ASPECTS OF MEDICAL THERAPY OF NEUROENDOCRINE DISORDERS

If we pay attention to the common opinion of men, we shall see that they are conscious of the eternity of their mind; but they confuse eternity with duration, and attribute it to imagination or memory, which they believe will remain after death.

- Spinoza; Ethica V, 34 -

ASPECTS OF MEDICAL THERAPY OF NEUROENDOCRINE DISORDERS

ASPECTEN VAN DE MEDICAMENTEUZE BEHANDELING
VAN NEUROENDOCRIENE ZIEKTEN

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
WOENSDAG 27 MEI 1992 OM 13.45 UUR

DOOR
AART JOHANNES VAN DER LELIJ
GEBOREN TE 'S GRAVENHAGE

PROMOTIE-COMMISSIE

Promotor:	Prof. Dr. S.W.J. Lamberts
Overige leden:	Prof. Dr. J.C. Birkenhäger
	Prof. J.H.P. Wilson
	Prof. Dr. G.H. Zeilmaker

In de drukkosten van dit proefschrift werd bijgedragen door Sandoz B.V. te Uden.

Het proefschrift werd gedrukt door Haveka B.V. te Alblasserdam.

ABBREVIATIONS

ACTH	adrenocorticotropic hormone (corticotropin)
CRH	corticotropin releasing hormone
CT	computer tomogram
DA	dopamine
FSH	follicle stimulating hormone
GHRH	growth hormone releasing hormone
GnRH	gonadotropin releasing hormone
GH	growth hormone
IGF-I	insulin-like growth factor-I
IRMA	immunoradiometric assay
LH	luteinizing hormone
m-RNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
PRL	prolactin
RIA	radioimmuno assay
Sm-C	somatomedin-C
TRH	thyrotropin releasing hormone
TSH	thyrotropin

CONTENTS:

CHAPTER 1.

SURVEY OF LITERATURE:

1.1 Introduction:

The pituitary gland is situated within the sella turcica, which is located as a recess of the anterior cranial fossa in the sphenoid. The pituitary gland itself is made up of an anterior lobe (adenohypophysis) and a posterior lobe (neurohypophysis). Acromegaly was the first pituitary disease to become recognized as a clinical entity, although initially it was not clear, whether the eosinophilic adenomas, causing pituitary enlargement were causative or just a manifestation of the syndrome itself. Following the documented clinical improvement of acromegalic patients after partial hypophysectomy, it was proven that the pituitary adenomas were etiologic [1]. Nowadays, the anterior pituitary can be divided in at least seven distinct hormone-secreting cell types [2].

Pituitary tumors can be classified according to their clinical presentation [3],to the immunocytochemical staining properties of the tumor or to the type of hormone(s) they hypersecrete in vivo [4]. Using immunocytochemical technics, the prevalence of different types of pituitary adenomas could be estimated in large series of surgical removed pituitary tumors [5 6]. (See Table 1)

Table 1:

TYPE OF PITUITARY TUMOR	PREVALENCE IN %
PROLACTINOMAS	25-30
GH SECRETING TUMORS	15-35
FSH/LH SECRETING TUMORS	4
ACTH SECRETING TUMORS	15
TSH SECRETING TUMORS	< 1
NON FUNCTIONING TUMORS (no or few hormone positive cells could be detected)	20-30

As mentioned above, pituitary adenomas originate in and are composed of adenohypophyseal cells. They are frequently occurring neoplasms. In unselected pituitaries of adults obtained post-mortem a prevalence from 6 up to 23 per cent is recorded [7 8 9]. They also represent some 15 % of the intracranial neoplasms [10]. While

most pituitary adenomas are histologically benign, slow growing tumors, some adenomas show a more rapid growth, with extension into the surrounding tissues, eventually causing damage to the optic nerve(s) and/or other cranial nerves [11].

Apart from their presence within the sella turcica, adenohypophyseal cells have been also demonstrated within the nasopharyngeal region. Erdheim first described the pharyngeal pituitary in 1904 [12]. Like the pituitary adenohypophysis, the pharyngeal hypophysis contains at least seven hormone-producing cell types [13]. Though transsphenoidal vascular connections exist, it is unclear whether the pharyngeal pituitary has secretory properties [14]. Its function is unknown.

In the following pages, some aspects of the treatment and problems which can occur during treatment of each of the pituitary adenomas, as mentioned in Table 1, will be described.

1.2 Prolactinomas:

1.2.1 Introduction:

Prolactin is a 21,500 Dalton single-chain polypeptide which can be found in the circulation in 50 kDa and 150 kDa molecular variants. The information is encoded on chromosome 6 and transcription can be enhanced or suppressed by a variety of hormonal factors. Secretion occurs in a pulsatile manner. Hypothalamic regulation of PRL mainly involves tonic inhibition by dopamine which is transported via the portal vessels. The physiological importance of various hypothalamic stimulating factors is still largely unknown [15]. Prolactin secreting tumors are the most common hormone secreting pituitary tumors [16]. Not only prolactinomas cause high serum PRL levels, but also pregnancy, hypothyroidism, liver cirrhosis, renal failure and the use of several drugs should be considered in its differential diagnosis. Iatrogenic hyperprolactinemia occurs during the use of:

* Antidepressant drugs (eg amitriptyline, imipramine)
* Antihypertensive drugs (eg methyldopa)
* Opiates
* Estrogens
* Neuroleptics (eg perphenazine, haloperidol)
* Dopamine receptor blocking agents (eg domperidone, metoclopramide).

Because serum PRL levels do correlate well with the size of the sellar mass, only large tumors (with extra sellar extension) with considerably elevated serum PRL levels can be consistent with the diagnosis of a prolactinoma [17]. If this is not the case, also "non-functioning" pituitary tumors, as well as mixed pituitary tumors and other sellar disorders with a large tumor mass can account for a mild hyperprolactinemia (less than 150 μg/L) [18].

1.2.2 Radiodiagnostics:

Presently high resolution CT scan is the most accurate method to obtain indirect information about pituitary anatomy. Contiguous images in the coronal and sagittal planes with 2-3 mm collimation are recommended. Magnetic resonance imaging is still a relatively new imaging procedure, which has the potential for improving the visualization of the sellar region. Because it is still relatively new, it is yet not possible to determine whether it is superior to the high resolution CT scan. Baker [19] reported in a comparative investigation of MRI and CT scans that 10% of studies were judged superior by MRI, 10% superior by CT and 80 % were of equal value. In visualization of optic nerves and cavernous sinus, MRI is superior to CT, while it is the other way around in the visualization of calcified tissue.

1.2.3 Treatment:

A number of reports indicate that PRL levels, as well as tumor size remain unaltered for several years in the majority of untreated women with microprolactinomas [20] [21] [22]. Because of other problems or complaints, treatment may be necessary. Galactorrhea, for instance (especially in women) is one reason [23] [24]. Also sexual dysfunction (in men) [25] [26], infertility [27] and/or menstrual disturbances can necessitate treatment. In women osteoporosis can develop, due to the hypogonadal state caused by abnormalities in the pulsatile secretion of GnRH [28] [29]. Although headaches often occur in patients with macroadenomas of the pituitary, there also appears to be an increased incidence of headaches in hyperprolactinemic women with normal radiologic evaluation of the sellar region, although no correlation between PRL levels and the severity of the headaches was found [30].

Disturbances of the visual system may occur in patients with a macroprolactinoma. This does not happen so often as in nonfunctioning adenomas because due to the other complaints mentioned above (especially in women), patients do seek medical help in an earlier stage [31].

If one decides to treat a patient, one can choose between surgical treatment, medical treatment, radiotherapeutic treatment or any combination of these modalities. Drug therapy is nowadays the treatment of choice, not only for microprolactinomas but also for macroprolactinomas. The main reason is that surgical cure is unlikely in these patients, while risk of tumor recurrence is high [32]. Prior to the advent of medical treatment, therapy usually consisted of surgical resection and/or radiotherapy. It

appeared that pituitary radiation does not cause a rapid reduction in serum PRL levels. It does prevent a progression of the disease over the long-term, however [33] [34] [35].

1.2.4 Bromocriptine:

Prolactin secretion is inhibited by dopamine and stimulated by estradiol. The dopamine necessary for inhibition is released from nerve terminals in the median eminence and posterior pituitary. Estradiol may act directly on the anterior pituitary or by modulation of the two dopaminergic systems [36]. In 1971, the first clinical study on the effect of the dopaminergic drug 2-Br-alpha-ergocryptine mesylate (bromocriptine) was performed [37]. Bromocriptine (mainly acting at D2 receptors) directly stimulates neuronal and pituitary cell membrane dopamine receptors [38] [39]. After ingestion, peak levels are reached after 2 hours. Absorption after oral administration is about 28 %. First-pass metabolism is approximately 94 %. Excretion in the feces is 98 %; urine excretion is 2 % of the dose. PRL levels remain suppressed for 9-14 hours after a single dose [40] [41].

Long-term treatment with 2.5 mg bromocriptine three times daily results in a further decrease of PRL levels [42]. After short-term parenteral (7 days) and long-term peroral (4-6 weeks) treatment, electron microscopic morphometric evaluation of the adenomas showed that a reduction in size of prolactinoma cells occurs within a few days (half-time to maximum shrinkage, 2.2 days). The total volume of cellular lysosomes decreased significantly to about one-half of the pretreatment value, while the number of stored secretory granules did not change significantly [43]. Bromocriptine is capable of improving visual disturbances and reducing tumor size objectively in patients with large tumors within several days or weeks [44]. It reduces tumor size by a mean of 50% or more in about 50% of the patients with a macroprolactinoma and decreases PRL levels to normal in about 70% of the patients [42]. Discontinuation of therapy may be associated with a rapid expansion of tumor size and increase in PRL levels, so most patients require dopaminergic therapy indefinitely [45].

1.2.5 Side Effects:

Side effects, which are roughly the same for all of the dopaminergic drugs, are (in order of importance): nausea, orthostatic hypotension, headaches, fatigue, nasal congestion, abdominal cramps and constipation [46]. One can try to minimize these

side-effects by beginning therapy with a low dose, given at bedtime with a snack and by increasing this dose over weeks until it achieves a therapeutic response.

The occurrence of side-effects, as well as the need for two or three daily doses of bromocriptine remain important problems in the long-term treatment of prolactinoma patients. Also bromocriptine appears unable to normalize plasma PRL levels in about 30% and to reduce tumor size in one-third of cases [46]. Therefore recently other dopaminergic drugs have been developed, including metergoline, lisuride, pergolide, mesulergine, terguride, dihydroergocristine, dihydroergocriptine, cabergoline and CV 205-502. Metergoline, dihydroergocristine and terguride were less effective than bromocriptine [47 48 49], while lisuride and mesulergine were more poorly tolerated than bromocriptine [50 51]. In the next chapters cabergoline, pergolide and CV 205-502 will be discussed in more detail.

1.2.6 Cabergoline:

An acute oral dose of 0.3-0.6 mg cabergoline [1-(6-allylergolin-8-yl)carbonyl-1-dimethylamino)propyl-3-
ethylurea] significantly lowers PRL levels in hyperprolactinemia for 7-14 days [52 53]. Given at weekly intervals for 8-9 weeks it induces normalisation of PRL levels in about two thirds of hyperprolactinemic women [53 54]. In a recent study by Ferrari et al. it was demonstrated that serum PRL levels fell to normal and remained so in 41 of 48 hyperprolactinemic women treated chronically with a weekly dose of cabergoline, ranging from 0.2-1.8 mg, usually administrated once or twice weekly; only three women needed treatment three times-weekly in order to achieve normal PRL concentrations [55]. Only two of 48 women did not satisfactorily lower their PRL levels, although there was a 50% reduction of PRL levels and resumption of menses, albeit only after several months of high dose cabergoline treatment. Five of six women with macroprolactinomas did show a marked tumor shrinkage. All patients were able to tolerate effective doses of cabergoline without important side-effects. Similar results were later reported by Ciccarelli et al. [56]. These results indicate that cabergoline is a well-tolerated new dopaminergic drug with long-lasting activity which represents potentially an advance in the chronic medical treatment of hyperprolactinemic states.

1.2.7 Pergolide:

In 1983 Kleinberg et al. reported the results of administration of pergolide mesylate to hyperprolactinemic patients [57]. This drug with dopaminergic properties produced long-lasting reductions in PRL levels after single doses of 50 μg. After 24 hours, the values remained depressed at a mean of 29% of the base-line value. After three months or more of pergolide therapy, PRL levels normalized in 37 of the 41 patients and remained slightly elevated in two. Menses resumed in 84% (16 of 19). 13 Patients had a macroprolactinoma. After treatment ten showed a tumor shrinkage (77%). The reduction in tumor size was marked in 7 of 10 patients. The majority of patients were found to have postural hypotension after the first dose of pergolide and also liver enzyme disturbances were recorded infrequently. In three patients the drug was discontinued after three weeks because of nausea and vomiting. Although pergolide is a potent inhibitor of PRL secretion [58 59], the drug has never found an important place in the treatment of hyperprolactinemic states, which is also related to the liver enzyme abnormalities found by Kleinberg [57]. In a blind study involving a total of 157 hyperprolactinemic patients the efficacy and tolerability of bromocriptine and pergolide were found to be the same, while also the incidence and type of side-effects to both dopamine agonists were similar [60].

1.2.8 CV 205-502:

CV 205-502 (an octahydrobenzo-[g]-quinoline), is a non-ergot alkaloid dopaminergic drug, which became recently available for therapy of hyperprolactinemic states [61]. It is a potent D-2 receptor agonist, with only a weak D-1 receptor activity. In studies with a single oral dose of 0.06 mg, CV 205-502 suppressed normal PRL secretion within two hours continuously for 36 hours [62]. On a weight basis, in several studies, this drug was far more potent than bromocriptine in suppressing both normal and pathological PRL secretion [63 64]. Vance et al. reported the results of a study in 26 hyperprolactinemic women [65]. They were treated with doses up to 0.09 mg daily. In 13 normal menstrual cycles returned (54%) and in 12 (80%) galactorrhea decreased or disappeared. Thirteen (54%) achieved normalisation of PRL levels. Newman et al. reported that CV 205-502 to a maximum of 0.14 mg daily was well tolerated in 10 hyperprolactinemic patients, who were previously proven to be intolerant to bromocriptine [66]. These promising results were confirmed in several studies, all showing the potent inhibitory effect of CV 205-502 on PRL secretion, as well as on tumor size,

both in micro- and macroprolactinoma patients and the relative small number of patients, which complained of side-effects so severe that drug therapy had to be discontinuated [67] [68] [69] [70].

