheral conversion of this hormone to estrone, as can be observed after administration of exogenous estradiol to postmenopausal women (42,43). The most important factor determining estrone levels, however, is the level of circulating androstenedione. Others did not find this correlation (2), but the group they studied consisted of only 40 women. Our finding implies that the peripheral concentration of the precursor hormone, androstenedione, is more important in determining estrone levels than is the amount of fat tissue in which the conversion takes place.

Estradiol levels were positively correlated with the BMI, estrone and androstenedione levels, and negatively with SHBG levels. The latter relationship was absent when tested multivariately. The positive correlation of obesity, "percent ideal weight", or the BMI with estradiol levels has been reported by several authors (2,13,17,25,32). Applying univariate regression analysis, we also found a very significant correlation. In multivariate analysis, however, the relationship between serum estradiol concentrations and the BMI was only marginally significant. Of greater importance are the levels of androstenedione and estrone, i.e. the hormones which can be converted into estradiol (39).

Prolactin levels were positively correlated with age and serum SHBG, androstenedione and estradiol concentrations. The significance of the first 3 correlations, however, was caused by PRL levels ranging from 37.5 to 147.9 µg/L in 5 women. Some of these women may have had symptomless microprolactinomas. Therefore, these relationships should be interpreted with caution. Estradiol levels were significantly correlated with PRL levels. This relationship may be due to interference of estradiol with the dopaminergic control of PRL secretion (44).

In summary, the most important conclusions from the present study are that: 1. Serum gonadotropin, α-subunit, LHß, but not PRL concentrations are negatively correlated with age, either because of a direct effect of aging on gonadotroph cells or

because of an effect of age on the hypothalamic regulation of these cells; 2. Gonadotropin and subunit levels are negatively correlated with the BMI; 3. Negative feedback of endogenous estradiol levels on gonadotropin secretion seems to be absent. This may have various causes: a. estradiol levels in postmenopausal subjects may be too low to elicit a response in gonadotropin levels; b. secretion of gonadotropins in postmenopausal women may be rather autonomous; c. in postmenopausal women, conversion of other steroids into estradiol in central nervous tissues may play a more important role in regulating the gonadotropin secretion than peripheral levels of estradiol; 4. Estrone and estradiol levels depend more on androstenedione and estrone levels, respectively, than on the BMI.

REFERENCES

1. Deslypere JP, Vermeulen A. Leydig cell function in normal men: effect of age, lifestyle, residence, diet, and activity. J Endocrinol Metab 1984;59:955-62.
2. Vermeulen A, Verdonck L. Sex hormone concentrations in post-menopausal women. Clin Endocrinol 1978;9:59-66.
3. Lyrenas S, Carlstrom K, Backstrom T, Von Schoultz B. A comparison of oestrogen levels after percutaneous and oral administration of oestradiol-17ß. Br J Obstet Gynaecol 1981;88:181-7.
4. Andreasson B, Bostofte E. Influence of 2 mg estradiol-17ß on circulating FSH, LH, total and unconjugated estradiol levels in post-menopausal women. Acta Obstet Gynecol Scand 1981;60:555-8.
5. Valkenburg HA, Haanen HCM. The epidemiology of low back pain. In: Proceedings of the symposium on idiopathic low back pain, Miami, Florida, 1980. White AA, Gordon SL, editors. Mosby Company, St Louis, USA, 1982:pp 9-22.
6. Van Hemert AM. Epidemiology of osteoporosis and prediction of fractures (dissertation). Erasmus University, Rotterdam, The Netherlands, 1989.
7. Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor HL. Indices of relative weight and obesity. J Chron Dis 1972;25:329-43.
8. Van Landeghem AA, Poortman J, Deshpande N, Di Martino L, Tarquini A, Thijssen JHH, Schwarz F. Plasma concentration gradient of steroid hormones across human mammary tumors in vivo. J Steroid Biochem 1981;14:741-7.
9. Hammond GL, Lahteenmaki PL. A versatile method for the detection of serum cortisol binding globulin and sex hormone binding globulin binding capacities. Clin Chim Acta 1983; 132:101-10.
10. Snedecor GW, Cochran WG. Statistical Methods, ed. 7. Iowa State University Press, Ames, USA, 1980;pp 360-1.

11. Id Ref 10, p 357.
12. Soules MR, Bremner WJ. The menopause and climacteric: endocrinologic basis and associated symptomatology. J Am Geriatr Soc 1982;30:547-61.
13. Meldrum DR, Davidson BJ, Tataryn IV, Judd HL. Changes in circuating steroids with aging in postmenopausal women. Obstet Gynecol 1981;57:624-8.
14. Macdonald PC, Edman CD, Hemsell DL, Porter JC, Siiteri PK. Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. Am J Obstet Gynecol 1978;130:448-55.
15. Veldhuis JD, Samojlik E, Evans WS, Rogol AD, Ridgeway EC, Crowley WF, Kolp S, Checinska E, Kirschner MA, Thorner MO, Stumpf P. Endocrine impact of pure estradiol replacement in postmenopausal women: alterations in anterior pituitary hormone release and circulating sex steroid hormone concentrations. Am J Obstet Gynecol 1986;155:334-9.
16. Longcope C, Hui SL, Johnston CC Jr. Free estradiol, free testosterone, and sex hormone-binding globulin in perimenopausal women. J Clin Endocrinol Metab 1987;64:513-8.
17. Davidson BJ, Gambone JC, Lagasse LD, Castaldo TW, Hammond GL, Siiteri PK, Judd HL. Free estradiol in postmenopausal women with and without endometrial cancer. J Clin Endocrinol Metab 1981;52:404-8.
18. Greenblatt RB, Colle ML, Mahesh VB. Ovarian and adrenal steroid production in the postmenopausal woman. Obstet Gynecol 1976;47:383-7.
19. Judd HL, Judd GE, Lucas WE, Yen SSC. Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. J Clin Endocrinol Metab 1974;39:1020-4.
20. Maruyama Y, Aoki N, Suzuki Y, Sinohara H, Yamamoto T. Variation with age in the levels of sex-steroid-binding plasma protein as determined by radioimmunoassay. Acta Endocrinol 1984;106:428-32.
21. Porter JC, Nansel DD, Gudelsky GA, Foreman MM, Pilotte NS, Parker Jr. CR, Burrows GH, Bates GW, Madden JD. Neuroendocrine control of gonadotropin secretion. Fed Proc 1980; 39:2896-2901.
22. Jarjour LT, Handelsman DJ, Raum WJ, Swerdloff RS. Mechanism of action of dopamine on the in vitro release of gonadotropin-releasing hormone. Endocrinology 1986;119: 1726-32.
23. Rasmussen DD. New concepts in the regulation of hypothalamic gonadotropin releasing hormone (GnRH) secretion. J Endocrinol Invest 1986;9:427-37.
24. Lightman SL, Jacobs HS, Maguire AK, McGarrick G, Jeffcoate SL. Climacteric flushing: clinical and endocrine response to infusion of naloxone. Br J Obstet Gynaecol 1981;88:919-24.
25. Grenman S, Ronnemaa T, Irjala K, Kaihola HL, Gronroos M. Sex steroid, gonadotropin, cortisol, and prolactin levels in healthy, massively obese women: correlation with abdominal fat cell size and effect of weight reduction. J Clin Endocrinol Metab 1986;63:1257-61.
26. Paradisi R, Venturoli S, Pasquali R, Capelli M, Porcu E, Fabbri R, Flamigni C. Effects of obesity on gonadotropin secretion in patients with polycystic ovarian disease. J

Endocrinol Invest 1986;9:139-44.
27. Zumoff B, Strain GW, Kream J, Levin J, Fukushima DK. Subnormal 24-hour mean plasma LH concentration and elevated plasma FSH/LH ratio in obese premenopausal women. J Reprod Med 1983;28:843-6.
28. Givens JR, Wiedemann E, Andersen RN, Kitabchi AE. ß-Endorphin and ß-lipotropin plasma levels in hirsute women: correlation with body weight. J Clin Endocrinol Metab 1980;50:975-6.
29. Flores F, Naftolin F, Ryan KJ. Estrogen formation by the isolated perfused rhesus monkey brain. Science 1973;180: 1074-5.
30. Weisz J, Gibbs C. Metabolites of testosterone in the brain of the newborn female rat after injection of tritiated testosterone. Neuroendocrinology 1974;14:72-86.
31. Weisz J, Gibbs C. Conversion of testosteron and androstenedione to estrogens in vitro by the brain of female rats. Endocrinology 1974;94:616-20.
32. Wittels EH. Obesity and hormonal factors in sleep and sleep apnea. Med Clin North Am 1985;69:1265-80.
33. Kopelman PG, Pilkington TRE, White N, Jeffcoate SL. Abnormal sex steroid secretion and binding in massively obese women. Clin Endocrinol 1980;12:363-9.
34. Peiris A, Kissebach A. Endocrine abnormalities in morbid obesity. Gastroenterol Clin North Am 1987;16:389-98.
35. Schneider G, Kirschner MA, Berkowitz R, Ertel NH. Increased estrogen production in obese men. J Clin Endocrinol Metab 1979;48:633-8.
36. Kirschner MA, Samojlik E, Silber D. A comparison of androgen production and clearance in hirsute and obese women. J Steroid Biochem 1983;19:607-14.
37. Zhang Y, Stern B, Rebar RW. Endocrine comparison of obese menstruating and amenorrheic women. J Clin Endocrinol Metab 1984;58:1077-83.
38. Hemsell DL, Grodin JM, Brenner PF, Siiteri PK, Macdonald PC. Plasma precursors of estrogen. II. Correlation of the extent of conversion of plasma androstenedione to estrone with age. J Clin Endocrinol Metab 1974;38:476-9.
39. Nimrod A, Ryan KJ. Aromatization of androgens by human abdominal and breast fat tissue. J Clin Endocrinol Metab 1975;40:367-72.
40. Grodin JM, Siiteri PK, Macdonald PC. Source of estrogen production in postmenopausal women. J Clin Endocrinol Metab 1973;36:207-14.
41. Schindler AE, Ebert A, Friedrich E. Conversion of androstenedione to estrone by human fat tissue. J Clin Endocrinol Metab 1972;35:627-30.
42. Fahraeus L, Larsson-Cohn U. Oestrogens, gonadotrophins and SHBG during oral and cutaneous administration of oestradiol-17ß to menopausal women. Acta Endocrinol 1982; 101:592-6.
43. Yen SSC, Martin PL, Burnier AM, Czekala NM, Greaney MO Jr., Callantine MR. Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17ß-estradiol in postmenopausal women. J Clin Endocrinol Metab 1975;40:518-21.
44. MacLeod RM. Regulation of prolactin secretion. In: Frontiers

of Neuroendocrinology, vol 4. Martini L, Ganong WF, editors. Raven Press, New York, USA, 1976:pp 169-94.