1.2.9 Complications of treatment with dopaminergic drugs:

As mentioned above, side effects like nausea and other abdominal problems sometimes can be a reason to discontinue drug therapy. Apart from these side-effects, also other very rare occurring complications of dopaminergic drug therapy of macroprolactinomas may occur: In patients with macroprolactinomas, which have infiltrated the surrounding tissues, especially in/through the sphenoidal bone, treatment with dopaminergic drugs which causes a reduction in tumor size, can result in leak of cerebrospinal fluid (liquorrhea), sometimes followed by a life-threatening meningitis [71] [72] [73].

1.3 Acromegaly:

1.3.1 Introduction:

As described above in the introduction, acromegaly was the first recognized distinct clinical syndrome with the pituitary as the source of the disorder. The hormone produced by the somatotrophs of the anterior pituitary is growth hormone. Chromosome 17-q contains the five gene clusters, responsible for human GH [74] [75]. The episodic secretion of GH is under dual hypothalamic control [76]. Without altering m-RNA levels, somatostatin is capable of suppressing GH secretion, while growth hormone-releasing hormone (GHRH) stimulates the secretion as well as induces gene transcription [77] [78] [79]. GH secretion is predominantly regulated by the fluctuations in somatostatin secretion; fluctuations, which are increased during sleep and fasting [80] [81]. Not only GHRH and somatostatin, but also IGF-I, thyroid hormone and estrogens contribute to the control of GH secretion [79]. Although human somatic growth is regulated almost primarily by GH, most of GH's growth-promoting effects are mediated by IGF-I, which is largely synthesized in the liver, but is also produced in the kidney, muscle, pituitary, chondrocytes and the gastro-intestinal tract [82] [83] [84]. As a negative-feedback mechanism, IGF-I suppresses GH-mRNA synthesis in the pituitary [85] [86]. Although most of GH's growth promoting actions are mediated by IGF-I, animal models suggest that GH and IGF-I act both independently and synergistically in skeletal and organ growth [87] [88].

Because of the apparent manifestation of the disease, acromegaly is usually diagnosed clinically. The incidence of acromegaly is approximately 3 per million and the prevalence is about 40 per million [89]. The somatotrophic cells are mainly situated in the lateral wings of the pituitary; their number or structure is not sex-related [90] [91]. The basic morphological abnormality associated with increased GH production is an increase in the number of GH producing adenohypophyseal cells. These adenomas are composed of acidophilic or chromophobic adenohypophyseal cells [92] [93]. Several disorders (Table 2) can cause hypersecretion of GH, inducing acromegaly in adults (and gigantism before epiphyseal closure).

Table 2 *

Excess GH secretion:

Pituitary:
- Densely granulated GH cell adenoma
- Sparsely granulated GH cell adenoma
- Mixed GH cell and PRL cell adenoma
- Mammosomatotroph adenoma
- Acidophil stem-cell adenoma
- Plurihormonal adenoma
- Somatotroph hyperplasia
- GH cell carcinoma

Ectopic pituitary tumor:
- Sphenoid or parapharyngeal sinus

Extrapituitary tumor:
- Pancreas
- Lung
- Ovary
- Breast

Excess GHRH secretion:

Eutopic:
- Hypothalamic hamartoma, ganglioneuroma

Ectopic:
- Carcinoid
  - Bronchus
  - Gastro-intestinal tract and pancreas
  - Undetermined
- Pancreatic islet cell
- Small-cell lung cancer
- Adrenal adenoma
- Pheochromocytoma

Excess growth factor secretion or action:

Acromegalism

* : Adapted from Melmed S. Acromegaly. N Eng J Med 1990;322: 966-977.

80% of the cases of acromegaly are caused by the common somatotroph adenoma, which can be either densely granulated, slow growing adenomas with large amounts of GH, or sparsely granulated, fast growing tumors [94].

Mixed GH cell-PRL cell adenomas are histologically benign, bimorphous and bihormonal tumors. They are composed of lactotrophic cells and somatotrophic cells [95 96 97]. In the tumor, densely- or sparsely granulated GH cells may be mixed with densely- or sparsely granulated PRL cells.

Acidophil stem cell adenomas are composed of immature cells, which are believed to be the precursors of both somatotrophs and lactotrophs, and they contain both hormones [98]. The degree of hyperprolactinemia, even in large tumors, is usually mild.

Mammosomatotroph cell adenomas are believed to be the mature variant of the stem cell adenomas, also containing both hormones, as was demonstrated e.g. by reverse hemolytic placque assay of individual tumor cells by several authors [99 100 101]. These patients have clinically acromegaly, while PRL levels are only slightly elevated [102].

A rare tumor is the unclassified plurihormonal adenoma, which can present itself with acromegaly. They produce two or more hormones, can be monomorphous or plurimorphous and can produce unusual combinations of hormones, such as GH and TSH, GH, PRL and TSH, GH, PRL and ACTH, or α-subunits [103].

Somatotroph hyperplasia is usually caused by an ectopic GHRH source and GH cell carcinomas are very rare (only five well-documented cases are reported [104]).

1.3.2 Relation between tumor-size, GH- and IGF-I levels:

In most studies a positive correlation between tumor size and basal GH levels has been found [105 106 107 108 109 110]. Up to levels of about 50-80 μg/L, GH concentration correlates well with serum IGF-I levels, suggesting that GH levels of about 60 μg/L do stimulate IGF-I production maximally. [111 112 113 114].

Whether a correlation between age and tumor volume exists, is unclear. Several authors have reported such a correlation [107 109], while others could not find such a relation [108].

1.3.3 Treatment:

As acromegaly is associated with an increase in the expected mortality rate [115], it has to be treated either surgically, medically or radiotherapeutically (or with any

combination of these).

1.3.4 Surgery:

If a GH level < 5 μg/L after transsphenoidal surgery is used as a criterium for cure, only 60% of the patients reach those GH levels, while only 30% of macroadenoma patients and very few of the patients with tumors with extrasellar extension turn out to be cured [116] [117] [118]. Considering the fact that 50% of the patients with GH levels < 5 μg/L after surgery, do still have elevated IGF-I levels [119], a real cure of acromegaly by surgery might be. rare So other means of therapy, or combinations of therapy are often necessary.

1.3.5 Radiotherapy:

When increased GH production still persists after surgery, or when there are contra-indications for surgery, external conventional (45 Gy), as well as proton-beam radiation (150 Gy) can be used as treatment. Both methods are capable of reducing GH levels in a majority of the patients to < 5 μg/L, although these GH levels are reached after 10 and 5 years respectively [120] [121]. Acquired hypopituitarism as a (late) complication of irradiation therapy occurs in more than 50% of the patients [122].

Medical therapy:

1.3.6 Bromocriptine:

As relatively large doses of bromocriptine are necessary to induce clinically beneficial effects [123] [124], this drug is not so often used as a primary therapy of acromegaly, but more often as an adjuvant therapy [125]. Barkan [119] reported that about 70% of the patients felt some improvement in the sense of well-being, although serum GH and IGF-I levels remained elevated. In less than 20% of the patients, bromocriptine is capable of reducing GH levels to less than 5 μg/L. In three reports, the administration of octreotide was found to be more effective in lowering GH levels than bromocriptine [126] [127] [128].

1.3.7 Octreotide:

Since the introduction of a cyclic octapeptide analogue of somatostatin, octreotide (Sandostatin; SMS 201-995), therapy of acromegaly has become much more successful [129] [130] [131]. Octreotide (50 μg as a single dose, subcutaneously), suppresses GH secretion within one hour. Maximal suppression of GH levels is reached after 3 hours. The response may be sustained up to 12 hours [132]. The drug reduces tumor volume in 50% of the patients [133] and even total resolution of a tumor has been reported [134]. The drug reduces the secretion of GH in 90% of the acromegalic patients and because the course of serum GH levels after a single-dose of octreotide correlates well with the serum GH levels reached during long-term treatment with SMS 201-995, octreotide has become the drug of first choice in the medical treatment of acromegaly [130] [135] [136] [137]. It is usually well tolerated, although it inhibits gallbladder contractility, causing the development of gallstones [138] [78] [139]. Some studies indicate that continuous subcutaneous infusion (or frequent subcutaneous injections) may be more effective than the usual regimen of three daily injections [140] [141]. Because serum IGF-I levels do correlate well with the mean 24-hour GH levels, IGF-I levels are widely used as a sensitive indicator of even mild hypersecretion of GH [137] [142]. Long-term therapy with octreotide reduced serum IGF-I levels in several groups of acromegalics by 37 to 81% [130] [143] [144]. One study suggested that pretreatment with octreotide of acromegalic patients results in better surgical cure rates [145].

1.4 Cushing's Syndrome:

1.4.1 Diagnostic tests:

Cushing's syndrome describes the clinical symptomatology related to longstanding hypercortisolism. The 1 mg overnight dexamethasone suppression test is an excellent screening test with almost no false-negative results [146] [147]. Although several authors have reported that urine sampling of cortisol in several ways is equal or superior to diagnose Cushing's syndrome, these assessments are mostly too time-consuming to be of great value in every day practice [148] [149] [150] [151].

Once the diagnosis of Cushing's syndrome is made and "iatrogenic" causes of Cushing's syndrome have been made unlikely (medication, alcohol abuse etc.), one must determine whether Cushing's syndrome is caused by pituitary overproduction of ACTH (Cushing's disease), ectopic production of ACTH (or CRH) or overproduction of cortisol by an adrenal adenoma or carcinoma. To differentiate between a pituitary/ectopic or adrenal cause, ACTH measurements can be of great help. Although new immunoradiometric assays (IRMA) for ACTH have been developed with a high sensitivity and specificity, these assays are often incapable of tracing "big" ACTH, which is secreted by most ectopic sources of ACTH, so radio immunological determinations are still preferred above immunoradiometric ones [152] [153]. To determine whether Cushing's syndrome has a pituitary or non-pituitary cause can be very difficult. A continuous dexamethasone infusion (1 mg/hr) for seven hours seems to be a superior diagnostic tool for this differential diagnostic problem with a decrease in serum cortisol levels of at least 190 nmol/l as criterium [154]. Only 2 out of 122 patients had false negative results and both had corticotrophin-releasing hormone (CRH) producing tumors. The CRH stimulation test is also a reliable test with high sensitivity and specificity to discriminate between pituitary and ectopic/adrenal causes [155] [156]. Pituitary adenomas have an exaggerated increase in ACTH and cortisol levels, while ectopic and adrenal Cushing's syndromes show no increase in both levels. This test is equally reliable as the long intravenous dexamethasone test [157] [158].

1.4.2 Radiodiagnostic techniques:

Several studies have shown that CT scanning is very unreliable in detecting ACTH producing pituitary micro-adenomas, with an overall sensitivity in localizing the adenoma of about 50% [159] [160] [161] [162] [163]. This is in part due to the small size of these adenomas, as well as by the fact that they have the same degree of enhancement with contrast as normal pituitary tissue [159] [163].

Magnetic Resonance imaging seems to be a better way in localizing pituitary ACTH secreting microadenomas, with a sensitivity of about 75%, although the number of patients examined is still relatively small [159] [164] [165] [166].

In case of an adrenal cause of Cushing's syndrome, CT scanning has a sensitivity of almost 100% and MR imaging offers no extra advantages over CT scanning in these patients [167].

1.4.3 Localizing by sampling of the inferior petrosal sinus:

By simultaneous sampling of both inferior petrosal sinuses and measuring the central-to-peripheral ACTH ratio, one can localize the adenoma in case of Cushing's disease either on the left- or right side of the pituitary gland with a sensitivity of about 85% (ACTH ratio of 2:1 or greater) [168] [169] [170] [171] [172] [173] [174]. If a ratio of 1.5:1 or less is found, one has to be suspicious of an ectopic production of ACTH.

1.4.5 Treatment:

1.4.6 Surgery:

Transsphenoidal surgery is the treatment of first choice in case of a pituitary dependent Cushing's syndrome (Cushing's disease) [175] [159]. In a study in 216 cases of Cushing's disease Mampalam et al [159] reported that an experienced neurosurgeon can identify an ACTH producing microadenoma in about 80% of the cases. The success of the operation depends on how rigid the criteria for cure have been. If only a decrease in serum cortisol levels is used, the success rate is higher than in series where serum cortisol levels became undetectable, so success rates between 25% and even 80% have been published [176] [177] [178] [159] [179].

1.4.7 Radiotherapy:

Although successful in children, conventional radiotherapy leads to biochemical and clinical improvement in only 15 to 25% of adult patients [180] [181]. Heavy-(alpha)-particle irradiation is effective in controlling hypercortisolism in 80% [182], while proton-beam therapy is effective in 65% of the patients [183]. Both therapies frequently result in hypopituitarism, while neurological complications occur relatively frequently.

1.4.8 Medical therapy:

Primary medical therapy of Cushing's disease is not successful, but short-term treatment is sometimes necessary. Medical treatment for the other forms of Cushing's syndrome is necessary even more often. For this purpose, ketoconazole, metyrapone and aminoglutethimide can be used.

1.4.9 Ketoconazole:

Ketoconazole is an imidazole derivative, which inhibits mitochondrial cytochrome P450 enzymes. The major site of action appears to be in the inhibition of 17-20 desmolase. A moderate blockade of 17-hydroxylase may be present, while there is a marked inhibition of 21- and/or 11-hydroxylase. In doing so, it inhibits the conversion of androgens to estrogens, of progesterone to androstenedione and testosterone, and of 11-deoxycorticosterone to corticosterone [184] [185] [186]. Started in doses of 800-1200 mg daily and with maintenance doses of 600-800 mg daily, ketoconazole is relatively well tolerated, although liver-enzyme disturbances can be a problem. It has been proven to be effective in the treatment of hypercortisolism in most types of Cushing's syndrome [187] [188] [189] [190] [191] [192] [193] [194] [195] [196].

1.4.10 Aminoglutethimide:

Although originally developed as an anticonvulsant drug, aminoglutethimide is now also used as a drug against hypercortisolism. It inhibits primarily the cholesterol side-chain cleavage enzyme which is involved in the synthesis of corticosteroids as well

as the aromatase enzyme which converts androgens to estrogens. It causes gastro-intestinal side-effects, somnolence and a transient rash in therapeutical doses of 1-2 gram daily. Steroid withdrawal symptoms are inevitable and replacement therapy by corticosteroids is required [197] [198]. It was subsequently shown that aminoglutethimide effectively suppresses cortisol secretion in patients with adrenal cancer [199] and Cushing's disease [200].

1.4.11 Metyrapone:

Metyrapone is an 11-ß-hydroxylase inhibitor, which is widely used as a differential diagnostic drug in patients with Cushing's syndrome [201]. Cortisol secretion is decreased, while the production of 11-deoxycortisol and dehydroepiandrosterone is increased. It has also been used in the treatment of Cushing's disease. In doses up to 2 grams daily, biochemical and clinical remission can be achieved [202] [203]. Nausea, vomiting and dizziness can occur. The drug can also cause hirsutism due to the overproduction of adrenal androgens, as well as hypertension, hypokalemic alkalosis and edema, due to the accumulation of 11-deoxycorticosterone.

1.4.12 Suramin:

Originally, this polyanionic compound has been used in the treatment of trypanosomiasis and AIDS. It appeared that the substance caused adrenal insufficiency. It is able to bind and inactivate growth factor and enzyme systems, critical to cellular homeostasis and proliferation [204] [205]. It exerts significant cytostatic and cytocidal effects against a variety of tumor cell lines in vitro [206] [207]. In pilot studies, it was used lymphomas with promising results in the treatment of metastatic adrenocortical carcinoma, prostatic cancer and nodular [208] [209].

1.4.13 Mifepristone (RU 486):

Synthesized in 1980 as a pure antagonist of progesterone receptors (without agonist activity), mifepristone was found to have also a high affinity for glucocorticoid receptors [210] [211] [212] [213] [214] [215]. After one single-dose oral gift of 400 mg, RU induces a response that lasts at least 34 hours. The blocking effects on the cortisol

receptor in man can be antagonized by high doses of dexamethasone [216]. Studies in patients with Cushing's syndrome, caused by ectopic ACTH production or adrenocortical cancer, showed that RU 486 in doses from 5 to 22 mg/kg body weight daily, caused a clear clinical improvement [217] [218] [219].

1.5 Thyrotropin (TSH)-secreting adenomas:

1.5.1 Introduction:

These rare tumors are seldomly found in patients with primary hypothyroidism, but they can present themselves also as thyrotoxicosis [220] [221]. In the latter case, these hyperthyroid patients often have elevated serum TSH levels, but also TSH levels in the normal range for euthyroid persons can be found [222]. The thyroid is almost always diffusely enlarged. Some patients do have symptoms of acromegaly, or galacthorrea [223] [224] [225]. As all glycoprotein producing tumors, TSH-secreting adenomas can cause an elevation of serum alpha-subunit concentrations, which may be independent of the alterations in the concentration of other pituitary hormones and it may even be the only tumor marker present [226].

1.5.2 Therapy:

1.5.3 Surgery and radiotherapy:

Although surgery is rarely curative and residual tumor may grow, transsphenoidal microsurgery is still the therapy of choice [227]. Whether there a place for (postoperative) radiotherapy is not known. There are no large controlled studies and even after radiation therapy, tumor growth may reoccur [228]. The results available indicate that transsphenoidal surgery alone has a cure rate for hyperthyroidism of almost 40%, while a combination of surgery and radiotherapy has a cure rate of 46% [229].

1.5 Medical therapy:

1.5.4 Dopaminergic Drugs:

The acute response of TSH producing tumors to dopaminergic drugs has been inconsistent [230]. In one case an increase in TSH levels was reported after administration of L-dopa [231]. There are only few reports on long-term treatment with bromocriptine, and they suggest no tumor shrinkage, nor a decrease in TSH levels [232]. In one study, pergolide seemed slightly superior to bromocriptine in reducing, but not normalizing TSH levels in the treatment of thyrotropin-secreting tumors [233]. In general, the treatment of TSH-secreting adenomas with dopaminergic drugs has not proven to be successful.

1.5.5 Octreotide:

Specific somatostatin receptors have been demonstrated on cell membranes of TSH secreting tumors [234 235]. Also, somatostatin has been shown to decrease TSH secretion [236 78 237 238]. In several studies therefore octreotide has been used in the treatment of thyrotropin-secreting adenomas and the drug was able to reduce TSH levels by 40 to 90% and alpha-subunits levels by 33 to 65% [236 239 240]. In one study, TSH suppression by octreotide lasted only for 30 weeks, suggesting an escape from the suppressive effect of SMS 201-995 [240]. Although promising effects, the results of larger series are necessary to determine the clinical efficacy of the drug.

1.6 Nonfunctioning tumors:

1.6.1 Introduction:

As mentioned above, approximately 25 to 30% of patients with a pituitary adenoma have no clinical evidence of hypersecretion of any hormone whatsoever. Using in vitro techniques however, most of these tumors show evidence of alpha-subunits alone, or together with gonadotrophins [241] [242] [243] [244] [245]. As these tumors are clinically nonfunctioning, they present themselves as macroadenomas, causing local pressure on the surrounding tissue and hypopituitarism [246] [247] [248] [249].

1.6.2 Therapy:

For these reasons, surgical decompression by transsphenoidal microsurgery is the treatment of choice and improvement of visual field abnormalities is seen in a majority of patients [250] [251]. Postoperative radiotherapy is advisable but tumor recurrence can be observed and the field of radiation must sometimes be wide, because of the tumor size and therefore long-term side effects of radiotherapy are not neglectable [252] [253].

The efficacy of bromocriptine treatment for nonfunctioning pituitary tumors is not clear. Although some authors do claim tumor shrinkage after long-term treatment with high (20 mg) doses of bromocriptine, others could not find a decrease in tumor volume during treatment with dopaminergic drugs [249] [254] [255] [256] [257].

1.7 References:

1. Cushing H. Partial hypophysectomy for acromegaly. Ann Surg 1909;1: 1002.

2. Labhart A, Froesch ER, Landolt AM, Prader A, Zackmann M, Zapf J. The adenohypophysis. In: A. Labhart (editor).Clinical Endocrinology: Theory and Practice (complete revised second edition) 1986; 81-86.

3. Junqueira LC, Carneiro J, Contopoulos AN. Basic Histology. Lange Medical Publishers, Los Altos 1975; 375-376.

4. Kovacs K, Horvath E, Ezrin C. Anatomy and histology of the normal and abnormal pituitary gland. In: De Groot L. et al., eds. Endocrinology. WB Saunders Company, Philadelphia 1989; 266-277.

5. Kovacs K, Horvath E, Ezrin C. Anatomy and histology of the normal and abnormal pituitary gland. In: De Groot L, et al., eds. Endocrinology. WB Saunders Company, Philadelphia 1989; 271-281.

6. Wilson CB, Dempsey LC. Transsphenoidal microsurgical removal of 250 pituitary adenomas. J Neurosurg 1978; 48: 13-22.

7. Burrow GN, Wortzman G, Rewcastle NB, Holgate RC, Kovacs K. Microadenomas of the pituitary and abnormal sellar tomograms in an unselected autopsy series. N Eng J Med 1981;304: 156-158.

8. McComb DJ, Ryan N, Horvath E, et al. Subclinical adenomas of the human pituitary. New light on old problems. Arch Pathol Lab Med 1983;107: 488-492.

9. Parent AD, Brown B, Smith EE. Incidental pituitary adenomas: A retrospective study. Surgery 1982;92: 880-884.

10. Kovacs K, Horvath E. Morphology of adenohypophyseal cells and pituitary adenomas. In: Imura H (ed): The pituitary gland. Comprehensive Endocrinology. New York, Raven Press, 1985: 25

11. Scheithauer BW, Kovacs K, Laws ER Jr, et al. Pathology of invasive pituitary tumors with special reference to functional classification. J Neurosurg 1986;65: 733-738.

12. Erdheim J, Uber Hypophysenganggeschwulste und Hirncholesteatome. Kaiser Akad Wiss Math Naturwiss 1904;113: 537-726.

13. Ciocca D, Puy L, Stati A. Identification of seven hormone-producing cell types in the human pharyngeal hypophysis. J Clin Endocrinol Metab 1985;60: 212-216.

14. Puy L, Ciocca D. Human pharyngeal and sellar pituitary glands: differences and similarities revealed by an immunocytochemical study. J Endocrinol 1986;108: 231-238.

15. Benker G, Jaspers C, Hausler G, Reinwein D. Control of prolactin secretion. Klin Wochenschr 1990;68: 1157-1167.

16. Burrow GN, Wortzman G, Rewcastle NB, Holgate RC, Kovacs K. Microadenomas of the pituitary and abnormal sellar tomograms in an unselected autopsy series. N Eng J Med 1981;304: 156-158.

17. Flier JS, Underhill LH. Diagnosis and management of hormone-secreting pituitary adenomas. N Eng J Med 1991;324: 822-831.

18. Vance ML, Evans WS, Thorner MO. Bromocriptine. Ann Int Med 1984;100: 78-91.

19. Baker HL, Berquist TH, Kispert DB, Reese DF, Houser OW, Earnest F 4th, Forbes GS, May GR. Magnetic resonance imaging in a routine clinical setting. Mayo Clin Proc 1985;60: 75-90.

20. Sisam DA, Sheenan JP, Sheeler LR. The natural history of untreated microprolactinomas. Fertil Steril 1987;48: 67-71.

21. Schlechte J, Dolan K, Sherman B, Chapler F, Luciano A. The natural history of untreated microprolactinemia: a perspective analysis. J Clin Endocrinol Metab 1989;68: 412-418.

22. March CM, Kletzky OA, Davajan V, et al. Longitudinal evaluation of patients with untreated prolactin-secreting adenomas. Am J Obstet Gynecol 1981;139: 835-844.

23. Nabarro JDN. Pituitary prolactinomas. Clin Endocrinol 1982;17: 129-133.

24. Thorner MO, McNeilly AS, Hagen C, et al. Long-term treatment of galactorrhea and hypogonadism with bromocriptine. Br Med J 1974;2: 419-422.

25. Carter JN, Tyson JE, Tolis G. Prolactin-secreting tumors and hypogonadism in 22 men. N Eng J Med 1978;299: 847-851.

26. Segal S, Yaffe H, Laufer N, et al. Male hyperprolactinemia effects on fertility. Fertil Steril 1979;32: 556-562.

27. Ben-David M, Schenker JG. Transient hyperprolactinemia: A correctable cause of idiopathic infertility. J Clin Endocrinol Metab 1983;57: 442-446.

28. Klibanski A, Greenspan SL. Increase in bone mass after treatment of hyperprolactinemic amenorrhea. N Eng J Med 1986;315: 542-546.

29. Jacobs HS, Franks S, Murray MAF, Hull MG, Steele SJ, Nabarro JD. Clinical and endocrine features of hyperprolactinaemic amenorrhea. Clin Endocrinol (Oxf) 1976;5: 439-454.

30. Kemmann E, Jones JR. Hyperprolactinemia and headaches. Am J Obstet Gynecol 1983;145: 665-670.

31. Trautman JC, Laws ER. Visual status after transsphenoidal surgery at the Mayo Clinic, 1971-1982. Am J Ophthalmol 1983;96: 200-205.

32. Serri O, Rasio E, Beauregard H, Hardy J, Somma M. Recurrence of hyperprolactinemia after selective transsphenoidal adenomectomy in women with prolactinoma. N Eng J Med 1983;309: 280-283.

33. Tran LM, Blount L, Horton D, Sadechi A, Parker RG. Radiation therapy of pituitary tumors: results in 95 cases. Am J Clin Oncol 1991;14: 25-29.

34. Rush SC, Newali J. Pituitary adenoma: the efficacy of radiotherapy as the sole treatment. Int J Radiat Oncol Biol Phys 1989;17: 165-169.

35. Sheline GE, Grossman A, Jones AE. Radiation therapy for prolactinomas. In: Black PM, Zervas NT, Ridgeway EC, et al (eds): Secretory Tumors of the Pituitary Gland. New York, Raven Press 1984; 93

36. Burgett RA, Garris PA, Ben-Jonathan N. Estradiol-induced prolactinomas: differential effects on dopamine in posterior pituitary and median eminence. Brain Res 1990;531: 143-147.

37. Corrodi H, Fuxe K, Hokfelt T, Lidbrink P, Ungersted U. Effect of ergot drugs on central catecholamine neurons: evidence for a stimulation of central dopamine neurons. J Pharm Pharmacol 1973;25: 409-412.

38. Benker G, Jaspers C, Hausler G, Reinwein D. Control of prolactin secretion. Klin Wochenschr 1990;68: 1157-1167.

39. Calabro MA, Macleod RM. Binding of dopamine to bovine anterior pituitary membranes. J Clin Endocrinol 1974;40: 363-370.

40. Schran HF, Bhuta SI, Schwarz HJ, Thorner MN. The pharmocokinetics of bromocriptine in man. In: Goldstein M, Calne DB, Lieberman A, Thorner MO, eds. Ergot Compounds & Brain Function: Neuroendocrine & Neuropsychiatric Aspects. New York: Raven Press; 1980: 125-139. (Advances in Biochemical Psychopharmacology; vol 23).

41. Muller EE, Panerai AE, Cocchi D, Mantegazza P. Endocrine profile of ergot alkaloids. Life Sci. 1977;21: 1545-1558.

42. Molitch ME, Elton RL, Blackwell RE, Caldwell B, Chang RJ, Jaffe R, Joplin G, Robbins RJ, Tyson J, Thorner MO. Bromocriptine as primary therapy for prolactin-secreting macroadenomas: Results of a prospective multicentre study. J Clin Endocrinol Metab 1985;60: 698-705.

43. Landolt AM, Osterwalder V, Landolt TA. Storage and release of secretory granules in human prolactinomas: modification by bromocriptine. J Endocrinol 1978;113: 495-499.

44. Thorner MO, Martin WH, Rogol AD, Morris JL, Perryman RL, Conway BP, Howards SS, Wolfman MG, MacLeod RM. Rapid regression of pituitary prolactinomas during bromocriptine treatment. J Clin Endocrinol Metab 1980;51: 438-445.

45. Thorner MO, Perryman RL, Rogol AD, Conway BP, MacLeod RM, Login IS, Morris JL. Rapid changes of prolactinoma volume after withdrawal and reinstitution of bromocriptine. J Clin Endocrinol Metab 1981;53: 480-483.

46. Vance ML, Evans WS, Thorner MO. Bromocriptine. Ann Int Med 1984;100: 78-91.

47. Crosignani PG, Ferrari C, Liuzzi A, Benco R, Mattei A, Rampini P, Dallabonzana D, Scarduelli C, Spelta B. Treatment of hyperprolactinemic states with different drugs: a study with bromocriptine, metergoline, and lisuride. Fertil Steril 1982;37: 61-67.