8. EXPERIMENTAL TREATMENTS FOR CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS.

8.1 GnRH ANALOGS.

Responses in serum gonadotropin and α-subunit concentrations to long-term GnRH analog treatment have been studied extensively in patients with prostatic carcinoma or precocious puberty (1-3). Suppressed serum LH concentrations, suppressed or unaltered FSH levels, and elevated α-subunit concentrations have been reported (1,3-5). The effect of long-term treatment with GnRH analogs on α-subunit production by the pituitary gonadotroph is unclear: the amount of α-subunit that is secreted as a part of LH or FSH diminishes, while free α-subunit secretion increases.

FSH and α-subunit are frequently secreted by clinically nonfunctioning and gonadotroph pituitary adenomas (6). Moreover, gonadotropin and gonadotropin-subunit release from gonadotroph and clinically nonfunctioning pituitary adenomas can be stimulated with GnRH, both **in vivo** and **in vitro** (6), implying the presence of GnRH receptors in these tumors. GnRH analogs might, therefore, suppress gonadotropin and subunit release by these tumors, which is a prerequisite for any experimental drug treatment.

To elucidate the effect of GnRH analogs on the α-subunit production by the pituitary gonadotroph, we studied the effects of long-term GnRH analog treatment on serum gonadotropin and α-subunit concentrations in a group of patients with metastatic prostatic carcinoma (Chapter 8.2).

REFERENCES

1. St-Arnaud R, Lachance R, Kelly SJ, Belanger A, Dupont A, Labrie F. Loss of luteinizing hormone bioactivity in patients with prostatic cancer treated with an LHRH agonist and a pure antiandrogen. Clin Endocrinol 1986;24:21-30.
2. Grant JBF, Ahmed SR, Shalet SM, Costello CB, Howell A, Blacklock NJ. Testosterone and gonadotrophin profiles in patients on daily or monthly LHRH analogue ICI 118630 (Zoladex) compared with orchiectomy. Br J Urol 1986;58:539-44.
3. Lahlou N, Roger M, Chaussain JL, Feinstein MC, Sultan C,

Toublanc JE, Schally AV, Scholler R. Gonadotropin and α-subunit secretion during long term pituitary suppression by D-Trp^{6}-luteinizing hormone-releasing hormone microcapsules as treatment of precocious puberty. J Clin Endocrinol Metab 1987;65:946-53.

4. Schroeder FH, Lock TMTW, Chadha DR, Debruyne FMJ, Karthaus HFM, de Jong FH, Klijn JGM, Matroos AW, de Voogt HJ. Metastatic cancer of the prostate managed with buserelin versus buserelin plus cyproterone acetate. J Urol 1987;137:912-8.
5. Ahmed SR, Brooman PJC, Shalet SM, Howell A, Blacklock NJ. Treatment of advanced prostatic cancer with LHRH analogue ICI 118630: clinical response and hormonal mechanisms. Lancet 1983;2:415-8.
6. Kwekkeboom DJ, de Jong FH, Lamberts SWJ. Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas in vivo and in vitro: relation to sex and effects of TRH, GnRH and bromocriptine. J Clin Endocrinol Metab 1989;68: 1128-35.

8.2 PROLONGED TREATMENT WITH THE GnRH ANALOG BUSERELIN SUPPRESSES LHβ PRODUCTION BY THE PITUITARY GONADOTROPH, WHILE α-SUBUNIT PRODUCTION DOES NOT CHANGE.

D.J. Kwekkeboom[1], S.W.J. Lamberts[1], J.H.M. Blom[2], F.H. Schroeder[2] and F.H. de Jong[1,3].

Departments of Medicine[1], Urology[2] and Biochemistry[3]
Erasmus University Rotterdam, The Netherlands.

Clinical Endocrinology (Oxf), in press.

ABSTRACT

Seven patients with metastatic prostatic cancer were treated with biodegradable implants of the GnRH analog buserelin and 6 were treated with buserelin intranasally. After 4 to 24 weeks of treatment mean serum testosterone concentrations were significantly lower in the patients treated with implants than in those treated intranasally (0.7 vs. 1.7 nmol/L respectively; $P<0.01$). Also, serum LH concentrations were significantly lower in the group treated with implants.

Serum α-subunit concentrations were significantly higher than pre-treatment values during buserelin treatment. However, the sum of the concentrations of α-subunit present either as free α-subunit or as a part of LH did not differ significantly from pre-treatment values after 8 weeks or more of buserelin treatment.

During buserelin treatment serum LH concentrations measured by radioimmunoassay (RIA) were higher than those measured by immunoradiometric assay (IRMA). Crossreactivity of α-subunit in the LH RIA accounted for many, but not all of the observed discrepancies.

We conclude that: 1. The principal long-term effect of prolonged buserelin administration on the pituitary gonadotroph is the suppression of LHß production, while α-subunit production is not affected. 2. The serum concentrations of bioactive LH are better reflected by LH concentrations measured by IRMA than by those measured by RIA. 3. Subcutaneous application of biodegradable buserelin implants is more effective in suppressing serum LH and testosterone concentrations than intranasal buserelin application.

INTRODUCTION

Repeated administration of GnRH analogs is an effective form of androgen suppressing therapy in metastatic prostatic cancer (1-10). Buserelin (D-Ser(TBU)^6-GnRH) is a highly active analog of GnRH (11). Administered as a nasal spray it produces, after an initial increase of serum gonadotropins, a sustained suppression of serum LH and testosterone concentrations.

Recently, we reported on the treatment of prostatic cancer with an implant preparation of buserelin and its effects on serum testosterone concentrations (12). The aim of the present study was to elucidate the differential effect of long-term buserelin administration on LH, FSH and α-subunit release from the pituitary gonadotroph. We also compared the outcomes of LH concentrations during buserelin treatment measured by immunoradiometric assay (IRMA) with those measured by radioimmunoassay (RIA) and related them to circulating α-subunit and testosterone concentrations. Additionally, we compared the effects of buserelin treatment on serum LH, FSH, α-subunit and testosterone concentrations in patients treated with buserelin implants with those in patients who received buserelin by means of a nasal spray.

PATIENTS AND METHODS

Patients and treatment schemes

Seven patients with histologically proven, advanced prostate

cancer (stage C or D) were treated with subcutaneous application of buserelin. These patients have been described elsewhere (12). After one week of treatment with cyproterone acetate (CPA) (50 mg 3 times daily), 4 patients received 3.3 mg and the other 3 received 6.6 mg of buserelin given in a 75:25 polylactide-glycolide co-polymer formulation by subcutaneous implantation in the anterior abdominal wall under local anaesthesia. The implantation was repeated every 4 weeks in the patients who received a 3.3 mg implant and every 8 weeks in those who received a 6.6 mg implant. The treatment with CPA was continued until the second implantation of buserelin.

Six other patients with histologically proven, advanced prostate cancer (stage C or D) were treated with 1.5 mg buserelin administered subcutaneously for the first 7 days, and thereafter with 0.4 mg buserelin three times daily intranasally (i.n.). These patients also have been described before (1).

Immunoassays

Serum testosterone was measured by RIA as described elsewhere (13). The sensitivity of the assay was 0.2 nmol/L; the interassay coefficient of variation was <10% for samples containing less than 2 nmol/L.

FSH and LH were measured using IRMA kits supplied by IRE-Medgenix, Brussels, Belgium. The sensitivity of these assays was 0.5 IU/L. The crossreactivity of α-subunit in the LH and FSH assays expressed in terms of the standard preparations used was, respectively, 0.027 and 0.005 IU/μg. LH was also measured by RIA using antibodies obtained from KABI (Stockholm, Sweden). The sensitivity of this assay was 1.0 IU/L. The crossreactivity of α-subunit in this assay was 0.152 IU/μg at the 50% binding intercept and increased to 0.55 IU/μg at the 90% binding intercept. α-Subunit was measured by RIA using antibodies purchased from UCB, Brussels, Belgium. The sensitivity of the assay was 0.3 μg/L. The crossreactivities of LH and FSH in the α-subunit assay were, respectively, 0.006 and 0.023 μg/IU. LH, FSH and α-subunit were expressed in terms of the MRC 68/40, MRC 78/549 and MRC 78/556 reference preparations, respectively. The

interassay coefficients of variation of the assays were, respectively, <14% for LH, <8% for FSH, and <11% for α-subunit.

Statistics

Hormone and α-subunit data were evaluated using analysis of variance (ANOVA). Log transformation of data was used to stabilise variance. For the comparison of treatment means the Newman Keuls method was applied (14). P values <0.05 were considered significant.

RESULTS

There were no significant differences in hormone or α-subunit concentrations between patients treated with a buserelin implantation every 4 or every 8 weeks. Therefore, the results in these two groups were combined. Serum testosterone, LH, FSH and α-subunit concentrations in the 7 patients treated with buserelin implants are shown in figures 1 to 4.

CPA significantly suppressed serum testosterone concentrations until 2 days after buserelin implantation. The mean serum testosterone concentration was lower, but did not differ significantly from the pre-treatment mean on days 3 and 5, while it was significantly lower from 1 week after buserelin implantation. After 3 weeks, all testosterone concentrations were lower than 1.5 nmol/L, 78% of the testosterone concentrations being smaller than or equal to 1.0 nmol/L. Serum LH concentrations significantly increased during the first 2 days after buserelin implantation. After 3 weeks, all LH concentrations except 4 in the same patient were undetectable (<0.5 IU/L). Serum FSH concentrations only significantly decreased during the second and third week after buserelin implantation.

Serum testosterone concentrations immediately before and after any buserelin implantation did not differ significantly, while serum FSH, LH and α-subunit concentrations increased significantly only after the first implantation.

Serum α-subunit concentrations were significantly higher than pre-treatment levels during the whole period of buserelin

treatment. The amount of α-subunit that is present in LH preparations can be estimated to be 6.2% μg/IU (15). We calculated the concentration of α-subunit present either as free α-subunit or in LH by adding 6.2% of the serum LH concentration to the serum α-subunit concentration. The concentration of α-subunit present as either free α-subunit or as a part of LH significantly increased between day 1 and week 4 of treatment, but did not differ significantly from pre-treatment values after 8 weeks or more of buserelin treatment (Table 1).

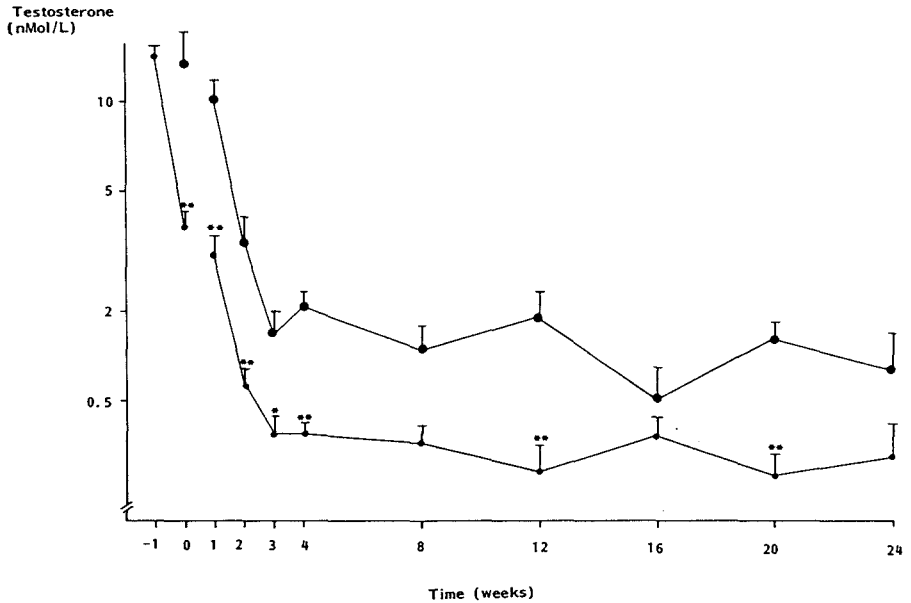

Figure 1. Mean (± SE) serum testosterone concentrations in patients with prostatic cancer treated with buserelin implants (lower symbols;n=7) or with buserelin i.n. (upper symbols; n=6). * : P<0.05 ** : P<0.01 vs. corresponding group treated i.n..

Serum LH concentrations measured by RIA, both with and without correction for α-subunit crossreactivity, were compared with those measured by IRMA (figure 5). In order to make a sound

comparison, the detection limit for both assays was set at the detection limit of the RIA (1.0 IU/L). From one week after buserelin implantation, LH concentrations measured by RIA differed significantly from those measured by IRMA on the majority of sampling days. After correction for crossreactivity of α-subunit in the LH RIA, no significant differences were found (Mann-Whitney U-tests). The correlation coefficients between serum testosterone concentrations and LH concentrations measured by RIA and IRMA were, respectively, 0.61 and 0.73 (Spearman Rank Correlation Coefficients; $P<0.01$ in both instances). After 2 to 24 weeks of buserelin treatment serum LH concentrations were at or below the detection limit in 34 of 36 samples as measured by IRMA, but only in 6 of 36 samples as measured by RIA. Serum α-subunit concentrations were strongly correlated with LH concentrations as measured by RIA in these 36 samples ($r_s=0.42$; $P<0.01$; Spearman Rank correlation test). After correction for α-subunit crossreactivity, LH concentrations measured by RIA were at or below the detection limit in 17 of 36 samples.

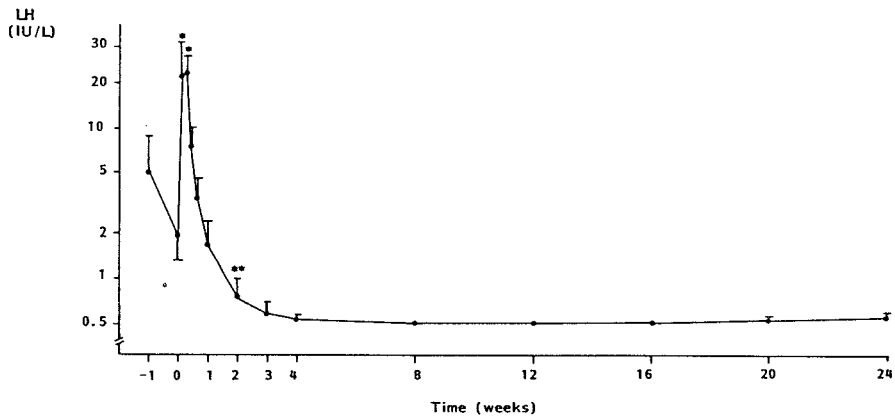

Figure 2. Mean (± SE) serum LH concentrations in 7 patients with prostatic cancer treated with buserelin implants. * : P<0.05 ** : P<0.01 vs. pre-treatment value.

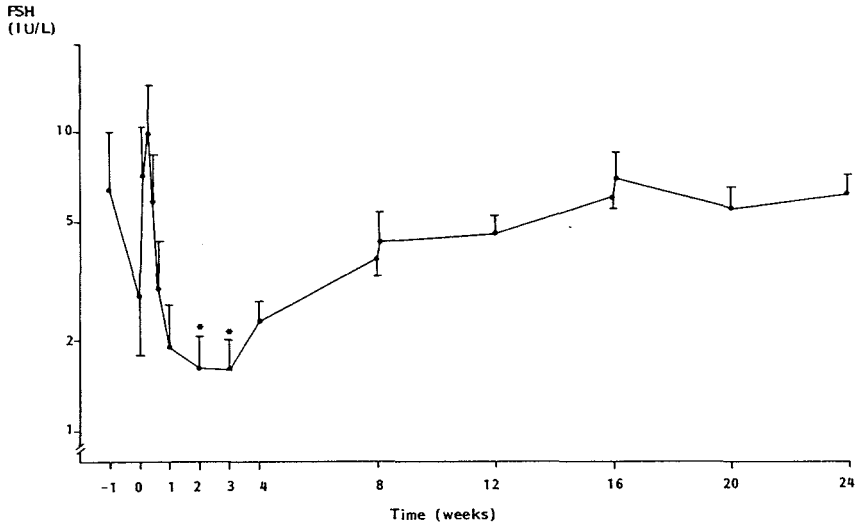

Figure 3. Mean (± SE) serum FSH concentrations in 7 patients with prostatic cancer treated with buserelin implants. * : P<0.05 ** : P<0.01 vs. pre-treatment value.

Serum LH, FSH, α-subunit and testosterone concentrations in the 6 patients treated with buserelin i.n. were compared with those in the 7 patients treated with buserelin implants. FSH concentrations did not differ significantly at any moment of the treatment between the two groups, and α-subunit concentrations were lower in the group treated with buserelin implants only after the first week of CPA administration (0.6 μg/L vs. 1.2 μg/L, respectively; P<0.01). As in the group treated with implants, serum LH concentrations in the group treated with buserelin i.n. were suppressed from week 2 onward. Serum LH concentrations from 4 to 24 weeks were undetectable in 9 of 28 samples in the group receiving buserelin i.n. (range <0.5-3.8 IU/L), and in 38 of 41 samples in the group treated with

buserelin implants (range <0.5-0.8 IU/L) (P<0.01; Mann-Whitney U-test). Serum testosterone concentrations in both groups are shown in figure 1. Mean testosterone concentrations from week 4 to week 24 were 1.7 ± 0.13 (SE) in the group receiving buserelin i.n. and 0.7 ± 0.04 in the group treated with buserelin implants (P<0.01; Student's t-test).

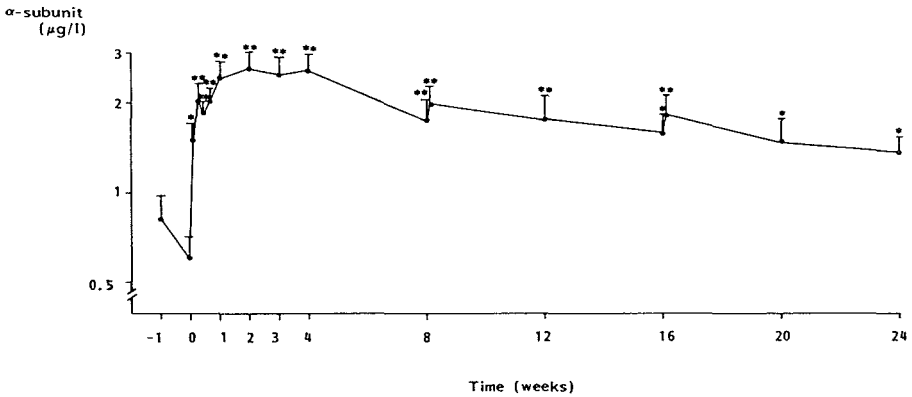

Figure 4. Mean (± SE) serum α-subunit concentrations in 7 patients with prostatic cancer treated with buserelin implants. * : P<0.05 ** : P<0.01 vs. pre-treatment value.

DISCUSSION

Repeated GnRH analog administration is an effective treatment of metastatic prostatic carcinoma (1-10). The effectiveness of the treatment, however, may depend on the route of administration of the drug. We compared the effects of buserelin administered intranasally and as a monthly or two-monthly implant and found that the latter 2 methods of application were more effective in suppressing serum LH and testosterone concentrations. The same has been shown for the depot preparation of another GnRH analog, ICI 118630 (Zoladex) (16). As suppression of serum testosterone

concentrations is the therapeutic goal of buserelin treatment, the administration of this drug by means of subcutaneous biodegradable implants is preferable to i.n. application.

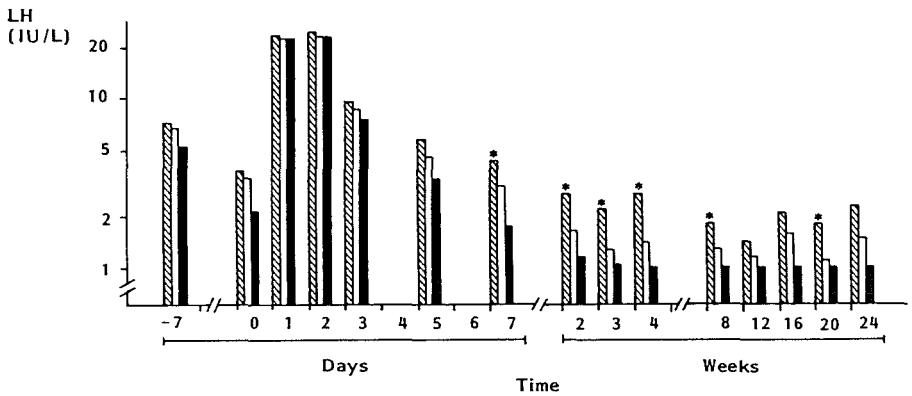

Figure 5. Mean serum LH concentrations in patients with prostatic cancer treated with buserelin implants. The detection limit of both LH assays was set at 1.0 IU/L. ▨ : LH measured by RIA □ : LH measured by RIA after correction for crossreactivity of α-subunit ■ : LH measured by IRMA. *: P<0.05 vs. LH IRMA concentrations (Mann-Whitney U-test).