48. Scarduelli C, Cavioni V, Galparoli C, Spellecchia D, Ferrari C, Crosignani PG. Clinical use of a new anti-prolactinemic drug: dihydroergocristine. J Obstet Gynecol 1987;7: 225-227.

49. Dallabonzana D, Liuzzi A, Oppizzi G, Cozzi R, Verde G, Chiodini P, Rainer E, Dorow R, Horowski R. Chronic treatment of pathological hyperprolactinemia and acromegaly with the new ergot derivative terguride. J Clin Endocrinol Metab 1986;63: 1002-1007.

50. Bouloux PMG, Besser GM, Grossman A, Moult PJA. Clinical evaluation of lisuride in the management of hyperprolactinemia. Br Med J 1987;294: 1323-1324.

51. Grossman A, Bouloux PMG, Loneragan R, Rees RH, Wass JAH, Besser GM. Comparison of the clinical activity of mesulergine and pergolide in the treatment of hyperprolactinemia. Clin Endocrinol(Oxf) 1985;22: 611-616.

52. Ferrari C, Barbieri C, Caldara R, Mucci M, Codecasa F, Paracchi A, Romano C, Boghen M, Dubini A. Long lasting prolactin lowering effect of cabergoline, a new dopamine agonist, in hyperprolactinemic patients. J Clin Endocrinol Metab 1986;63: 941-945.

53. Mattei GB, Ferrari C, Baroldi P, Cavioni V, Paracchi A, Galparoli C, Romano C, Spellecchia D, Gerevini G, Crosignani PG. Prolactin-lowering effect of acute and once weekly repetitive oral administration of cabergoline at two dose levels in hyperprolactinemic patients. J Clin Endocrinol Metab 1988;66: 193-198.

54. Melis GB, Gambacciani M, Paoletti AM, Beneventi F, Mais V, Baroldi P, Fioretti P. Dose-related prolactin inhibitory effects of the new long-acting dopamine agonist cabergoline in normal cycling, puerperal, and hyperprolactinemic women. J Clin Endocrinol Metab 1987;65: 541-545.

55. Ferrari C, Mattei A, Melis GB, Paracchi A, Muratori M, Faglia G, Sghedoni D, Crosignani PG. Cabergoline: long-acting oral treatment of hyperprolactinemic disorders. J Clin Endocrinol Metab 1989;68: 1201-1205.

56. Ciccarelli E, Giusti M, Miola C, Potenzoni F, Sghedoni D, Camanni F, Giordano G. Effectiveness and tolerability of long term treatment with cabergoline, a new long-lasting ergoline derivative, in hyperprolactinemic patients. J Clin Endocrinol Metab 1989;69: 725-728.

57. Kleinberg DL, Boyd III AE, Wardlaw S, Frantz AG, George A, Bryan N, Hilal S, Greising J, Hamilton D, Seltzer T, Sommers CJ. Pergolide for the treatment of pituitary tumors secreting prolactin or growth hormone. N Eng J Med 1983;309: 704-709.

58. Mattox JH, Bernstein J, Buckman MT. Control of hyperprolactinemia with pergolide. Int J Fertil 1986;30: 39-43.

59. Kletsky OA, Borenstein R, Mileikowsky GN. Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia. Am J Obstet Gynecol 1986;154: 431-435.

60. Lamberts SWJ, Quik RF. A comparison of the efficacy and safety of pergolide and bromocriptine in the treatment of hyperprolactinemia. J Clin Endocrinol Metab 1991;72: 635-641.

61. Venetikou MS, Burrin JM, Woods CA, Yeo TH, Brownell J, Adams EF. Effects of two novel dopaminergic drugs, CV 205-502 and ECP 201-403 on prolactin and growth hormone secretion by human pituitary tumours in vitro. Acta Endocrinol 1987;116: 287-292.

62. Gaillard RC, Nordmann R, Petcher TJ, Brownell J. A novel octahydrobenzo[g]quinoline, CV 205-502, with potent dopamine agonist properties. In: Molinatti GM, Martini L. (eds) Endocrinol 1985 (1986) Elsevier Science Publ. BV. Amsterdam; p. 305-308

63. 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 test. J Clin Endocrinol Metab 1989;68: 329-335.

64. 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: 321-326.

65. Vance ML, Cragun C, Reimnitz C, Chang R, Rashef E, Blackwell R, Miller M, Molitch M. CV 205-502 treatment of hyperprolactinaemia. J Clin Endocrinol Metab 1989;68: 336-339.

66. Newman CB, Hurley AM, Kleinberg DL. Effect of CV 205-502 in hyperprolactinaemic patients intolerant of bromocriptine. Clin Endocrinol 1989;31: 391-400.

67. van 't Verlaat JW, Croughs RJ, Brownell J. Treatment of macroprolactinomas with a new non-ergot, long-acting dopaminergic drug, CV 205-502. Clin Endocrinol (Oxf) 1990;33: 619-624.

68. Barnett PS, Dawson JM, Butler J, Coskeran PB, Maccabe JJ, McGregor AM. CV 205-502, a new non-ergot dopamine agonist, reduces prolactinoma size in man. Clin Endocrinol (Oxf) 1990;33: 307-316.

69. Serri O, Beauregard H, Lesage J, Pedneault L, Comtois R, Jilwan N, Somma M, Vachon L, Brownell J. Long term treatment with CV 205-502 in patients with prolactin-secreting pituitary macroadenomas. J Clin Endocrinol Metab 1990;71: 682-687.

70. Duranteau L, Chanson P, Lavionne A, Horlait S, Lubetzki J, Kuhn JM. Effect of the new dopaminergic agonist CV 205-502 on plasma prolactin levels and the tumour size in bromocriptine-resistant prolactinomas. Clin Endocrinol (Oxf) 1991;34: 25-29.

71. Klibanski A, Zervas NT. Diagnosis and management of hormone-secreting pituitary adenomas. N Eng J Med 1991;324: 822-831.

72. Davis JR, Sheppard MC, Heath DA. Giant invasive prolactinomas: a case report and review of nine further cases. Q J Med 1990;74: 227-238.

73. Hildebrandt G. CSF rhinorrhea after dopamine agonist treatment for invasive macroprolactinoma. Surg Neurol 1990;34: 133.

74. Miller WL, Eberhardt NL. Structure and evolution of the growth hormone gene family. Endocr Rev 1983;4: 97-130.

75. Cooke NE, Ray J, Watson MA, Estes PA, Kuo BA, Liebhaber SA. Human growth hormone gene and its highly homologous growth hormone variant gene display different splicing patterns. J Clin Invest 1988;82: 270-275.

76. Thorner MO, Vance ML. Growth hormone. J Clin Invest 1988;82: 745-747.

77. Barinaga M, Bilezikjian LM, Vale WW, Rosenfeld MG, Evans RM. Independent effects of growth hormone releasing factor on growth hormone release and transcription. Nature 1985;314: 279-281.

78. Reichlin S. Somatostatin. N Eng J Med 1983;309: 1495-1501.

79. Frohman LA, Jansson JO. Growth hormone-releasing hormone. Endocr Rev 1986;7: 223-253.

80. Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL, Thorner MO. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 1987;64: 51-58.

81. Ho KY, Veldhuis JD, Johnson ML, Furlanetto R, Evans WS, Alberti KG, Thorner MO. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. J Clin Invest 1988;81: 968-975.

82. Van Wijk JJ. The somatomedins: biological actions and physiological control mechanisms. In: Li CH, ed. Growth factors, Vol. 12 of hormone proteins and peptides. New York Academic Press 1984;81: 125.

83. D'Ercole AJ, Stiles AD, Underwood LE. Tissue concentrations of somatomedin-C: further evidence of multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc Natl Acad Sci USA 1984;81: 935-939.

84. Fagin JA, Brown A, Melmed S. Regulation of pituitary insulin-like growth factor-I messenger ribonucleic acid levels in rats harbouring somatomammotrophic tumors: implications for growth hormone autoregulation. Endocrinol 1988;122: 2204-2210.

85. Yamashita S, Weiss M, Melmed S. Insulin-like growth factor-I regulates growth hormone secretion and messenger ribonucleic acid levels in human pituitary tumor cells. J Clin Endocrinol Metab 1986;63: 730-735.

86. Yamashita S, Melmed S. Insulin-like growth factor-I regulation of growth hormone gene transcription in primary rat pituitary cells. J Clin Invest 1987;79: 449-452.

87. Shea BT, Hammer RE, Brinster RL. Growth allometry of the organs in giant transgenic mice. Endocrinol 1987;123; 1924-1930.

88. Mathews LS, Hammer RE, Behringer RR, D'Ercole AJ, Bell GI, Brinster RL, Palmiter RD. Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology 1988;123: 2827-2833.

89. Alexander L, Appleton D, Hall R, Ross WM, Wilkinson R. Epidemiology of acromegaly in the newcastle region. Clin Endocrinol (Oxf) 1980;12: 71.

90. Phifer RF, Spicer SS, Hennigar GG. Histochemical reactivity and staining properties of functionally defined cell types in the human adenohypophysis. Am J Pathol 1973;73: 569-576.

91. Kovacs K, Horvath E. The pituitary. In: Silverberg SG. (ed) Principles and Practice of surgical pathology. John Wiley & Son. New York 1983.

92. Adelman LS. Pathology of pituitary adenomas. In: Post KD, Jackson IM, Reichlin S. (eds) The pituitary adenoma. Plenum, New York 1980; 47.

93. Kovacs K, Horvath E. Pathology of pituitary adenomas. Bull Los Angeles Neurol Soc 1977;42: 92-101.

94. Melmed S, Braunstein GD, Horvath E, Ezrin C, Kovacs K. Pathophysiology of acromegaly. Endocr Rev 1983;4: 271-290.

95. Horvath E, Kovacs K. Pathology of the pituitary gland. In: Ezrin C, Horvath E, Kaufman B, Kovacs K, Weiss MH. (eds) Pituitary Diseases. CRC Press, Boca Raton, Fl 1980: 1-83.

96. Kovacs K, Horvath E. Pituitary adenomas: pathologic aspects. In: Tolis G, Labrie F, Martin JB, Naftolin F. (eds) Clinical Neuroendocrinology. A Pathophysiological Approach. Raven Press, New York 1979: 367.

97. Kovacs K, Horvath E, Ezrin C. Pituitary adenomas. Pathol Annu 12 (part 2) 1977: 341-382.

98. Horvath E, Kovacs K, Singer W, Ezrin C, Kerenyi NA. Acidophil stem cell adenoma of the human pituitary. Arch Pathol Lab Med 1977;101: 594

99. Lloyd RV, Anagnostou D, Cano M, Barkan AL, Chandler WF. Analysis of mammosomatotrophic cells in normal and neoplastic human pituitary tissues by the reverse hemolytic placque assay and immunocytochemistry. J Clin Endocrinol Metab 1988;66: 1103-1110.

100. Hofland LJ. Functional heterogeneity among cell types in the normal pituitary gland and in human and rat pituitary tumors. Thesis. Erasmus University nov 1989.

101. Robert F, Pelletier G, Serri O, Hardy J. Mixed growth hormone and prolactin-secreting human pituitary adenomas: a pathologic, immunocytochemical, ultrastructural and immunoelectron microscopic study. Hum Pathol 1988;19: 1327-1334.

102. Horvath E, Kovacs K, Killinger DW, Smyth HS, Platts ME, Weiss MH, Ezrin C. Mammosomatotroph cell adenoma of the human pituitary (abstract). Proc Annu Meet Electron Microsc Soc Am 1980;38: 726.

103. Labhart A, Froesch ER, Landolt AM, Prader A, Zackmann M, Zapf J. The adenohypophysis. In: Labhart A. (ed). Clinical Endocrinology: Theory and practice (complete revised second edition) 1986: 135-136.

104. Mountcastle RB, Roof BS, Mayfield RK, Mordes DB, Biggs PJ, Rawe SE. Pituitary adenocarcinoma in an acromegalic patient: response to bromocriptine and pituitary testing: a review of the literature on 36 cases of pituitary carcinoma. Am J Med Sci 1989;298: 109-118.

105. Wright AD, Mclachlan MSF, Doyle FH, Fraser TR. Serum growth hormone levels and size of pituitary tumour in untreated acromegaly. Br Med J 1969;4: 582-584.

106. De Pablo F, Eastman RC, Roth J, Gorden P. Plasma prolactin in acromegaly before and after treatment. J Clin Endocrinol Metab 1981;53: 344-352.

107. Klijn JGM, Lamberts SWJ, de Jong FH, van Dongen KJ, Birkenhager JC. Interrelationships between tumoursize, age, plasma growth hormone and incidence of extra sellar extension in acromegalic patients. Acta Endocrinol (Copenh) 1980;95: 289-297.

108. Dons RF, Rieth KG, Gorden P, Roth J. Size and erosive features of the sella turcica in acromegaly as predictors of therapeutic response to supervoltage irradiation. Am J Med 1983;74: 69-72.

109. Nabarro. Acromegaly. Clin Endocrinol 1987;26: 481-512.

110. Smals AEM, Pieters GFFM, Smals AGH, Kloppenborg PWC. Sex difference in the relation between sellar volume and basal and GH-releasing hormone stimulated GH in acromegaly. Acta Endocrinol (Copenh) 1986;88: 224-236.

111. Lamberts SWJ, Uitterlinden P, Verleun T. Relationship between growth hormone and somatomedin-C levels in untreated acromegaly, after surgery and radiotherapy and during medical treatment with sandostatin (SMS 201-995). Eur J Clin Invest 1987;17: 354-359.

112. Gianella-Neto D, Wajchenberg BL, Mendonca BB, Almeida SF, Macchione M, Spencer EM. Criteria for the cure of acromegaly: comparison between basal growth hormone and somatomedin-C plasma concentrations in active and non- active acromegalic patients. J Endocrinol Invest 1988;11: 57-60.

113. Oppizzi G, Petroncini MM, Dallabonzana D, Cozzi R, Verde G, Chiondini PG, Liuzzi A. Relationship between somatomedin-C and growth hormone levels in acromegaly: basal and dynamic evaluation. J Clin Endocrinol Metab 1986;63: 1348-1353.

114. Barkan AL, Beitins IZ, Kelch RP. Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. J Clin Endocrinol Metab 1988;67: 69-73.