A sustained rise of α-subunit concentrations after prolonged GnRH analog treatment has been reported by several groups (4,17). We also found that serum α-subunit concentrations were significantly higher than pre-treatment concentrations during the whole period of buserelin treatment. The α-subunit secretion from the pituitary, however, also depends on the amounts of LH, FSH and TSH that are released. As LH concentrations were significantly suppressed after 2 weeks of buserelin treatment, it may be questioned whether the α-subunit secretion from this moment was increased. We investigated whether the secretion of α-subunit from the pituitary gonadotroph was increased by estimating the concentrations of α-subunit secreted as either free α-subunit or

as a part of LH. After 8 weeks or more of buserelin treatment, these concentrations did not differ significantly from pre-treatment values. Also, FSH concentrations and, therefore, the α-subunit concentrations secreted as a part of FSH, did not differ significantly from pre-treatment values after 8 weeks or more of buserelin treatment. This indicates that prolonged GnRH analog treatment does not affect the α-subunit production in the pituitary gonadotroph, and that the principal long-term effect of this therapy is the suppression of LHß production. Therefore, it is not surprising that the administration of GnRH analogs in patients with gonadotropin and α-subunit secreting pituitary adenomas does not reduce tumoral hormone and subunit secretion, as has recently been reported (18,19).

Table 1. Mean serum concentrations (± SE) of α-subunit present as either free α-subunit or as a part of LH in 7 patients with prostatic cancer treated with buserelin implants (first implant week 0); CPA treatment started week -1.

Time	α-Subunit (µg/L)
Week -1	1.5 ± 0.5
Day 0	0.9 ± 0.2
Day 2	3.9 ± 0.5*a
Week 1	2.7 ± 0.3*
Week 2	2.8 ± 0.3*
Week 3	2.7 ± 0.3*
Week 4	2.7 ± 0.3*
Week 8	1.9 ± 0.3
Week 12	2.0 ± 0.3
Week 16	1.7 ± 0.3
Week 20	1.7 ± 0.3
Week 24	1.5 ± 0.3

*** : significantly different from pre-treatment concentration**
a : highest mean concentration

We found that during buserelin treatment serum LH concentrations measured by IRMA decreased more profoundly than those measured by RIA. Crossreactivity of α-subunit in the LH RIA accounted for the majority of the observed differences. However, after correction for crossreactivity of α-subunit, serum LH concentrations measured by RIA were higher than those measured by IRMA in about 50% of the samples. Other groups have suggested that biologically inactive forms of LH that are measured by RIA,

but not by IRMA, may be responsible for this discrepancy (4,17). Moreover, changes in glycosylation of LH during GnRH agonist therapy have been reported (20). Serum testosterone concentrations showed a better correlation with LH concentrations measured by IRMA than with LH concentrations measured by RIA. After 2 weeks or more of buserelin treatment, 78% of the serum testosterone concentrations were lower than or equal to 1.0 nmol/L (castration levels), while 94% of the serum LH concentrations as measured by IRMA were at or below the detection limit and the majority of LH concentrations as measured by RIA were higher than the detection limit. Therefore, the LH concentrations measured by IRMA seem to reflect the concentrations of bioactive LH better than those measured by RIA.

We conclude that: 1. The principal long-term effect of prolonged buserelin administration on the pituitary gonadotroph is the suppression of LHß production, while α-subunit production is not affected. 2. The serum concentrations of bioactive LH are better reflected by LH concentrations measured by IRMA than by those measured by RIA. 3. Subcutaneous application of biodegradable buserelin implants is more effective in suppressing serum LH and testosterone concentrations than intranasal buserelin application.

REFERENCES

1. Schroeder FH, Lock TMTW, Chadha DR, Debruyne FMJ, Karthaus HFM, de Jong FH, Klijn JGM, Matroos AW, de Voogt HJ. Metastatic cancer of the prostate managed with buserelin versus buserelin plus cyproterone acetate. J Urol 1987;137:912-8.
2. Klijn JGM, de Voogt HJ, Schroeder FH, de Jong FH. Combined treatment with buserelin and cyproterone acetate in metastatic prostatic carcinoma. Lancet 1985;2:493.
3. Presant CA, Soloway MS, Klioze SS, Kosola JW, Yakabow AL, Mendez RG, Kennedy PS, Wyres MR, Naessig VL, Ford KS. Buserelin as primary therapy in advanced prostatic carcinoma. Cancer 1985;56:2416-9.
4. St-Arnaud R, Lachance R, Kelly SJ, Belanger A, Dupont A, Labrie F. Loss of luteinizing hormone bioactivity in patients with prostatic cancer treated with an LHRH agonist and a pure antiandrogen. Clin Endocrinol 1986;24:21-30.
5. Labrie F, Dupont A, Belanger A, Lacoursiere Y, Raynaud JP, Husson JM, Gareau J, Fazekas ATA, Sandow J, Monfette G, Girard JG, Emond J, Houle JG. New approach in the treatment

of prostate cancer: complete instead of partial withdrawal of androgens. The Prostate 1983;4:579-94.
6. Santen RJ, Demers LM, Max DT, Smith J, Stein BS, Glode LM. Long term effects of administration of a gonadotropin-releasing hormone superagonist analog in men with prostatic carcinoma. J Clin Endocrinol Metab 1984;58:397-400.
7. Tolis G, Ackman D, Stellos A, Mehta A, Labrie F, Fazekas ATA, Comaru-Schally AM, Schally AV. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. Proc Natl Acad Sci USA 1982;79:1658-62.
8. Ahmed SR, Brooman PJC, Shalet SM, Howell A, Blacklock NJ. Treatment of advanced prostatic cancer with LHRH analogue ICI 118630: clinical response and hormonal mechanisms. Lancet 1983;2:415-8.
9. Parmar H, Phillips RH, Lightman SL, Edwards L, Allen L, Schally AV. Randomised controlled study of orchidectomy vs long-acting D-Trp-6-LHRH microcapsules in advanced prostatic carcinoma. Lancet 1985;2:1201-5.
10. Waxman JH, Wass JAH, Hendry WF, Whitfield HN, Besser GM, Malpas JS, Oliver RTD. Treatment with gonadotrophin releasing hormone analogue in advanced prostatic cancer. Br Med J 1983; 286:1309-12.
11. Borgmann V, Hardt W, Schmid-Gollwitzer M, Adenauer H, Nagel R. Sustained suppression of testosterone production by the luteinising-hormone-releasing-hormone agonist buserelin in patients with advanced prostate carcinoma. A new therapeutic approach? Lancet 1982;1:1097-9.
12. Blom JHM, Hirdes WH, Schroeder FH, de Jong FH, Kwekkeboom DJ, van 't Veen AJ, Sandow J, Krauss B. Pharmacokinetics and endocrine effects of the LHRH analogue buserelin after subcutaneous implantation of a slow release preparation in prostatic cancer patients. Urol Res 1989;17:43-6.
13. Verjans HL, Cooke BA, de Jong FH, de Jong CMM, van der Molen HH. Evaluation of a radioimmunoassay for testosterone estimation. J Steroid Biochem 1973;4:665-76.
14. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980:235.
15. Kwekkeboom DJ, de Jong FH, Lamberts SWJ. Confounding factors in the interpretation of gonadotropin- and gonadotropin-subunit release from cultured human pituitary adenomas. In press, J Steroid Biochem.
16. Grant JBF, Ahmed SR, Shalet SM, Costello CB, Howell A, Blacklock NJ. Testosterone and gonadotrophin profiles in patients on daily or monthly LHRH analogue ICI 118630 (Zoladex) compared with orchiectomy. Br J Urol 1986;58:539-44.
17. Lahlou N, Roger M, Chaussain JL, Feinstein MC, Sultan C, Toublanc JE, Schally AV, Scholler R. Gonadotropin and α-subunit secretion during long term pituitary suppression by D-Trp^6-luteinizing hormone-releasing hormone microcapsules as treatment of precocious puberty. J Clin Endocrinol Metab 1987;65:946-53.
18. Klibanski A, Jameson JL, Biller BMK, Crowley WF Jr., Zervas NT, Rivier J, Vale WW, Bikkal H. Gonadotropin and α-subunit responses to chronic gonadotropin-releasing hormone analog

administration in patients with glycoprotein hormone-secreting pituitary tumors. J Clin Endocrinol Metab 1989; 68:81-6.
19. Sassolas G, Lejeune H, Trouillas J, Forest MG, Claustrat B, Lahlou N, Loras B. Gonadotropin-releasing hormone agonists are unsuccesful in reducing tumoral gonadotropin secretion in two patients with gonadotropin-secreting pituitary adenomas. J Clin Endocrinol Metab 1988;67:180-5.
20. Bhasin S, Robinson R, Peterson M, Stein BS, Handelsman D, Rajfer J, Heber D, Swerdloff RS. Molecular heterogeneity and biologic activity of luteinizing hormone after gonadotropin-releasing hormone agonist treatment. Fertil Steril 1984; 42:318-9.

8.3 GnRH ANALOGS AND CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS.

From the preceding chapter it is clear that the production of FSH and α-subunit by the pituitary gonadotroph is not suppressed by the GnRH analog buserelin. Therefore, it is unlikely that the production of FSH and α-subunit, which are secreted by the vast majority of clinically nonfunctioning and gonadotroph pituitary adenomas **in vitro**, can be suppressed by this drug.

Not surprisingly, recent reports on the effects of prolonged treatment with GnRH agonists in patients with a gonadotroph adenoma indicate that serum α-subunit concentrations were higher than pre-treatment values, while the response in serum LH and FSH concentrations was variable (1,2).

Recently, Daniels et al. (3) studied the effects of the GnRH agonist buserelin on gonadotropin and subunit secretion from 3 gonadotroph pituitary adenomas **in vitro** for 28 days. In all 3 tumors, α-subunit secretion was stimulated by the GnRH analog during the whole period of culturing, while FSH and LH secretion were stimulated in 2 and were not significantly different from the secretion of untreated cells in the third adenoma.

In conclusion, GnRH analog treatment **in vivo** does not suppress α-subunit and FSH production by either the normal pituitary gonadotroph or the gonadotroph adenoma cell, and neither does GnRH analog treatment suppress gonadotropin and α-subunit secretion from gonadotroph adenomas **in vitro**. For these reasons, clinical trials on the effect of buserelin treatment in patients with a gonadotroph or clinically nonfunctioning pituitary adenoma are not advised.

REFERENCES

1. Klibanski A, Jameson JL, Biller BMK, Crowley WF Jr., Zervas NT, Rivier J, Vale WW, Bikkal H. Gonadotropin and α-subunit responses to chronic gonadotropin-releasing hormone analog administration in patients with glycoprotein hormone-secreting pituitary tumors. J Clin Endocrinol Metab 1989;68:81-6.
2. Sassolas G, Lejeune H, Trouillas J, Forest MG, Claustrat B,

Lahlou N, Loras B. Gonadotropin-releasing hormone agonists are unsuccesful in reducing tumoral gonadotropin secretion in two patients with gonadotropin-secreting pituitary adenomas. J Clin Endocrinol Metab 1988;67:180-5.

3. Daniels M, Newland P, Dunn J, Kendall-Taylor P, White MC. Long-term effects of a gonadotrophin-releasing hormone agonist ([D-Ser(But)^{6}]GnRH(1-9)nonapeptide-ethylamide) on gonadotrophin secretion from human pituitary gonadotroph cell adenomas in vitro. J Endocr 1988;118:491-6.

8.4 DOPAMINE AGONISTS IN THE MANAGEMENT OF CLINICALLY NONFUNCTIONING AND GONADOTROPH PITUITARY ADENOMAS.

INTRODUCTION

The administration of bromocriptine can lower serum gonadotropin and α-subunit concentrations in patients with a gonadotroph or α-subunit secreting tumor (1-6). Prolonged bromocriptine treatment led to an improvement of visual field defects or a reduction in tumor mass in 4 of 6 patients reported in the literature (3,5,6). No tumor size reduction was observed after the administration of bromocriptine for weeks to several months in 26 patients with a clinically nonfunctioning pituitary adenoma (7-9). A decrease in tumor size has been reported in 2 such patients who were treated with 7.5 mg bromocriptine daily for 4 months and with 20 mg bromocriptine daily for 25 months, respectively (10,11).

The effectivity of prolonged dopamine agonist treatment in patients with a clinically nonfunctioning or gonadotroph pituitary tumor may depend on the period of treatment and on the administered dose.

In Chapters 5 and 6 it was reported that bromocriptine can lower the **in vitro** release of gonadotropins and subunits from clinically nonfunctioning and gonadotroph pituitary adenomas. However, the intracellular hormone concentrations of tumorcells incubated with or without bromocriptine for 24 to 72 h did not differ significantly. To investigate whether the period of treatment with bromocriptine influences the gonadotropin and subunit release and intracellular hormone concentrations in these tumors **in vitro**, we culture these tumors for longer periods. The preliminary results of 2 of these cultures are presented here.

To investigate whether prolonged treatment with dopamine agonists can lower gonadotropin and subunit secretion from clinically nonfunctioning and gonadotroph pituitary adenomas **in vivo** and whether this treatment can lead to tumor size reduction, we treat patients with such tumors who do not have major visual field defects with the dopamine agonist CV 205-502 for several

months. In comparison with bromocriptine, CV 205-502 has a more potent and long acting dopaminergic effect on the pituitary (12). Also, side effects of bromocriptine which may necessitate the discontinuation of the treatment, such as nausea, emesis and hypotension, are virtually absent during CV 205-502 treatment (13), enabling the administration of high daily doses.

PATIENTS AND METHODS

Patients

In vivo and **in vitro** responses to hormones and drugs were studied in a man with a gonadotroph pituitary adenoma and in a woman with a clinically nonfunctioning pituitary adenoma. In both the presence of a pituitary tumor was verified by histologic examination of the removed tissue. A third patient, a man with a clinically nonfunctioning pituitary adenoma, was treated with CV 205-502.

In vivo investigations

The responses to 200 μg TRH were studied on seperate days. Blood samples were collected before and 10, 20, 30, 60 and 120 minutes after iv TRH injection.

Bromocriptine (2.5 mg) or a placebo was given orally and blood samples were collected hourly for 12 hours on other days.

The patient who was treated with CV 205-502 received 75 μg CV 205-502 daily in the first 2 weeks and 150 μg CV 205-502 in the subsequent weeks.

In vitro investigations

Surgically removed pituitary tumor tissue was washed several times, incubated with dispase, and the cells were dispersed using a Dounce type homogenizer. The tumor cells were seperated from blood cells by discontinuous Ficoll-Isopaque gradient centrifugation, and then were suspended in Eagle's Minimum Essential Medium (MEM) containing 10% fetal calf serum (FCS) and cultured at 37°C in Costar Transwell cell culture chambers inserted in Costar multiwell plates, at a concentration of 200.000 cells per

Transwell. After renewal of the media on day 4 or 5 and on subsequent days when the media were renewed, the cells were incubated in quadruplicate with or without TRH (100 nmol/L; Hoechst, Frankfurt am Main, Germany), GnRH (100 nmol/L; Hoechst), buserelin (100 nmol/L; Hoechst), or bromocriptine (10 nmol/L; Sandoz, Basel, Switzerland). At the end of the incubations, cells were lysed in distilled water containing 1 g/L bovine serum albumin by repeated freezing and thawing and the hormone concentrations in these lysates were measured.

Immunoassays

Prolactin and GH were measured using radioimmunoassay (RIA) kits obtained, respectively, from IRE-Medgenix, Brussels, Belgium, and Sorin, Milano, Italy. TSH was measured using a kit obtained from Behring, Marburg, FRG. The sensitivity of the assay was 0.1 mU/L and the crossreactivities of subunits and gonadotropins were <1% and <0.01%, respectively.

FSH and LH were measured using immunoradiometric assay kits supplied by IRE-Medgenix, Brussels, Belgium. The sensitivity of these assays was 0.5 IU/L. α-Subunit and LHß were measured by RIA using antibodies purchased from UCB, Brussels, Belgium. The sensitivity of the α-subunit assay was 0.3 µg/L and that of the LHß assay was 1.0 µg/L. LH was expressed in terms of the MRC 68/40 reference preparation, FSH in terms of the MRC 78/549 reference preparation, and α-subunit and LHß in terms of MRC 78/554 and 78/556, respectively.

The crossreactivities on a weight/weight basis were: LH assay -FSH 0.5%, α-subunit 0.4%, LHß <0.5%; FSH assay -LH and α-subunit 0.06 %, LHß <0.01%; α-subunit assay -LH 3.9%, FSH 20.0%, LHß 0.2%; LHß assay -LH 53.4%, FSH 1.1%, and α-subunit 1.8%. The potencies of the preparations used to determine crossreactivity were for LH 6.6 IU/µg and for FSH 8.7 IU/µg. The intra- and interassay coefficients of variation of the assays were, respectively, <5% and <15% for LH, <3% and <8% for FSH, <6% and <11% for α-subunit, and <7% and <13% for LHß.

Statistics

In vitro hormone release was evaluated using one way analysis of variance (ANOVA). Log transformation of data was used to stabilise variance. For the comparison of treatment means the Newman Keuls method was applied (14). P values <0.05 were considered significant.

RESULTS

The patient data and the in vivo hormone levels are presented in Table 1.

Table 1. Patient and hormone data in 3 patients with a clinically nonfunctioning or gonadotroph pituitary adenoma.

Patient	Sex	Age	LH (IU/L)	FSH (IU/L)	α-subunit (μg/L)	LHβ (μg/L)	α-subunit/ gonadotropin ratio (%)	GH (μg/L)	PRL (μg/L)	TSH (mU/L)	T_4 (nmol/L)
1	M	59	1.3*	16.5*	4.0*	< 1	22.5*	2.0	4.2	0.46	95
2	F	56	4.1*	31.6	1.1*	< 1*	3.1	2.2	27.8*	0.72	114
3	M	57	2.6	7.6	1.0	< 1	9.8	< 0.5	6.1	2.21	83
Normal values											
Men			1.9 - 9.2	1.6 - 11.1	0.4 - 1.1	< 1	3.8 - 16.0	< 5	< 12	0.2 - 4.2	60 - 140
Postmenopausal women			17.5 - 86.6	26.2 - 107.7	1.3 - 4.0	1.4 - 5.4	1.4 - 3.3	< 5	< 15	0.2 - 4.2	60 - 140

***: Abnormal concentration.**

A TRH test was performed in patients 1 and 3. In both patients the response in TSH levels after TRH administration was normal. In response to TRH administration serum LH, FSH, and α-subunit concentrations increased by maximally 58%, 4% and 19% in patient 1, and by 38%, 28% and 56% in patient 3. A normal response to metyrapone was present in patients 2 and 3. The effect of bromocriptine administration on serum gonadotropin and α-subunit concentrations in patients 2 and 3 is shown in figures 1 and 2. Serum FSH concentrations decreased during bromocriptine administration in both, while serum α-subunit concentrations decreased to a lesser extent in patient 3.

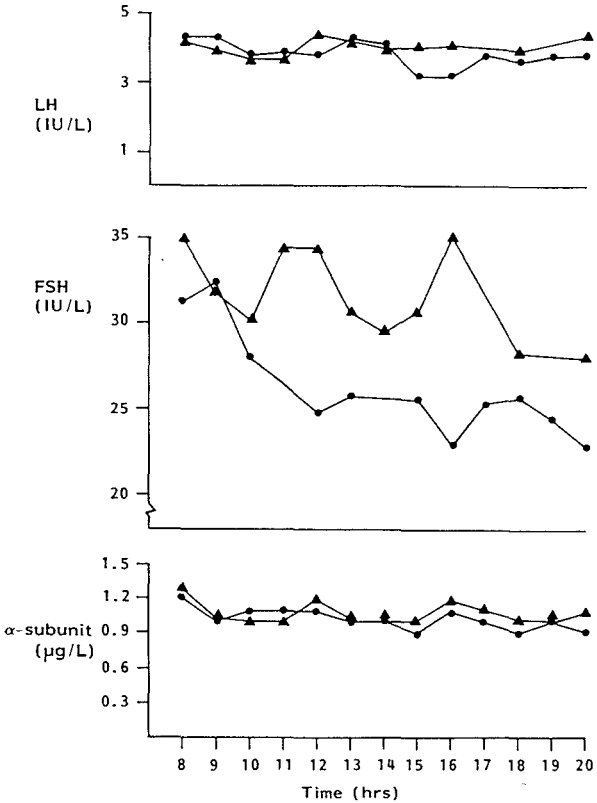

Figure 1. Serum gonadotropin and α-subunit concentrations in patient 2. ▲ :after administration of a placebo at 800 h. ● :after administration of bromocriptine (2.5 mg) at 800 h.