115. Wright AD, Hill DM, Lowy C, Fraser TR. Mortality in acromegaly. Q J Med 1970;39: 1-16.

116. Laws ER, Carpenter SM, Scheithauer BW, Randall RV. Long-term results of transsphenoidal surgery for the management of acromegaly. In: Robbins R, Melmed S, (eds). Acromegaly: a century of scientific and clinical progress. Plenum Press, New York. 1987: 241-248.

117. Serri O, Somma M, Comtois R, Rasio E, Beauregard H, Jilwan N, Hardy J. Acromegaly: biochemical assessment of cure after long-term follow-up of transsphenoidal selective adenectomy. J Clin Endocrinol Metab 1985;61: 1185-1189.

118. Ross DA, Wilson CB. Results of transsphenoidal microsurgery for growth hormone-secreting pituitary adenoma in a series of 214 patients. J Neurosurg 1988;68: 854-867.

119. Barkan AL. Acromegaly diagnosis and therapy. Endocrinol Metab Clin 1989;18: 277-310.

120. Eastman RC, Gorden P, Roth J. Conventional supervoltage irradiation is an effective treatment for acromegaly. J Clin Endocrinol Metab 1979;48: 931-940.

121. Kliman B, Kjellberg RN, Swisher B, Butler W. Long-term effects of proton-beam therapy for acromegaly. In: Robbins RJ, Melmed S, (eds) Acromegaly: a century of scientific and clinical progress. Plenum Press, New York 1987: 221-228.

122. Snyder PJ, Fowble BF, Schatz NJ, Savino PJ, Gennarelli TA. Hypopituitarism following radiation therapy of pituitary adenomas. Am J Med 1986;81: 457-462.

123. Liuzzi A, Chiondini PG, Botalla A, Cremascoli G, Muller EE, Silvestrini F. Decreased plasma growth hormone (GH)levels in acromegalics following CB 154 (2-Br-alpha-ergocriptine) administration. J Clin Endocrinol Metab 1974;38: 910-912.

124. Lamberts SWJ, Liuzzi A, Chiondini PG, Verde S, Klijn JG, Birkenhager JC. The value of plasma prolactin levels in the prediction of the responsiveness of growth hormone secretion to bromocriptine and TRH in acromegaly. Eur J Clin Invest 1982;12: 151-155.

125. Wass JA, Thorner MO, Morris DV, Rees LH, Mason AS, Jones AE, Besser GM. Long-term treatment of acromegaly with bromocriptine. Br Med J 1977;1: 875-878.

126. Chiodini PG, Cozzi R, Dallabonzana D, Oppizzi G, Verde G, Petroncini M, Liuzzi A, del Pozo E. Medical treatment of acromegaly with SMS 201-995, a somatostatin analogon: a comparison with bromocriptine. J Clin Endocrinol Metab 1987;64: 447-453.

127. Lamberts SWJ, Zweens M, Verschoor L, del Pozo E. A comparison among the growth hormone-lowering effects in acromegaly os the somatostatin analogon SMS 201-995, bromocriptine, and the combination of both. J Clin Endocrinol Metab 1986;63: 16-19.

128. Halse J, Harris AG, Kvistborg A, Kjartansson O, Hanssen E, Smiseth O, Djosland O, Hass G, Jervell J. A randomized study of SMS 201-995 versus bromocriptine treatment in acromegaly: clinical and biochemical effects. J Clin Endocrinol Metab 1990;70: 1254-1261.

129. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, Petcher TJ. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci 1982;31: 1133-1140.

130. Lamberts SWJ, Uitterlinden P, Verschoor L, van Dongen KJ, del Pozo E. Long-term treatment of acromegaly with the somatostatin analogue SMS 201-995. N Eng J Med 1985;313: 1576-1580.

131. Lamberts SWJ. The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of its analogs in the treatment of human pituitary tumors. Endocr Rev 1988;9: 417-436.

132. Plewe G, Beyer J, Krause U, Neufeld M, del Pozo E. Long-acting and selective suppression of growth hormone secretion by somatostatin analogue SMS 201-995 in acromegaly. Lancet 1984;2: 782-784.

133. Barkan AL, Lloyd RV, Chandler WF, Hatfield MK, Gebarski SS, Kelch RP, Beitins IZ. Preoperative treatment of acromegaly with long-acting somatostatin analog SMS 201-995: shrinkage of invasive pituitary macroadenomas and improved surgical remission rate. J Clin Endocrinol Metab 1988;67: 1040-1048.

134. Barakat S, Melmed S. Reversible shrinkage of a growth hormone-secreting pituitary adenoma by a long-acting somatostatin analogue, octreotide. Arch Intern Med 1989;149: 1443-1445.

135. Lamberts SW, Uitterlinden P, Schuijff PC, Klijn JG. Therapy of acromegaly with sandostatin: the predictive value of an acute test, the value of serum somatomedin-C measurements in dose adjustment and the definition of a biochemical 'cure'. Clin Endocrinol (Oxf) 1988;29: 411-420.

136. Comi RJ, Gorden P. The response of serum growth hormone levels to the long-acting somatostatin analog SMS 201-995 in acromegaly. J Clin Endocrinol Metab 1987;64: 37-42.

137. Barnard LB, Grantham WG, Lamberton P, O'Dorisio TM, Jackson IMD. Treatment of resistant acromegaly with a long-acting somatostatin analogue (SMS 201-995). Ann Intern Med 1986;105: 856-861.

138. Ho KY, Weissberger AJ, Marbach P, Lazarus L. Therapeutic efficacy of the somatostatin analog SMS 201-995 (octreotide) in acromegaly: effects of dose and frequency and long-term safety. Ann Intern Med 1990;112: 173-181.

139. van Liessum PA, Hopman WPM, Pieters GFFM, et al. Postprandial gallbladder motility during long term treatment with the long-acting somatostatin analog SMS 201-995 in acromegaly. J Clin Endocrinol Metab 1989;69: 557-562.

140. Christensen SE, Weeke J, Orskov H, et al. Continuous subcutaneous pump infusion of somatostatin analogue SMS 201-995 versus subcutaneous injection schedule in acromegalic patients. Clin Endocrinol (Oxf) 1987;27: 297-306.

141. Wang C, Lam KSL, Arceo E, Chan FL. Comparison of the effectiveness of 2-hourly versus 8-hourly injections of a somatostatin analog (SMS 201-995) in the treatment of acromegaly. J Clin Endocrinol Metab 1989;69: 670-677.

142. Lamberts SWJ, Uitterlinden P, delPozo E. SMS 201-995 induces a continuous decline in circulating groth hormone and somatomedin-C levels during therapy of acromegalic patients for over two years. J Clin Endocrinol Metab 1978;65: 703-710.

143. Barkan A, Lloyd RV, Chandler WF, et al. Treatment of acromegaly with SMS 201-995 (sandostatin): clinical, biochemical and morphological study. In: Lamberts SWJ. (ed) Sandostatin in the treatment of acromegaly. New York, Springer, 1988; 103-108.

144. Harris AG, Prestele H, Herold K, et al. Long-term efficacy of sandostatin (SMS 201-995, octreotide) in 178 acromegalic patients: results from the International Multicentre Acromegaly Study Group, In: Lamberts SWJ. (ed) Sandostatin in the treatment of acromegaly. New York, Springer, 1988; 117-125.

145. Barkan AL, Lloyd RV, Chandler WF, et al. Preoperative treatment of acromegaly with long-acting somatostatin analog SMS 201-995: shrinkage of invasive pituitary macroadenomas and improved surgical remission rate. J Clin Endocrinol Metab 1988;67: 1040-1048.

146. Liddle GW. Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab 1960;20: 1539-1560.

147. Crapo L. Cushing's syndrome: a review of diagnostic tests. Metabolism 1979;28: 955-977.

148. Contreras LN, Hane S, Tyrrell JB. Urinary cortisol in the assessment of pituitary-adrenal function: utility of 24-hours and spot determinations. J Clin Endocrinol Metab 1986;62: 965-969.

149. Laudat MH, Billaud L, Thomopoulos P, Vera O, Yllia A, Luton JP. Evening urinary free corticoids: a screening test in Cushing's syndrome and incidentally discovered adrenal tumours. Acta Endocrinol (Copenh) 1988;119: 459-464.

150. Phillipou G. Investigation of urinary steroid profiles as a diagnostic method in Cushing's syndrome. Clin Endocrinol (Oxf) 1982;16: 433-439.

151. Zadik Z, de Lacerda L, de Camargo LA, Hamilton BP, Migeon CJ, Kowarski AA. A comparative study of urinary 17-hydroxycorticosteroids, urine free cortisol, and the integrated concentration of plasma cortisol. J Clin Endocrinol Metab 1980;51: 1099-1101.

152. Kaye TB, Crapo L. The Cushing's syndrome: An update on diagnostic tests. Ann Int Med 1990;112: 434-444.

153. Raff H, Findling JW. A new immunoradiometric assay for corticotrophin evaluated in normal subjects and patients with Cushing's syndrome. J Clin Endocrinol Metab 1989;35: 596-600.

154. Biemond P, de Jong FH, Lamberts SWJ. Continuous dexamethasone infusion for seven hours in patients with the Cushing's syndrome. A superior differential diagnostic test. Ann Intern Med 1990;112: 738-742.

155. Chrousos GP, Schulte HM, Oldfield EH, Gold PW, Cutler GB Jr, Loriaux DL. The corticotrophin-releasing factor stimulation test. An aid in the evaluation of patients with Cushing's syndrome. N Eng J Med 1984;310: 622-626.

156. Nakahara M, Shibasaki T, Shizume K, Kiyosawa Y, Odagiri E, Suda T, Yamaguchi H, Tsushima T, Demura H, Maeda T. Corticotrophin-releasing factor test in normal subjects and patients with hypothalamic-pituitary-adrenal disorders. J Clin Endocrinol Metab 1983;57: 963-968.

157. Nieman LK, Chrousos GP, Oldfield EH, Avgerinos PC, Cutler GB Jr, Loriaux DL. The ovine corticotropin-releasing hormone stimulation test and the dexamethasone suppression test in the difficult diagnosis of Cushing's syndrome. Ann Int Med 1986;105: 862-867.

158. Hermus AR, Pieters GF, Pesman GJ, Smals AG, Benraad TJ, Kloppenborg PW. The corticotropin-releasing-hormone test versus the high-dose dexamethasone test in the differential diagnosis of Cushing's syndrome. Lancet 1986;2: 540-544.

159. Mampalam TJ, Tyrrell JB, Wilson CB. Transsphenoidal microsurgery for Cushing disease: a report of 216 cases. Ann Int Med 1988;109: 487-493.

160. Pojunas KW, Daniels DL, Williams AL, Thorsen MK, Haughton VM. Pituitary and adrenal CT of Cushing's syndrome. Am J Rad 1986;146: 1235-1238.

161. Teasdale E, Teasdale G, Mohsen F, MacPherson P. High-resolution computed tomography in pituitary microadenoma: is seeing believing? Clin Radiol 1986;37: 227-232.

162. Saris SC, Patronas NJ, Doppman JL, Loriaux DL, Cutler GB Jr, Nieman LK, Chrousos GP, Oldfield EH. Cushing's syndrome: pituitary CT scanning. Radiology 1987;162: 775-777.

163. Marcovitz S, Wee R, Chan J, Hardy J. The diagnostic accuracy of preoperative CT scanning in the evaluation ofpituitary ACTH-secreting adenomas. Am J Rad 1987;149: 803-806.

164. Doppman JL, Frank JA, Dwyer AJ, Oldfield EH, Miller DL, Nieman LK< Chrousos GP, Cutler GB Jr, Loriaux DL. Gadolinium DTPA enhanced MR imaging of ACTH-secreting microadenomas of the pituitary gland. J Comp Assist Tomogr 1988;12: 728-735.

165. Dwyer AJ, Frank JA, Doppman JL, Oldfield EH, Hickey AM, Cutler GB, Loriaux DL, Schiable TF. Pituitary adenomas in patients with Cushing's disease: initial experience with Gd-DTPA-enhanced MR imaging. Radiology 1987;163: 421-426.

166. Peck WW, Dillon WP, Norman D, Newton TH, Wilson CB. High-resolution MR imaging of pituitary microadenomas at 1.5 T: experience with Cushing's disease. Am J Rad 1989;152: 145-151.

167. Fig LM, Gross MD, Shapiro B, Ehrmann DA, Freitas JE, Schteingart DE, Glazer GM, Francis IR. Adrenal localization in the adrenocorticotropic hormone-independent Cushing's syndrome. Ann Int Med 1988;109: 547-553.

168. Oldfield EH, Chrousos GP, Schulte HM, Schaaf M, McKeever PE, Krudy AG, Cutler GB Jr, Loriaux DL, Doppman JL. Preoperative lateralization of ACTH-secreting pituitary microadenomas by bilateral and simultaneous inferior petrosal venous sinus sampling. N Eng J Med 1985;312: 100-103.

169. Findling JW, Kehoe ME, Shaker JL, Raff H. Routine inferior petrosal sinus sampling in the differential diagnosis of adrenocorticotropin(ACTH)-dependent Cushing's syndrome: early recognition of the occult ectopic ACTH syndrome. J Clin Endocrinol Metab 1991;73: 408-413.

170. Grant SJ, Stiel JN, Sorby WA, Henniker AJ. Venous ACTH sampling in Cushing's syndrome. Med J Aust 1983;1: 336-337.

171. Snow RB, Patterson RH Jr, Horwith M, Saint Louis L, Fraser RA. Usefulness of preoperative inferior petrosal vein sampling in Cushing's disease. Surg Neurol 1988;29: 17-21.

172. Schulte HM, Allollio B, Gunther RW, Benker G, Windeck R, Winkelmann W, Reinwein D. Bilateral and simultaneous sinus petrosus inferior catheterization in patients with Cushing's syndrome: plasma-immunoreactive-ACTH-concentrations before and after administration of CRF. Horm Metab Res Suppl 1987;16: 66-67.

173. Landolt AM, Valavanis A, Girard J, Eberle AN. Corticotrophin-releasing factor-test used with bilateral, simultaneous inferior petrosal sinus blood-sampling for the diagnosis of pituitary-dependent Cushing's disease. Clin Endocrinol (Oxf) 1986;25: 687-696.

174. Miller DL, Doppman JL. Petrosal sinus sampling: technique and rationale. Radiology 1991;178: 37-47.

175. Melby JC. Therapy of Cushing disease: a consensus for pituitary microsurgery. Ann Int Med 1988;109: 445-446.

176. Bigos ST, Somma M, Rasio E, Eastman RC, Lanthier A, Johnston HH, Hardy J. Cushing's disease: management by transsphenoidal pituitary microsurgery. J Clin Endocrinol Metab 1980;50: 348-354.