The cultured tumors of both patient 1 and 2 released LH, FSH, α-subunit and LHß, while no PRL, GH or TSH could be detected in the media or cells. The basal release per 200.000 cells/day and from the the cultured adenoma cells of patient 1 decreased during the culture period from 6.3 to 1.6 mIU for LH, from 6.3 to 3.0

mIU for FSH, and from 12.0 to 4.3 ng for α-subunit (Figure 3). Because of shortage of medium LHß release could not be evaluated on all sampling days.

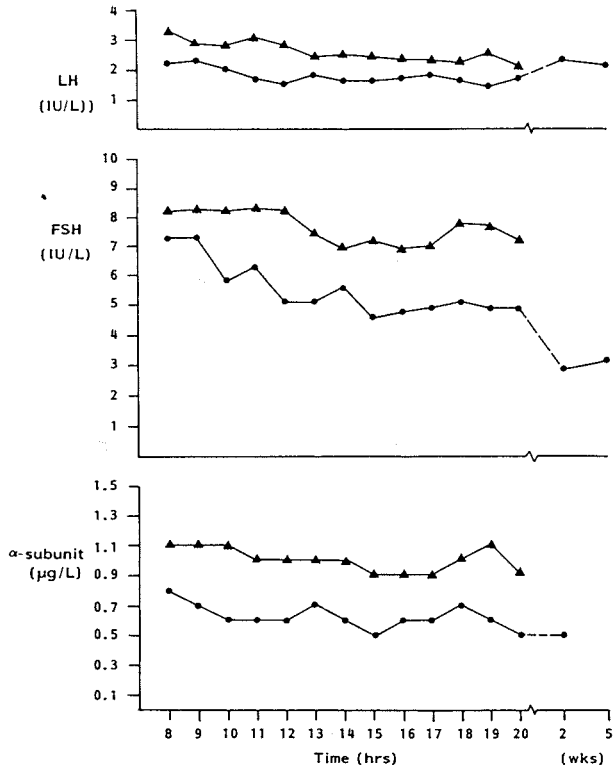

Figure 2. Serum gonadotropin and α-subunit concentrations in patient 3. ▲ : after placebo; ● : after bromocriptine (2.5 mg, orally) at 800 h. Time in weeks refers to weeks in which CV 205-502 was administered.

The basal release per 200.000 cells/day from the cultured adenoma cells of patient 2 decreased during the culture period from 2.9 to 1.9 mIU for FSH and from 0.9 to 0.4 ng for α-subunit

(Figure 4). LHß and LH release could be detected only until the 7^{th} and 11^{th} day of culturing and have therefore been omitted.

Bromocriptine inhibited gonadotropin and α-subunit release from the adenomatous cells from patient 1 from the 5^{th} day of culturing, but not during the first 3 days of incubation (figure 3). Intracellular hormone or α-subunit concentrations after 12 days of culturing were not affected by bromocriptine.

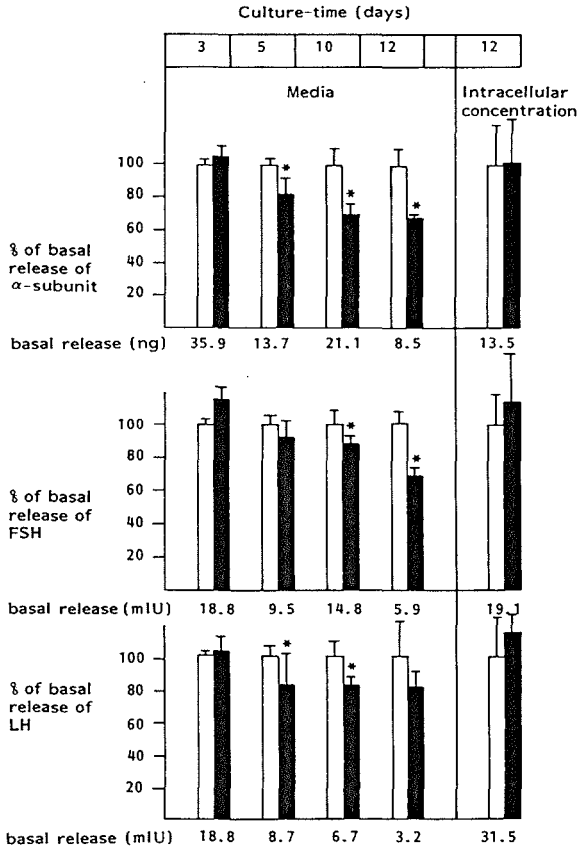

Figure 3. In vitro effects of bromocriptine on the gonadotropin and α-subunit release from the adenoma of patient 1. Means (±SE) of quadruplicate incubations are shown. Release is per 200.000 cells. □ : Control; ■ : Bromocriptine 10 nmol/L.

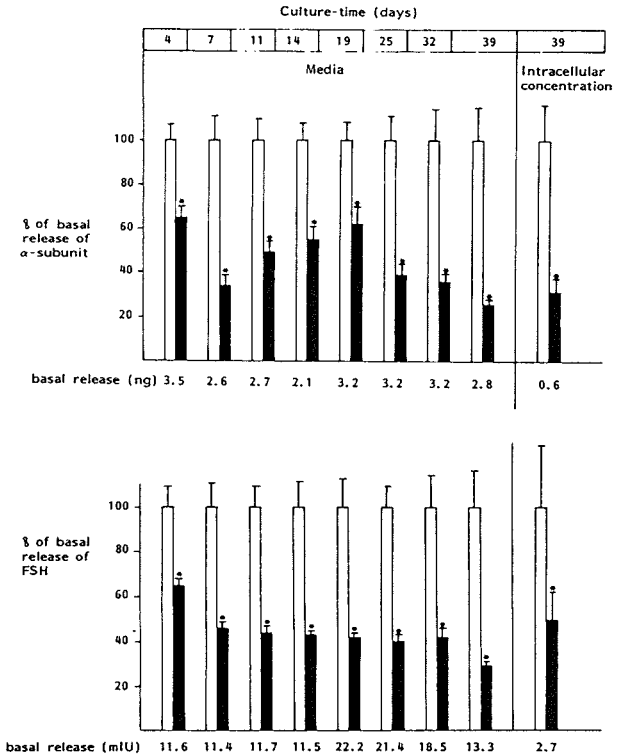

Figure 4. In vitro effects of bromocriptine on the gonadotropin and α-subunit release from the adenoma of patient 2. Means (±SE) of quadruplicate incubations are shown. Release is per 200.000 cells. □ : Control; ■ : Bromocriptine 10 nmol/L.

TRH, GnRH and buserelin stimulated α-subunit and gonadotropin release to a variable extent during the 12 days of culturing (data not shown). The percentual release of FSH and α-subunit from the tumor from patient 1 decreased significantly as the period of incubation with bromocriptine lasted longer (r= -0.86

and r= -0.87, respectively; P<0.01 in both instances), while the inhibition of percentual LH release showed no significant time-dependent trend (r= -0.46). Also, the percentual stimulation of FSH and α-subunit release by GnRH increased significantly, while no significant time-dependent trend could be demonstrated for the effects of TRH or buserelin (data not shown).

Bromocriptine inhibited FSH and α-subunit release from the tumorcells from patient 2 (figure 4). Intracellular FSH and α-subunit concentrations were significantly decreased after 39 days of incubation with bromocriptine. TRH, GnRH and buserelin stimulated FSH and α-subunit secretion throughout the 39 days in which the cells were cultured (data not shown). The percentual release of FSH and α-subunit from the adenoma cells of patient 2 decreased significantly as the period of incubation with bromocriptine lasted longer (r= -0.74 and r= -0.60, respectively; P<0.01 in both instances). Also, as the culture lasted longer, the percentual stimulation of FSH and α-subunit release by GnRH and buserelin, but not by TRH, increased significantly (data not shown).

The effects of prolonged administration of CV 205-502 on serum gonadotropin and α-subunit concentrations in patient 3 are shown in figure 2. FSH and α-subunit levels decreased, while serum LH concentrations were not greatly affected by the treatment.

DISCUSSION

We previously reported that bromocriptine can lower the **in vitro** release of gonadotropins and subunits from clinically nonfunctioning and gonadotroph pituitary adenomas. However, the intracellular hormone concentrations of tumorcells incubated with or without bromocriptine for 24 to 72 h did not differ significantly (nonpublished data). The present results indicate that it may require long-term culturing with dopamine agonists to lower intracellular hormone concentrations in these tumors **in vitro**.

As cells were cultured longer, an increasing inhibitory action of bromocriptine on FSH and α-subunit release from a cultured

clinically nonfunctioning adenoma and from a cultured gonadotroph pituitary adenoma was found. This effect might be due to an inhibitory effect of bromocriptine on cell proliferation. This does not seem likely, however, as very few mitoses are observed in these adenomas. It is more probable that bromocriptine during prolonged incubation has an increasing inhibitory effect on the synthesis of gonadotropins and subunits, eventually resulting in decreased intracellular concentrations of these glycoproteins.

From the **in vivo** tests with bromocriptine in 2 patients with a clinically nonfunctioning pituitary adenoma it can be concluded that the suppression of serum gonadotropin and α-subunit concentrations by this drug is not limited to patients with a pituitary adenoma which causes clinically recognizable hypersecretion of LH, FSH or α-subunit.

Prolonged administration of CV 205-502 caused a sustained suppression of serum FSH and α-subunit concentrations in a patient with a clinically nonfunctioning pituitary adenoma. It may be presumed that this is due to a suppressed secretion of FSH and α-subunit from the clinically nonfunctioning tumor, as the administration of CV 205-502 to normal subjects does not lead to appreciable changes in serum gonadotropin concentrations (15).

The intracellular hormone concentrations in the tumor from patient 2 were decreased after prolonged incubation with bromocriptine. This might imply that secretory granules and intracellular structures involved in the synthesis of glycoprotein hormones, such as the endoplasmatic reticulum, shrink during prolonged bromocriptine treatment. Whether this leads to appreciable tumor volume reduction in patients with a clinically nonfunctioning or gonadotroph pituitary adenoma during treatment with CV 205-502 **in vivo**, is the subject of a study that has only just started.

REFERENCES

1. Berezin M, Olchovsky D, Pines A, Tadmor R, Lunenfeld B. Reduction of follicle-stimulating hormone (FSH) secretion in FSH-producing pituitary adenoma by bromocriptine. J Clin Endocrinol Metab 1984;59:1220-3.
2. Chapman AJ, Macfarlaine IA, Shalet SM, Beardwell CG, Dutton

J, Sutton ML. Discordant serum α-subunit and FSH concentrations in a woman with a pituitary tumour. Clin Endocrinol 1984;21:123-9.
3. Vance ML, Ridgway EC, Thorner MO. Follicle-stimulating hormone- and α-subunit-secreting pituitary tumor treated with bromocriptine. J Clin Endocrinol Metab 1985;61:580-4.
4. Lamberts SWJ, Verleun T, Oosterom R, Hofland L, van Ginkel LA, Loeber JG, van Vroonhoven CCJ, Stefanko SZ, de Jong FH. The effects of bromocriptine, thyrotropin-releasing hormone, and gonadotropin-releasing hormone on hormone secretion by gonadotropin-secreting pituitary adenomas in vivo and in vitro. J Clin Endocrinol Metab 1987;64:524-30.
5. Klibanski A, Deutsch PJ, Jameson JL, Ridgway EC, Crowley WF, Hsu DW, Habener JF, Black PMcL. Luteinizing hormone secreting pituitary tumor: biosynthetic characterization and clinical studies. J Clin Endocrinol Metab 1987;64:536-42.
6. Klibanski A, Shupnik MA, Bikkal HA, Black PMcL, Kliman B, Zervas NT. Dopaminergic regulation of α-subunit secretion and messenger ribonucleic acid levels in α-secreting pituitary tumors. J Clin Endocrinol Metab 1988;65:96-102.
7. Barrow DL, Tindall GT, Kovacs K, Thorner MO, Horvath E, Hoffman JC Jr. Clinical and pathological effects of bromocriptine on prolactin-secreting and other pituitary tumors. J Neurosurg 1984;60:1-7.
8. Grossman A, Ross R, Charlesworth M, Adams CBT, Wass JAH, Doniach I, Besser GM. The effect of dopamine agonist therapy on large functionless pituitary tumours. Clin Endocrinol 1985;22:679-86.
9. Zarate A, Moran C, Kleriga E, Loyo M, Gonzalez-Angulo A, Aquilar-Parada E. Bromocriptine therapy as pre-operative adjunct of non-functional pituitary macro-adenomas. Acta Endocrinol 1985;108:445-50.
10. Johnston DG, Hall K, McGregor A, Ross WM, Kendall-Taylor P, Hall R. Bromocriptine therapy for "nonfunctioning" pituitary tumors. Am J Med 1981;71:1059-61.
11. Wass JAH, Williams J, Charlesworth M, Kingsley DPE, Halliday AM, Doniach I, Rees LH, McDonald WI, Besser GM. Bromocriptine in management of large pituitary tumours. Br Med J 1982; 284:1908-11.
12. Venetikou MS, Burrin JM, Woods CA, Yeo TH, Brownell J, Adams EF. Effects of two novel dopaminergic drugs, CV 205-502 and CQP 201-403, on prolactin and growth hormone secretion by human pituitary tumours in vitro. Acta Endocrinol (Copenh) 1987;116:287-92.
13. Rasmussen C, Bergh T, Wide L, Brownell J. CV 205-502: a new long-acting drug for inhibition of prolactin hypersecretion. Clin Endocrinol 1987;26:321-6.
14. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames, Iowa State University Press, 1980:235.
15. Gaillard RC, Abeywickrama K, Brownell J, Muller AF. Specific effect of CV 205-502, a potent non-ergot dopamine agonist, during a combined anterior pituitary function test. J Clin Endocrinol Metab 1989;68:329-35.

9. DISCUSSION OF THE MAJOR CONCLUSIONS.

The clinical classification of pituitary adenomas according to the hormones which they hypersecrete **in vivo** distinguishes gonadotroph from clinically nonfunctioning adenomas. Yet in postmenopausal women most pituitary tumors which have the immunocytochemical and ultratstructural features of gonadotroph adenomas do not cause elevated serum gonadotropin concentrations (1). These tumors obviously are not clinically recognized as gonadotroph adenomas because of the high serum gonadotropin concentrations that occur normally in postmenopausal women. The classification of pituitary adenomas according to immunocytochemical and ultrastructural features also distinguishes between nonfunctioning and gonadotroph adenomas. Yet both gonadotroph and virtually all nonfunctioning pituitary adenomas contain and release gonadotropins or their subunits **in vitro** (Chapter 5). Apparently, clinically nonfunctioning pituitary adenomas contain and secrete such small amounts of gonadotropins and gonadotropin-subunits, that tumorcells do not immunostain and that **in vivo** serum concentrations of these glycoproteins are not elevated.

The **in vitro** production of gonadotropins and their subunits is a common feature in both clinically nonfunctioning and gonadotroph pituitary adenomas. There are, however, more similarities:

-In both tumortypes hormone or subunit secretion can be stimulated by TRH and can be suppressed by bromocriptine, both **in vivo** and **in vitro** (Chapters 5 and 8.4).

-Both tumortypes are diagnosed in elderly patients.

-Immunocytochemistry may or may not reveal cells that are positive for the gonadotropins or their subunits in both gonadotroph and clinically nonfunctioning pituitary adenomas (1-4).

-On electron microscopic examination, there are striking differences between the 2 tumor types. The RER, moderately to well developed in gonadotroph adenomas, is poorly developed in clinically nonfunctioning adenomas. Secretory granules are more numerous in gonadotroph than in clinically nonfunctioning adenomas. An abundance of microtubules is observed in gonado-

troph, but not in clinically nonfunctioning pituitary adenomas. Both, however, may be shown to consist of cells with secretory granules and numerous free ribosomes, while oncocytic transformation may be observed in both (1,5,6).

As the similarities are of greater clinical importance than the differences, clinically nonfunctioning and gonadotroph pituitary adenomas should be regarded as one clinical entity.

The etiology of clinically nonfunctioning and gonadotroph pituitary adenomas is unclear. There are, however, indications that primary hypogonadism might play a role in the development of these tumors:

In aging men, an age-associated decrease in plasma testosterone levels is accompanied by an increase in serum gonadotropin concentrations (7). By contrast, in aging postmenopausal women circulating sex steroid concentrations do not decrease, while serum gonadotropin concentrations decline with age (Chapter 7.2). In men gradual age-dependent hypogonadism develops, while in postmenopausal women age-independent hypogonadism is present. Hormone production by the pituitary gonadotroph cells declines in aging postmenopausal women, and increases in aging men. Whether this sex-related difference in gonadotroph cell activity is reflected in the fact that gonadotroph pituitary adenomas are diagnosed for the most part in men is speculative, as high serum gonadotropin concentrations that occur normally in postmenopausal women may mask the secretion of gonadotropins by a gonadotroph pituitary tumor.

Circulating sex steroid concentrations do not influence serum gonadotropin levels in postmenopausal women (Chapter 7.2), while hormone production by the pituitary gonadotroph cell is less responsive to the administration of estradiol and drugs than in premenopausal women. In old men gonadotropin secretion is less influenced by the administration of antiopioids than in young men (8). So, compared to young subjects, in both aging men and aging women pituitary gonadotroph cell activity is less affected by drug administration. Whether these relatively autonomously secreting gonadotroph cells which are associated with aging, may develop into clinically nonfunctioning or gonadotroph adenoma

cells, is a tempting speculation.

In conclusion, hypogonadism is present in both aging men and aging women and could play a role in the development of gonadotroph and clinically nonfunctioning pituitary adenomas. On the other hand, relatively autonomously secreting gonadotroph cells which are present in aging subjects might develop into clinically nonfunctioning or gonadotroph pituitary adenomas, independent of circulating sex steroid concentrations. Lastly, a hypothalamic cause of these adenomas should be considered.

The accepted therapy of clinically nonfunctioning and gonadotroph pituitary adenomas is transsphenoidal adenomectomy. Because of operative and postoperative complications, medicinal therapy also receives attention. A prerequisite for any drug therapy is tumor volume reduction. Therefore, hormone secretion by the tumor should decrease, whether caused by a reduction in the number of cells or by a decreased tumorcell volume. As gonadotroph and virtually all clinically nonfunctioning pituitary adenomas produce LH, FSH, α-subunit, LHß or a combination of these, it is essential that any drug to be administered suppresses the production of these hormones and subunits.

GnRH agonists can suppress LH production by the gonadotroph cells. In men with prostatic carcinoma, however, prolonged treatment with these drugs does not suppress the production of α-subunit or FSH (Chapter 8.2). Neither can GnRH agonists suppress the production of α-subunit or FSH from gonadotroph or clinically nonfunctioning adenomas, either **in vivo** or **in vitro** (9). For these reasons, trials on the effect of GnRH agonists in patients with a clinically nonfunctioning or gonadotroph pituitary adenoma are not recommended.

During long-term culturing the suppressive effect of the dopamine agonist bromocriptine on gonadotropin and subunit secretion from cells from clinically nonfunctioning or gonadotroph pituitary adenomas increases as incubations last longer (Chapter 8.4). Intracellular hormone concentrations in cultured cells from a clinically nonfunctioning adenoma were decreased after several weeks of incubation with bromocriptine. Preliminary results on the effect of prolonged treatment with a dopamine

agonist in a patient with a clinically nonfunctioning pituitary adenoma who was not operated on, show a decreased secretion of FSH and α-subunit. Whether this suppressive effect on the secretion and synthesis of gonadotropins and α-subunit leads to appreciable tumor volume reduction has yet to be seen.

REFERENCES

1. Horvath E, Kovacs K. Gonadotroph adenomas of the human pituitary: sex-related fine-structural dichotomy. Am J Pathol 1984;117:429-40.
2. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 1983;59:585-9.
3. Kovacs K, Horvath E, Ryan N, Ezrin C. Null cell adenoma of the human pituitary. Virchows Arch [Pathol Anat] 1980;387:165-74.
4. Landolt AM, Heitz PU. Alpha-subunit-producing pituitary adenomas. Virchows Arch [Pathol Anat] 1986;409:417-31.
5. Trouillas J, Girod C, Sassolas G, Claustrat B, Lheritier M, Dubois MP, Goutelle A. Human pituitary gonadotropic adenoma; histological, immunocytochemical, and ultrastructural and hormonal studies in eight cases. J Pathology 1981;135:315-36.
6. Landolt AM, Oswald UW. Histology and ultrastructure of an oncocytic adenoma of the human pituitary. Cancer 1973;31: 1099-1105.
7. Deslypere JP, Vermeulen A. Leydig cell function in normal men: effect of age, lifestyle, residence, diet, and activity. J Endocrinol Metab 1984;59:955-62.
8. Vermeulen A, Deslypere JP, De Meirleir K. A new look to the andropause: altered function of the gonadotrophs. J Steroid Biochem 1989;32:163-5.
9. Daniels M, Newland P, Dunn J, Kendall-Taylor P, White MC. Long-term effects of a gonadotrophin-releasing hormone agonist ([D-Ser(But)^6]GnRH(1-9)nonapeptide-ethylamide) on gonadotrophin secretion from human pituitary gonadotroph cell adenomas in vitro. J Endocr 1988;118:491-6.