177. Boggan JE, Tyrrell JB, Wilson CB. Transsphenoidal microsurgical management of Cushing's disease: report of 100 cases. J Neurosurg 1983;59: 195-200.

178. Salassa RM, Laws ER Jr, Carpenter PC, Northcutt RC. Transsphenoidal removal of pituitary microadenoma in Cushing's disease. Mayo Clin Proc 1978;53: 24-28.

179. Melby JC. Therapy of Cushing's disease: a consensus for pituitary microsurgery. Ann Int Med 1988;109: 445-446.

180. Orth DN, Liddle GW. Results of treatment in 108 patients with Cushing's syndrome. N Eng J Med 1971;285: 243-247.

181. Tran LM, Blount L, Horton D, Sadeghi A, Parker RG. Radiation therapy of pituitary tumors: results in 95 cases. Am J Clin Oncol 1991;14: 25-29.

182. Linfoot JA. Heavy ion therapy: Alpha particle therapy of pituitary tumors. In: Linfoot JA (ed): Recent advances in the diagnosis and treatment of pituitary tumors. New York, Raven Press 1979: 245-267.

183. Kjellberg RN, Kliman B. Lifetime effectiveness- a system of therapy for pituitary adenomas, emphasing Bragg peak proton hypophysectomy. In: Linfoot JA (ed): recent advances in the diagnosis and treatment of pituitary tumors. New York, Raven Press 1979: 269-288.

184. Pont A, Graybill JR, Craven PC, Galgiani JN, Dismukes WE, Reitz RE, Stevens DA. High-dose ketoconazol therapy and adrenal and testical function in humans. Arch Int Med 1984;144: 2150-2153.

185. Bruynseels J, De Coster R, Van Rooy P, Wouters W, Coene MC, Snoeck E, Raeymakers A, Freyne E, Sanz G, Vanden Bussche G, et al. R 75251, a new inhibitor of steroid biosynthesis. Prostate 1990;16: 345-357.

186. Trachtenberg J, Zadra J. Steroid synthesis inhibition by ketoconazol: sites of action. Clin Invest Med 1988;11: 1-5.

187. Tabarin A, Navarranne A, Guerin J, Corcuff JB, Parneix M, Roger P. Use of ketoconazol in the treatment of Cushing's disease and ectopic ACTH syndrome. Clin Endocrinol (Oxf) 1991;34: 63-69.

188. Khanderia U. Use of ketoconazol in the treatment of Cushing's syndrome. Clin Pharm 1991;10: 12-13.

189. Sonino N. The use of ketoconazole as an inhibitor of steroid production. N Eng J Med 1987;317: 812-818.

190. Hoffman DM, Brigham B. The use of ketoconazol in ectopic adrenocorticoptopic hormone syndrome. Cancer 1991;1: 1447-1449.

191. Verhelst JA, Druwe P, van Erps P, Denis LJ, Mahler C. Use of ketoconazol in the treatment of a virilizing adrenocortical carcinoma. Act Endocrinol (Copenh) 1989;121: 229-234.

192. Jimenez Reina L, Leal-Cerro A, Garcia J, Garcia-Luna PP, Astorga R, Bernal G. In vitro effects of ketoconazol on corticotrope cell morphology and ACTH secretion of two pituitary adenomas from patients with Nelson's syndrome. Act Endocrinol (Copenh) 1989;121: 185-190.

193. Sinnaeva LJ, Becks GP. Preoperative ketoconazole therapy for adrenocortical carcinoma. Can Med Assoc J 1989;15: 131-133.

194. Terzolo M, Panarelli M, Piovesan A, Torta M, Paccotti P, Angeli A. Ketoconazole treatment in Cushing's disease. Effect on the circadian profile of plasma ACTH and cortisol. J Endocrinol Invest 1988;11: 717-721.

195. Farwell AP, Devlin JT, Stewart JA. Total suppression of cortisol excretion by ketoconazole in the therapy of the ectopic adrenocorticotropic hormone syndrome. Am J Med 1988;84: 1063-1066.

196. Lamberts SW, Bons EG, Bruining HA, de Jong FH. Differential effects of the imidazole derivatives etomidate, ketoconazole and miconazole and of metyrapone on the secretion of cortisol and its precursors by human adrenocortical cells. J Pharmacol Exp Ther 1987;240: 259-264.

197. Child DF, Durke CW, Burley DM et al. Drug control of Cushing's syndrome: combined aminoglutethimide and metyrapone therapy. Acta Endocrinol (Copenh) 1979;82: 330-341.

198. Shaw MA, Nicholls PJ, Smith HJ. Aminogluthetimide and ketoconazole: historical perspectives and future prospectives. J Steroid Biochem 1988;31: 137-146.

199. Schteingart DE, Cash R, Conn JW. Aminogluthetimide and metastatic adrenal cancer. Maintained reversal (six months) if Cushing's syndrome. JAMA 1966;198: 1007-1011.

200. Schteingart DE, Conn JW. Effects of aminogluthetimide upon adrenal function and cortisol metabolism in Cushing's syndrome. J Clin Endocrinol Metab 1967;27: 1657-1661.

201. Howlett TA, Drury PL, Perry L, Doniach I, Rees LH, Besser GM. Diagnosis and management of ACTH-dependent Cushing's syndrome: comparison of the features in ectopic and pituitary ACTH production. Clin Endocrinol 1986;24: 699-713.

202. Jeffcoate WJ, Rees JH, Tomlin S. Metyrapone in long-term management of Cushing's disease. Br Med J 1988;296: 227-230.

203. Kramlinger KG, Peterson GC, Watson PK , Leonard LL. Metyrapone for depression and delirium secondary to Cushing's syndrome. Psychosomatics 1985;26: 67-71.

204. Stein CA, La Rocca RV, Thomas R, McAtee N, Myers CE. Suramin: an anticancer drug with a unique mechanism of action. J Clin Oncol 1989;7: 499-508.

205. Ashby H, Di Mattina M, Linehan WM, Robertson CN, Queenan JT, Albertson BD. The inhibition of human adrenal steroidogenic activities by suramin. J Clin Endocrinol Metab 1989;68: 505-508.

206. La Rocca RV, Stein CA, Danesi R, Jamis-Dow CA, Weiss GH, Myers CE. Suramin in adrenal cancer: modulation of steroid hormone production, cytotoxicity in vitro, and clinical antitumor effect. J Clin Endocrinol Metab 1990;71: 497-504.

207. Marzouk HF, Zuyderwijk J, Uitterlinden P, de Jong FH, Lamberts SW. Suramin prevents ACTH-stimulated corticosterone release by dispersed adrenocortical cells. Endocrinol 1990;126: 666-668.

208. La Rocca RV, Stein CA, Danesi R, Myers CE. Suramin, a novel antitumor compound. J Ster Biochem Mol Biol 1990;37: 893-898.

209. Marzouk HF, Hofland LJ, den Holder FH, van Koetsveld PM, Steenbergen J, Zuiderwijk J, Abou-Hashim EM, el-Kannishy MH, de Jong FH, Lamberts SW. Effects of suramin on hormone release by cultured rat anterior pituitary cells. Mol Cell Endocrinol 1990;72: 95-102.

210. Philibert D, Moguilewski M, Mary I. Pharmacological profile of RU 486 in animals. In: Baulieu E-E, Segal SJ, (eds) The antiprogestin steroid RU 486 and human fertility control. New York Plenum Press 1985: 49-68.

211. Philibert D, Deraedt R, Teutsch G. RU 486: a potent antiglucocorticoid in vivo. [Abstract] International Congress of Pharmacology, Tokyo, Japan. 1981: 1463.

212. Proux-Ferland L, Cote J, Philibert D, Deraedt R. Potent antiglucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat. [Abstract]. J Steroid Biochem 1982;17: xvii

213. Teutsch G. Analogues of RU 486 for the mapping of the progestin receptor: synthetic and structural aspects. In: Baulieu E-E, Segal SJ, (eds). The antiprogestin steroid RU 486 and human fertility control. New York Plenum Press 1985: 27-47.

214. Heikinheimo O, Kontula K, Croxatto H, Spitz I, Luukainen T, Lahteenmakis P. Plasma concentrations and receptor binding of RU 486 and its metabolites in humans. J Steroid Biochem 1987;26: 279-284.

215. Nieman L, Choate T, Chrousos G, Healy DL, Morin M, Renquist D, Merriam GR, Spitz IM, Bardin CW, Baulieu EE. The progesterone antagonist RU 486. A potential new contraceptive agent. N Eng J Med 1987;316: 187-191.

216. Raux-Demay MC, Pierret T, Bouvier d'Yvoire M, Bertagna X, Girard F. Transient inhibition of RU 486 antiglucocorticoid action by dexamethasone. J Clin Endocrinol Metab 1990;70: 230-233.

217. Bertagna X, Bertagna C, Laudat NH, Husson JM, Girard F, Luton ZP. Pituitary-adrenal response to the antiglucocorticoid action of RU 486 in Cushing's syndrome. J Clin Endocrinol Metab 1986;63: 639-624.

218. Nieman LK, Chrousos GP, Kellner C, Spitz IM, Nisula BC, Cutler GB, Merriam GR, Bardin CW, Loriaux DL. Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU 486. J Clin Endocrinol Metab 1985;61: 536-540.

219. Nieman LK, Udelsman R, Loriaux DL, Chrousos CP. Antiglucocorticoids: basic and clinical studies. In: D'Agata R, Chrousos GP, (eds) Recent Advances in adrenal regulation and function. New York, Raven Press 1987: 235-258.

220. McComb DJ, Ryan N, Horvath E, Kovacs K. Subclinical adenomas of the human pituitary. Arch Pathol Lab Med 1983;107: 488-494.

221. Wilson CB. A decade of pituitary microsurgery; The Herbert Olivecrona lecture. J Neurosurg 1984;61: 817-822.

222. Smallridge RC, Smith CE. Hyperthyroidism due to thyrotropin-secreting pituitary tumors: diagnostic and therapeutic consideration. Arch Inter Med 1983;143: 503-507.

223. Afrasiabi A, Valenta L, Gwinup G. A TSH-secreting pituitary tumour causing hyperthyroidism: Presentation of a case and review of the literature. Act Endocrinol (Copenh) 1979;92: 448-459.

224. Carlson HE, Linfoot JA, Braunstein GD, Kovacs K, Young RT. Hyperthyroidism and acromegaly due to a thyrotropin- and growth hormone-secreting pituitary tumor: Lack of hormonal response to bromocriptine. Am J Med 1983;74: 915-919.

225. Beck-Peccoz P, Piscitelli G, Amr S, Ballabio M, Bassetti M, Giannattasio G, Spada A, Nissim M, Weintraub BD, Faglia G. Endocrine, biochemical, and morphological studies of a pituitary adenoma secreting growth hormone, thyrotropin (TSH), and alpha-subunit: evidence for secretion of TSH with increased bioactivity. J Clin Endocrinol Metab 1986;62: 704-711.

226. 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: 1254-1259.

227. Nelson AT Jr, Tucker HS Jr, Becker DP. Residual anterior pituitary function following transsphenoidal resection of pituitary macroadenomas. J Neurosurg 1984;61: 577-580.

228. Ebersold MJ, Quast LM, Laws ER Jr, Scheithauer B, Randall RV. Long-term results in transsphenoidal removal of nonfunctioning pituitary adenomas. J Neurosurg 1986;64: 713-719.

229. Smallridge RC. Thyrotropin-secreting pituitary tumors. Endocrinol Metab Clin North Am 1987;16: 765-792.

230. Smallridge Endocrinol Metab Clin North Am 1987;16: 765

231. Chanson P, Orgiazzi J, Derome PJ, Bression D, Jedynak CP, Trouillas J, Legentil P, Racadot J, Peillon F. Paradoxal responses of thyrotropin to L-dopa and presence of dopaminergic receptors in a thyrotropin-secreting pituitary adenoma. J Clin Endocrinol Metab 1984;542-546.

232. Wollesen F, Andersen T, Karle A. Size-reduction of extrasellar pituitary tumors during bromocriptine treatment. Ann Int Med 1982;96: 281-285.

233. Sriwatanakul K, McCormick K, Woolf P. Thyrotropin (TSH)-induced hyperthyroidism: Response of TSH to dopamine and its agonists. J Clin Endocrinol Metab 1984;58: 255-259.

234. Ikuyama S, Nawata H, Kato K, Karashima T, Ibayashi H, Nakagaki H. Specific somatostatin receptors on human pituitary adenoma cell membranes. J Clin Endocrinol Metab 1985;61: 666-670.

235. Reubi JC, Heitz PU, Landolt AM. Visualization of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: Evidence for different tumor subclasses. J Clin Endocrinol Metab 1987;65: 65-69.

236. Comi JR, Gesundheit N, Murray L, Gorden P, Weintraub BD. Response of thyrotropin-secreting pituitary adenomas to a long-acting somatostatin analogue. N Eng J Med 1987;317: 12-15.

237. Ridgway EC, Klibanski A, Martorana MA, Milbury P, Kieffer JD, Chin WW. The effect of somatostatin on the release of thyrotropin and its subunits from bovine anterior pituitary cells in vitro. Endocrinology 1983;112: 1937-1941.

238. Siler TM, Yen SSC, Vale W, et al. Inhibition by somatostatin on the release of TSH induced in man by thyrotropin-releasing factor. J Clin Endocrinol Metab 1974;38: 742-747.

239. Sassolas G, Serusclat P, Claustrat B, Trouillas J, Merabet S, Cohen R, Souquet JC. Plasma alpha-subunit levels during the treatment of pituitary adenomas with the somatostatin analogue (SMS 201-995). Horm Res 1988;29: 124-128.

240. Wemeau JL, Dewailly D, Leroy R, D'Herbomez M, Mazzuca M, Decoulx M, Jaquet P. Long term treatment with the somatostatin analogue SMS 201-995 in a patient with a thyrotropin- and growth hormone-secreting pituitary adenoma. J Clin Endocrinol Metab 1988;66: 636-639.

241. Kwekkeboom, deJong 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: 1128-1135.

242. 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: 1011-1014.

243. Black PM, Hsu DW, Klibanski A, Kliman B, Jameson JL, Ridgway EC, Hedley-Whyte ET, Zervas NT. Production of glycoprotein hormone subunits in clinically nonfunctioning pituitary tumors. J Neurosurg 1987;66: 244-248.