SUMMARY

In comparison with other types of pituitary tumors clinically nonfunctioning and gonadotroph pituitary adenomas are diagnosed in elderly patients. In most cases they cause visual complaints (Chapter 3). Gonadotroph adenomas are characterized by elevated serum concentrations of FSH or, occasionally, of LH. Clinically nonfunctioning pituitary adenomas are characterized by the absence of hypersecretion of any pituitary hormone **in vivo**.

Cultured cells from gonadotroph and virtually all clinically nonfunctioning adenomas produce and contain FSH, LH, or the subunits of these hormones, alone or in combination (Chapter 5). The secretion of these hormones and subunits from both clinically nonfunctioning and gonadotroph pituitary adenomas can be stimulated by GnRH and TRH and can be suppressed by bromocriptine, both **in vivo** and **in vitro** (Chapter 5). As the similarities between both tumortypes are of greater clinical importance than the differences, clinically nonfunctioning and gonadotroph pituitary adenomas should be regarded as one clinical entity.

The etiology of clinically nonfunctioning and gonadotroph pituitary adenomas is unclear. There are, however, indications that primary hypogonadism might play a role in the development of these tumors. Hypogonadism is present in postmenopausal women and in aging men. On the other hand, serum gonadotropin concentrations in postmenopausal women are not influenced by circulating endogenous estradiol levels (Chapter 7.2), and, compared to young subjects, the pituitary gonadotroph cell activity is less affected by drug administration in both aging men and aging women. These relatively autonomously secreting gonadotroph cells which are associated with aging might develop into clinically nonfunctioning or gonadotroph adenomas, independent of circulating sex steroid levels. Lastly, a hypothalamic cause of these tumors should be considered.

The accepted therapy of clinically nonfunctioning and gonadotroph pituitary adenomas is transsphenoidal adenomectomy. Because of operative and postoperative complications, medicinal

therapy also receives attention. A prerequisite for any drug therapy is tumor volume reduction. Therefore, hormone secretion by the tumor should decrease, whether caused by a reduction in the number of cells or by a decreased tumorcell volume. As gonadotroph and virtually all clinically nonfunctioning pituitary adenomas produce LH, FSH, α-subunit, LHß or a combination of these, it is essential that any drug to be administered suppresses the production of these hormones and subunits.

GnRH agonists can suppress LH production by the gonadotroph cells. In men with prostatic carcinoma, however, prolonged treatment with these drugs does not suppress the production of α-subunit or FSH (Chapter 8.2). Neither can GnRH agonists suppress the production of α-subunit or FSH from gonadotroph or clinically nonfunctioning adenomas, either **in vivo** or **in vitro**. For these reasons, GnRH agonist treatment in patients with a clinically nonfunctioning or gonadotroph pituitary adenoma is not recommended.

During long-term culturing the suppressive effect of the dopamine agonist bromocriptine on gonadotropin and subunit secretion from cells from clinically nonfunctioning or gonadotroph pituitary adenomas increases as incubations last longer (Chapter 8.4). Intracellular hormone concentrations in cultured cells from a clinically nonfunctioning adenoma were decreased after several weeks of incubation with bromocriptine. Preliminary results on the effect of prolonged treatment with a dopamine agonist in a patient with a clinically nonfunctioning pituitary adenoma who was not operated on, show a decreased secretion of FSH and α-subunit. Whether this suppressive effect on the secretion and synthesis of gonadotropins and α-subunit leads to appreciable tumor volume reduction has yet to be seen.

SAMENVATTING

Klinisch niet-functionerende en gonadotrofe hypofyseadenomen komen in vergelijking met andere hypofysetumoren voor bij oudere patienten en veroorzaken in het merendeel van de gevallen visusklachten (Hoofdstuk 3). Gonadotrofe hypofyseadenomen zijn klinisch te herkennen aan verhoogde bloedspiegels van FSH en soms LH. Klinisch niet-functionerende hypofyseadenomen kenmerken zich door de afwezigheid van hypersecretie van enig hypofysevoorkwabshormoon **in vivo**.

In kweek gebrachte cellen van gonadotrofe en vrijwel alle klinisch niet-functionerende hypofyseadenomen produceren en bevatten FSH, LH, of de subunits van deze hormonen, afzonderlijk of in combinatie (Hoofdstuk 5). De secretie van deze hormonen en subunits door zowel klinisch niet-functionerende als gonadotrofe hypofyseadenomen kan **in vivo** en **in vitro** gestimuleerd worden met GnRH en TRH en geremd worden met bromocriptine (Hoofdstuk 5). Aangezien de overeenkomsten tussen beide tumortypen van groter klinisch belang zijn dan de verschillen, moeten klinisch niet-functionerende en gonadotrofe hypofyseadenomen als één klinische groep beschouwd worden.

De etiologie van klinisch niet-functionerende en gonadotrofe hypofyseadenomen is onduidelijk. Er zijn echter aanwijzingen dat primair hypogonadisme een rol kan spelen bij het ontstaan van deze tumoren. Hypogonadisme is aanwezig bij postmenopausale vrouwen en bij oudere mannen. Anderzijds worden de gonadotrofine spiegels bij postmenopausale vrouwen niet beinvloed door endogene plasma oestradiol spiegels (Hoofdstuk 7.2), en vertonen de gonadotrofine spiegels bij zowel oudere mannen als oudere vrouwen een kleinere respons op toediening van medicamenten dan bij jonge personen. De relatief autonoom secernerende gonadotrofe hypofysecellen die bij verouderende mannen en vrouwen aanwezig zijn, zouden zich kunnen ontwikkelen tot gonadotrofe of klinisch niet-functionerende hypofyseadenomen, onafhankelijk van circulerende spiegels van geslachtssteroiden. Een hypothalame oorzaak van deze tumoren kan evenmin worden uitgesloten.

De gangbare therapie voor klinisch niet-functionerende en

gonadotrofe hypofyseadenomen is transsphenoidale adenomectomie. Gezien het operatierisico en postoperatieve complicaties, staat medicamenteuze therapie in de belangstelling. Aangezien gonadotrofe en vrijwel alle klinisch niet-functionerende hypofyseadenomen LH, FSH, α-subunit, LHß, of een combinatie van deze glycoproteinen produceren, is een primaire eis voor een toe te passen medicament dat het de productie van deze hormonen en subunits remt. De aan een dergelijke therapie te stellen eis is immers dat ze tumorvolume reductie veroorzaakt; of dit nu door een vermindering van het celaantal of van het celvolume tot stand komt, in beide gevallen zal de hormoonsecretie minder worden.

GnRH agonisten kunnen de productie van LH door de gonadotrofe cellen remmen. Bij mannen met prostaatcarcinoom leidt langdurige behandeling met deze geneesmiddelen echter niet tot een verminderde productie van α-subunit of FSH (Hoofdstuk 8.2). Evenmin verminderen GnRH agonisten de productie van α-subunit of FSH door gonadotrofe of klinisch niet-functionerende hypofyseadenomen **in vivo** of **in vitro**. Om deze redenen is de behandeling van klinisch niet-functionerende en gonadotrofe hypofyseadenomen met GnRH agonisten niet geïndiceerd.

De dopamine agonist bromocriptine remt de secretie van gonadotrofines en subunits **in vitro** bij de meerderheid van klinisch niet-functionerende en gonadotrofe hypofyseadenomen. Tijdens langdurige incubatie van adenoomcellen met bromocriptine neemt het remmend effect van dit geneesmiddel op de gonadotrofine- en α-subunit secretie toe (Hoofdstuk 8.4). Incubatie van gekweekte cellen van een klinisch niet-functionerend adenoom met bromocriptine gedurende meerdere weken leidde tevens tot vermindering van de intracellulaire hormoonconcentraties. De voorlopige gegevens omtrent de langdurige behandeling met een dopamine agonist van een niet geopereerde patient met een klinisch niet-functionerend hypofyseadenoom tonen remming van de FSH en α-subunit secretie. Of dit remmend effect op de secretie en de synthese van gonadotrofines en subunits gepaard gaat met tumorvolume reductie, moet worden afgewacht.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotroph hormone
ANOVA	Analysis of variance
b	Slope
BMI	Body mass index
BSA	Bovine serum albumin
CG	Chorionic gonadotropin
CPA	Cyproterone acetate
CT scan	Computer(ized) tomographic scan
CV	Coefficient of variation
Et al.	And others
F	Female
FCS	Fetal calf serum
FRG	Federal republic of Germany
FSH	Follicle stimulating hormone
g	Gram
GH	Growth hormone
GnRH	Gonadotropin releasing hormone
h	Hour(s); (height)
i.e.	That is
i.n.	Intranasal(ly)
IRMA	Immunoradiometric assay
IU	International unit
iv	Intravenous(ly)
kg	Kilogram
L	Liter
LH	Luteinizing hormone
M	Male
MEM	Minimum essential medium
mg	Milligram
mIU	Milli international unit
mL	Milliliter
MRC	Medical research council
mU	Milli unit
ng	Nanogram
nmol	Nanomol

P	Probability
PAS	Perjodic acid Schiff
pmol	Picomol
PRL	Prolactin
r	Correlation coefficient
r_s	Spearman rank correlation coefficient
RER	Rough endoplasmatic reticulum
RIA	Radioimmunoassay
SD	Standard deviation
SE	Standard error of the mean
SHBG	Sex hormone binding globulin
T	Testosterone
T_4	Thyroxine
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
U	Unit
vs.	Versus
ug	Microgram
°C	Degrees Celsius

CURRICULUM VITAE

Dirk Jan Kwekkeboom
geboren 23-9-1958
te Goes

1970-1976	VWO, Baudartius Lyceum, Zutphen.
1976-1985	Studie Geneeskunde, Universiteit van Amsterdam.
1985	Artsexamen, Universiteit van Amsterdam.
1986-1989	Assistent in opleiding, afdeling Inwendige Geneeskunde III, Erasmus Universiteit Rotterdam.

ACKNOWLEDGMENTS

I am indebted to

-M. Leunisse and L. van der Zwan for the effort they took in collecting patient data.

-the department of neurosurgery for providing tumor material.

-C.C.J. van Vroonhoven for performing immunocytochemical studies in the removed tumor material.

-the representatives of sa IRE-Medgenix nv, who generously supplied the IRMA material used in the studies.

-the Rode Kruis Bloedbank for supplying blood samples.

-my colleagues for their help and interest and for the atmosphere they created.

-my promotor and co-promotor for their interest, help and criticism.