244. Jameson JL, Lindell CM, Hsu DW, Habener JF, Ridgway EC. Expression of chorionic gonadotropin beta-subunit like messenger ribonucleic acid in an alpha-subunit secreting pituitary adenoma. J Clin Endocrinol Metab 1986;62: 1271-1274.

245. 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 LHRH and TRH. Clin Endocrinol 1983;19: 325-329.

246. 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: 937-941.

247. Peterson RE, Kourides IA, Horwoth 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: 692-698.

248. 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: 1169-1175.

249. Johnston DG, Hall K, McGregor A, Ross WM, Kendall-Taylor P, Hall R. Bromocriptine therapy for "nonfunctioning" pituitary tumors. Am J Med 1981;71: 1059-1061.

250. Ebersold MJ, Quast LM, Laws ER Jr, Scheithauer B, Randall RV. Long-term results in transsphenoidal removal of nonfunctioning pituitary adenomas. J Neurosurg 1986;64: 713-718.

251. Nelson AT, Tucker HSG, Becker DP. Residual pituitary function following transsphenoidal resection of pituitary macroadenomas. J Neurosurg 1984;61: 577-581.

252. Wilson CB, Dempsey LC, Transsphenoidal microsurgical removal of 250 pituitary adenomas. J Neurosurg 1978;48: 13-22.

253. Klibanski A, Ridgway EC, Zervas NT. Pure alpha subunit-secreting pituitary tumors. J Neurosurg 1983;59: 585-589.

254. Wass JAH, Williams J, Charlesworth 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.

255. 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.

256. 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: 679-686.

257. Zarate A, Moran C, Kleriga E, Loyo M, Gonzalez-Angulo A, Aquilar-Parada E. Bromocriptine therapy as pre-operative adjunct of non-functioning pituitary macro-adenomas. Acta Endocrinol (Copenh) 1985;108: 445-450.

CHAPTER 2.

AIMS AND SCOPE OF THE THESIS:

From the survey of the literature in the preceding chapter, especially dealing with prolactinomas, acromegaly, and patients with Cushing's syndrome, several questions arise. These questions are listed below with a reference to the chapter where these questions will be discussed in detail.

1. Is it possible to predict the sensitivity of tumorous GH release in acromegalic patients to treatment with either bromocriptine or octreotide? We investigated, whether sex, age, tumor size or other variables can be used to predict this sensitivity **(Chapter 3).**

2. While both bromocriptine and octreotide have been proven to be usefull in the treatment of acromegaly, one wonders whether long-term treatment with a combination of these drugs might be useful in clinical practice too.
(Chapter 4)

3. Octreotide therapy results in pituitary tumor shrinkage in about half of acromegalic patients. However, the degree of shrinkage is often non-impressive. Which factors play a role in the mechanism of octreotide-mediated tumor shrinkage? **(Chapter 5)**

4. After transsphenoidal surgery, GH levels of less than 5 μg/L are reached in only 60% of patients (and in less than 30% of the patients with extrasellar extension of the adenoma). Is it possible to improve surgical results by pretreatment of acromegalic patients with octreotide for several months?
(Chapter 6)

5. The occurence of side-effects, as well as the need for two or three daily doses of bromocriptine remain important problems in the long-term treatment of prolactinomas. Can new dopaminergic drugs, like CV 205-502, be used in macroprolactinoma patients, as well as in patients intolerant to bromocriptine?
(Chapter 7)

6. Is there an explanation for the fact that some patients with huge adenohypophyseal tumors, presenting themselves primarily with pharyngeal problems due to the extension of the adenoma, do have normal or almost normal pituitary function?
(Chapter 8)

7. In patients with malignant Cushing's syndrome, psychiatric disturbances can be severe. Is it possible to improve the mental status in these patients, using the glucocorticoid receptor blocking drug mifepristone? **(Chapter 9)**

CHAPTER 3.

THE SENSITIVITY OF GROWTH HORMONE SECRETION TO MEDICAL TREATMENT IN ACROMEGALIC PATIENTS: INFLUENCE OF AGE AND SEX.

A.J. van der Lely [1], A.G. Harris [2], S.W.J. Lamberts [1].

1: Department of Medicine, Erasmus University, Rotterdam, The Netherlands.

2: Division of Clinical Pharmacology, Cedars-Sinai Medical Centre, UCLA School of Medicine, Los Angeles, California, USA.

Accepted for publication in Clinical Endocrinology.

Summary

OBJECTIVE: To investigate the relationship between age, sex, pituitary tumour volume, serum GH, PRL and IGF-I levels with the responsiveness of GH to TRH, bromocriptine and octreotide in patients with acromegaly.

DESIGN: Retrospective study. Correlations were determined between all variables using univariate regression analysis.

PATIENTS: 100 previously untreated acromegalic patients. 60 males (age 23-76;mean 49 yrs) and 40 females (age 25-83;mean 51 yrs).

MEASUREMENTS: Tumour volumes, fasting morning samples of GH, PRL and IGF-I, mean 24 hr GH levels and the acute GH responses to TRH, bromocriptine and octreotide.

RESULTS: Tumour size was related to serum and mean 24 hour GH levels, but not to IGF-I. Circulating IGF-I and GH levels were only related for the group of patients whose fasting and unsuppressed GH level was 40 μg/L or less were taken into consideration. Older patients tended to have lower circulating GH and IGF-I levels. There was a close homogeneity in the responsiveness of tumorous GH secretion to TRH, bromocriptine and octreotide. An elevated serum PRL level predicted a stronger inhibitory reaction of bromocriptine on GH. The sensitivity of GH release to octreotide was highest in elderly (especially male) acromegalics, as well as in patients with lower IGF-I levels.

CONCLUSIONS: Hormone secretion by GH secreting pituitary tumours, as well as circulating IGF-I levels tend to be lower in elderly patients. These tumours are more sensitive to octreotide, especially in elderly male patients. This suggest that octreotide might be used especially successful as a primary medical therapy in elderly (male) acromegalics.

INTRODUCTION:

Medical treatment of acromegaly has changed in recent years. Bromocriptine was reported to be only effective in a minority of patients (Wass *et al.*, 1977; Lindholm *et al.*, 1981; Barkan, 1989). The recent introduction of the somatostatin analog, octreotide, in the chronic treatment of acromegalic patients turned out to be more beneficial in the majority of them (Lamberts *et al.*, 1985, 1986, 1988). A comparative study of the effect of an acute, single administration of octreotide and bromocriptine on GH secretion in acromegalics demonstrated a more powerful inhibitory effect of the somatostatin analog in most patients, while GH release was inhibited by bromocriptine to a similar, or in exceptional cases greater extent in a minority of them (Lamberts *et al.*, 1986; Chiodini *et al.*, 1987). The role of medical therapy as a primary treatment of acromegaly is still under discussion (Melmed, 1990). Neither bromocriptine, nor octreotide have been recommended yet as a primary therapy for this disease. There are no studies available involving a large number of acromegalics determining whether sex, age, tumour size or other variables might play a role in predicting the sensitivity of tumorous hormone release to medical treatment.

In the present study we investigated the relationship between age, sex, pituitary tumour volume, baseline serum GH, PRL and IGF-I levels as well as the acute responsiveness of GH secretion to TRH, bromocriptine and octreotide in 100 consecutive, previously untreated patients.

PATIENTS, METHODS AND MATERIALS

In this study 100 previously untreated acromegalic patients were investigated: 60 males (age 23-76;mean 49 yrs) and 40 females (age 25-83;mean 51 yrs). Tumour volumes, fasting morning GH, PRL and IGF-I levels, mean 24 hr GH levels (of 24 samples in 24 hours) and the reactions of GH release to TRH, bromocriptine and octreotide were investigated. Oral consent for all investigations was obtained from all patients. All of the patients had an elevated IGF-I level and all had a demonstrable tumour. Fifty of the patients have been reported earlier in a study to investigate the effect of octreotide, bromocriptine and a combination of both (Wagenaar *et al.*, 1991) Because some patients had been extensively investigated elsewhere before referral to our hospital, not all parameters are available for all patients (see results Table I). All the parameters used, however, were gathered in our laboratory.

Tumour volumes were measured by multiplying the maximal height, width and

depth of the tumour images on CT scan examination (Philips Tomoscan 200; Thickness of slices 2 mm).

Early morning fasting serum GH, PRL and IGF-I concentrations were measured with specific radioimmunoassays. GH was measured with a commercial kit supplied by Medgenix Diagnostics, Brussels, Belgium (inter-assay variation 6.4%; intra-assay variation 6.2%). PRL by Medgenix Diagnostics, Brussels, Belgium (inter-assay variation 6.8%; intra-assay variation 6.4%). IGF-I by Medgenix Diagnostics, Brussels, Belgium (inter-assay variation 9.0%; intra-assay variation 5.2%).The mean 24 hr GH level was calculated from 24 GH determinations taken hourly during a 24 hr period ("control day"). During the TRH test (200 μg intravenously; Hoechst, Amsterdam, the Netherlands), the maximal GH level reached 10,20,30,60 and 120 minutes after its administration was expressed as a percentage of the baseline level. Bromocriptine
(2.5 mg) was given orally at 8.00 am and GH levels were measured hourly till 8.00 pm. The area under the curve (AUC) of these 13 GH measurements was expressed as a percentage of the AUC determined on the control day. Octreotide (50 μg) was administered subcutaneously at 8.30 am at least two days later and GH levels were followed hourly until 9.00 pm. The AUC of these 14 serum GH determinations was expressed as a percentage of the curve on the control day. We used the AUC of the GH concentrations in stead of the nadir, because to our opinion this method reflects the amount of suppression better than the nadir would do.

Statistics: As the data were normally distributed, correlations were determined between all variables using univariate linear regression analysis and were considered significant when p values were less than 0.05.

RESULTS

Tumour size showed a positive correlation with basal ($p<0.01$), as well as mean 24 hr GH levels ($p<0.01$; Table I). Neither tumour volume, nor basal or mean 24 hr GH levels correlated with the serum IGF-I concentrations. However, if only patients with basal GH levels of less than 40 μg/L were considered, a positive correlation with the circulating IGF-I concentration was found ($p<0.05$). Baseline GH and mean 24 hr GH levels showed a close correlation ($p<0.001$).

In male patients there was a negative relationship between the age of the acromegalic patients and the circulating GH ($p<0.05$) and IGF-I levels ($p<0.05$). However, we did not observe such a correlation between tumour volume and age, also not if only the maximal height of the adenomas was taken into consideration. Baseline

circulating GH and PRL levels were statistically significantly correlated ($p<0.01$). In every patient an oral glucose test was performed. No correlations between the change in GH after glucose and the response to TRH, bromocriptine or octreotide were found, however.

The responses of the circulating GH levels to TRH, bromocriptine and octreotide showed that the pituitary adenomas of female patients expressed a more pronounced reaction of GH to TRH, than males ($p<0.05$). The degree of stimulation of GH by TRH was neither related to baseline GH or PRL levels, nor to tumour size or age. There was no difference in the PRL levels between male and female patients. Elevated circulating PRL levels were accompanied by a significantly higher sensitivity of GH secretion to bromocriptine. Also the stimulatory effect of TRH on GH secretion was closely related to the inhibitory effect of bromocriptine ($p<0.01$).

The sensitivity of GH secretion to octreotide was higher in male patients ($p<0.05$), while GH release was more suppressed in reaction to 50 μg octreotide in elderly patients ($p<0.05$; Fig.1), with a relatively low circulating IGF-I level ($p<0.05$).

There was a distinct pattern suggesting a homogeneity of the responsiveness of GH secretion to the three stimuli investigated. Apart from the relation between the responses to TRH and bromocriptine mentioned above, the responses to TRH and octreotide were also related ($p<0.05$), as well as those to bromocriptine and octreotide ($p<0.01$; Fig. 2). However, the sensitivity of GH secretion to these stimuli was not related to tumour volume, nor to baseline or mean 24 hr GH levels.

DISCUSSION

This study once again confirms that bigger pituitary adenomas secrete higher quantities of GH (Wright *et al.*, 1969; Klijn *et al.*, 1980; De Fablo *et al.*, 1981; Dons *et al.*, 1983; Nabarro, 1987; Smals *et al.*, 1988). As previously observed, circulating GH and IGF-I levels are only related up to a certain cut-off level (in our case a serum GH concentration of 40 μg/L). This suggests that this is the circulating GH concentration which stimulates IGF-I production maximally. This observation is in agreement with that of some (Lamberts *et al.*, 1987; Giannella-Neto *et al.*, 1988; Oppizzi *et al.*, 1986; Barkan *et al.*, 1988), but not all investigators (Rieu *et al.*, 1982; Lindholm *et al.*, 1987). It probably explains that acromegaly of similar clinical severity can occur in patients with widely varying serum GH concentrations (Stonifer *et al.*, 1981; Giannella-Neto *et al.*, 1988), and that a considerable reduction of high circulating GH levels is often not accompanied by marked clinical improvement (Rieu *et al.*, 1982).

Tumour size was not negatively correlated with age, nor with the sex of our acromegalic patients. Previous studies had shown that older patients and male patients tend to have smaller pituitary tumours (Klijn *et al.*, 1980; Smals *et al.* 1988). However, our study clearly shows that the severity of the disease, as it is related to the circulating IGF-I level and to a lesser extent to the circulating GH levels, tends to be less in elderly, especially male acromegalic patients.

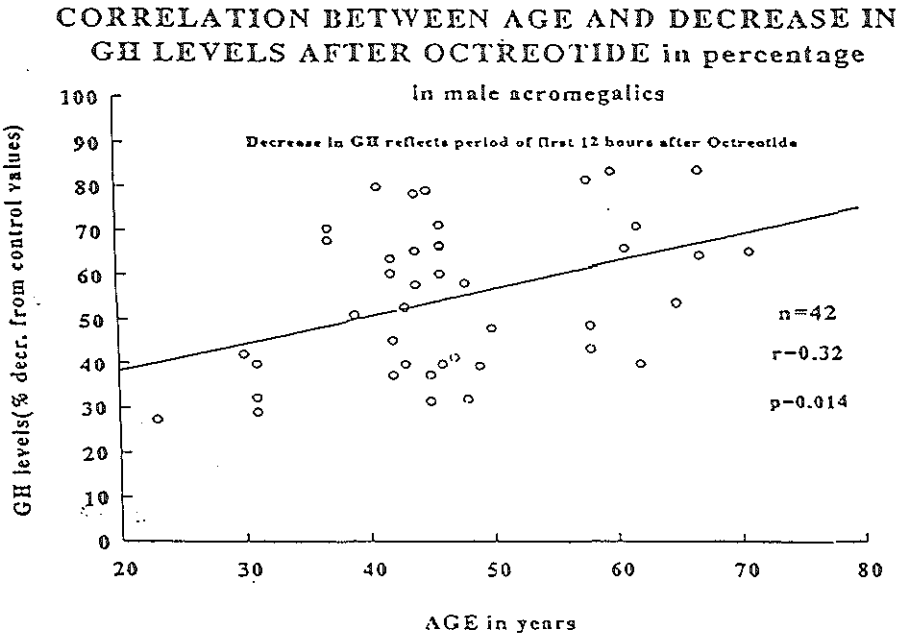
Very high circulating GH levels in acromegaly tend to be accompanied by hyperprolactinaemia. Hyperprolactinaemia in acromegaly can be caused by co-secretion of hormones by mixed GH/PRL pituitary tumours or in the case of very big tumours by pressure on the pituitary stalk interfering with the normal inhibition of PRL release by dopamine (Vance *et al.*, 1984; Flier & Underhill, 1991). The fact that in our patients circulating PRL levels were not related to tumour volume probably relates to this dual cause of hyperprolactinaemia. The value of measuring circulating PRL levels in acromegaly is only confined to its predictive value with regard to the coexistent higher sensitivity of GH secretion to bromocriptine (Liuzzi *et al.*, 1974; Lamberts *et al.*, 1982; Barkan, 1989).

Our study clearly shows that there is a homogeneity in the responsiveness of tumorous hormone secretion to TRH, bromocriptine and octreotide. Such a homogeneity in the responses to TRH and bromocriptine, and to bromocriptine and octreotide have been previously observed by some groups of investigators (Oppizzi *et al.*, 1980 and 1986; Lamberts *et al.*, 1986), but it was not reported by others (Hanew *et al.*, 1980; Pieters *et al.*, 1982). It should be mentioned, however, that the number of patients studied observed in these studies was considerably lower than that of our study. In particular, the close correlation between the responsiveness of GH release to octreotide and bromocriptine might be of clinical importance. We recently showed that a combination of both drugs might be of value, especially in those patients in whom circulating GH levels tend to "escape" within 5-7 hour after each octreotide injection during long-term therapy with the drug with three subcutaneous injections (Lamberts *et al.*, 1985; Wagenaar *et al.*, 1991). The most important conclusion from our study is, however, that the activity of the disease tends to be lowest in elderly patients, and that especially elderly male patients show the highest sensitivity to the GH release inhibitory effects of octreotide. It has been reported that the effect of a single dose of octreotide correlates well with the long-term effect of octreotide therapy in acromegalic patients (Lamberts *et al*, 1988). This suggests that octreotide might be especially successful as a primary medical therapy especially in elderly acromegalics.

Table I. The statistical analysis between patient data, tumour size, circulating hormones and their response to TRH, bromocriptine and octreotide.

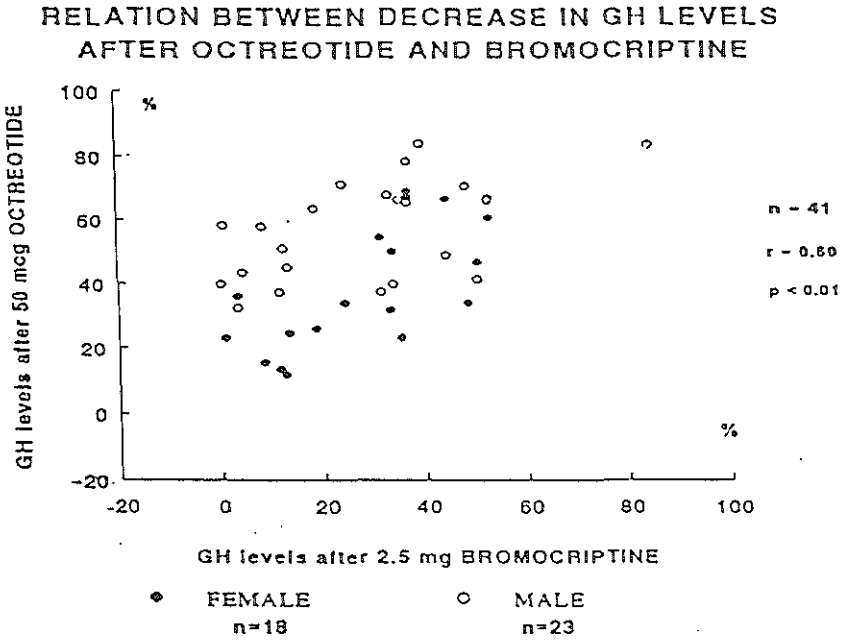
PARAMETERS		Number of patients	Correlation Coefficient	p-value
tumor volume	basal GH	100	0.46	<0.01
tumor volume	mean 24 h GH	70	0.48	<0.01
basal GH < 40 μg/L	IGF-I	39	0.29	<0.05
basal GH	mean 24 h GH	67	0.95	<0.001
age	basal GH	100	-0.21	<0.05
age	IGF-I	53	-0.32	<0.05
basal GH	basal PRL	90	0.20	<0.05
females	% stim. GH to TRH	25	0.17	<0.05
basal PRL	% decr. GH to bromocr.	48	0.27	<0.05
% stim. GH to TRH	% decr. GH to bromocr.	52	0.43	<0.01
males	% decr. GH to octreotide	42	0.30	<0.05
age	% decr. GH to octreotide	72	0.29	<0.05
IGF-I	% decr. GH to octreotide	39	-0.30	<0.05
% stim. GH to TRH	% decr. GH to octreotide	68	0.23	<0.05
% decr. GH to bromocr.	% decr. GH to octreotide	41	0.60	<0.01

Fig.1:



Correlation between age and the decrease in GH levels after 50 µg octreotide subcutaneously in 42 male acromegalics. The decrease in GH level reflects the area under the curve of the first 12 hours after octreotide administration as percentage of the curve on the control day. (r=0.32; p=0.014)

Fig.2:



Correlation between decrease of GH levels after Octreotide and Bromocriptine in 18 female (O) and 23 male (O) acromegalic patients. The decrease in GH level reflects the area under the curve of the first 12 hours after the administration of each of the drugs as percentage of the area under the curve on a control day. Both decreases correlate well: $r=0.60$, $p<0.01$. Note that men show a more profound decrease in GH levels after Octreotide, while women tend to show a more profound decrease after Bromocriptine administration.

References:

1. Barkan AL. (1989) Acromegaly: diagnosis and therapy. *Endocrinology Metabolism Clinics* ,48, 277-310.

2. Barkan AL, Beitins IZ, Kelch RP. (1988) Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. *Journal of Clinical Endocrinology and Metabolism*,67, 69-73.

3. Chiodini PG, Cozzi R, Dallabonzana D, Oppizzi G, Verde G, Petroncini M, Liuzzi A, Del Pozo E. (1987) Medical treatment of acromegaly with SMS 201-995, a somatostatin analogue: a comparison with bromocriptine. *Journal of Clinical Endocrinology and Metabolism*,64, 447-453.

4. De Fablo F, Eastman RC, Roth J, Gorden P. (1981) Plasma prolactin in acromegaly before and after treatment. *Journal of Clinical Endocrinology and Metabolism*,53, 344-352.

5. Dons RF, Rieth KG, Gorden P, Roth J. (1983) Size and erosive features of the sella turcica in acromegaly as predictors of therapeutic response to supervoltage irradiation. *American Journal of Medicine*,74, 69-72.

6. Flier JS, Underhill LH. (1991) Diagnosis and management of hormone-secreting pituitary adenomas. *New England Journal of Medicine*,324, 822-831.

7. Giannella-Neto D, Wajchenberg BL, Mendonca BB, Almeida SF,Macchione M, Spencer EM. (1988) Criteria for the cure of acromegaly: comparison between basal growth hormone and somatomedin C plasma concentrations in active and non-active acromegalic patients. *Journal of Endocrinological Investigation*,11, 57-60.

8. Hanew K, Kokubun M, Sasaki A, Moiri T, Yoshinasa K. (1980) The spectrum of pituitary growth hormone responses to pharmacological stimuli in acromegaly. *Journal of Clinical Endocrinology and Metabolism*,51, 292-297.

9. Klijn JGM, Lamberts SWJ, de Jong FH, van Dongen KJ, Birkenhager JC. (1980) Interrelationships between tumoursize, age, plasma growth hormone and incidence of extra sellar extension in acromegalic patients. *Acta Endocrinologica (Copenh)*,95, 289-297.

10. Lamberts SWJ, Liuzzi A, Chiodini PG, Verde S, Klijn JG, Birkenhager JC. (1982) The value of plasma prolactin levels in the prediction of the responsiveness of growth hormone secretion to bromocriptine and TRH in acromegaly. *European Journal of Clinical Investigation*,12, 151-155.

11. Lamberts SWJ, Uitterlinden P, Verschoor L, van Dongen KJ, del Pozo E. (1985) Long-term treatment of acromegaly with the somatostatin analogue SMS 201-995. *New England Journal of Medicine*,313, 1576-1580.

12. Lamberts SWJ, Zweens M, Verschoor L, del Pozo E. (1986) A comparison among the growth hormone-lowering effects in acromegaly of the somatostatin analogue SMS 201-995, bromocriptine and the combination of both. *Journal of Clinical Endocrinology and Metabolism*,63, 16-19.

13. Lamberts SWJ, Uitterlinden P, Verleun T. (1987) Relationship between growth hormone and somatomedin-C levels in untreated acromegaly, after surgery and radiotherapy and during medical therapy with sandostatin (SMS 201-995). *European Journal of Clinical Investigation*,17, 354-359.

14. Lamberts SWJ, Uitterlinden P, Schuiff PC, Klijn JG. (1988) Therapy of acromegaly with Sandostatin: predictive value of an acute test, the value of serum somatomedin-C measurements in dose adjustment and the definition of a biochemical 'cure'. *Clinical Endocrinology* ,29, 411-420.

15. Lamberts SWJ. (1988) The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of analogues in the treatment of human pituitary tumors. *Endocrinology Reviews*,9, 417-436.

16. Lindholm J, Riishede J, Vestergaard S, Hummer L, Faber O, Hagen C. (1981) No effect of bromocriptine in acromegaly: a controlled trial. *New England Journal of Medicine* ,304, 1450-1454.

17. Lindholm J, Giwercman B, Giwercman A, Astrup J, Bjerre P, Skakkebaek NE. (1987) Investigation of the criteria for assessing the outcome of treatment in acromegaly. *Clinical Endocrinology*,27, 553-562.

18. Liuzzi A, Chiodini PG, Botalla A, Cremascoli G, Muller EE, Silvestrini F. (1974) Decreased plasma growth hormone (GH) levels in acromegalics following CB 154 (2-Br-alpha-ergocriptine) administration. *Journal of Clinical Endocrinology and Metabolism*,38, 910-912.

19. Melmed S. Acromegaly. (1990) *New England Journal of Medicine*,322, 966-977.

20. Nabarro JDN. (1987) Acromegaly. *Clinical Endocrinology*,26, 481-512.

21. Oppizzi G, Botalla L, Verde G, Cozzi R, Liuzzi A, Chiodini PC.(1980) Homogeneity in the growth hormone lowering effect of dopamine and somatostatin in acromegaly. *Journal of Clinical Endocrinology and Metabolism*,51, 616-620.

22. Oppizzi G, Petroncini MM, Dallabonzana D, Cozzi R, Verde G, Chiodini PG, Liuzzi A. (1986) Relationship between somatomedin-C and growth hormone levels in acromegaly: basal and dynamic evaluation. *Journal of Clinical Endocrinology and Metabolism*,63, 1348-1353.

23. Pieters GFFM, Romeijn JE, Smals AGH, Kloppenborg PWC. (1982) Somatostatin sensitivity and growth hormone responses to releasing hormones and bromocriptine in acromegaly. *Journal of Clinical Endocrinology and Metabolism*,54, 942-947.

24. Rieu M, Girard F, Bricaire H, Binoux M. (1982) The importance of insulin-like growth factor (somatomedin) measurements in the diagnosis and surveillance of acromegaly. *Journal of Clinical Endocrinology and Metabolism*,55, 147-153.

25. Smals AEM, Pieters GFFM, Smals AGH, Kloppenborg PWC. (1988) Sex difference in the relation between sellar volume and basal and GH-releasing hormone stimulated GH in acromegaly. *Acta Endocrinologica (Copenh)*,117, 387-391.

26. Stonesifer LD, Jordan RM, Kohler PO. (1981) Somatomedin-C in treated acromegaly: poor correlation with growth hormone and clinical response. *Journal of Clinical Endocrinology and Metabolism*,53, 931-938.

27. Vance ML, Evans WS, Thorner MO. (1984) Bromocriptine. *Annals of Internal Medicine*,100, 78-91.

28. Wagenaar AH, van der Lely AJ, Harris AG, Lamberts SWJ. (1991) The dynamics of the acute effects of octreotide, bromocriptine and the combination of both drugs on growth hormone secretion in acromegaly. *Acta Endocrinologica (Copenh)*;152: 637-642.

29. Wass JA, Thorner MO, Morris DV, Rees LH, Stuart Mason A, Jones AE, Besser GM. (1977) Long-term treatment of acromegaly with bromocriptine. *British Medical Journal* ,1, 875-878.

30. Wright AD, McLachlan MBF, Doyle FH, Fraser TR. (1969) Serum growth hormone levels and size of pituitary tumour in untreated acromegaly. *British Medical Journal*,4, 582-584.

CHAPTER 4.

DYNAMICS OF THE ACUTE EFFECTS OF OCTREOTIDE, BROMOCRIPTINE AND BOTH DRUGS IN COMBINATION ON GROWTH HORMONE SECRETION IN ACROMEGALY.

Wagenaar AH [1], Harris AG [1], van der Lely AJ [2], Lamberts SWJ [2].

1 Department of Neuroendocrinology, Clinical Research, Sandoz Pharma Ltd, Basel Switzerland.

2 Department of Medicine, Erasmus University Hospital, 3015 GD, Rotterdam, The Netherlands.

This article has been published in the Acta Endocrinol (Copenh) 1991;152: 637-642.

Summary:

The separate and combined GH-lowering effects of single doses of octreotide and bromocriptine were assessed in 51 acromegalics, on 4 occasions each days apart. Patients received sequentially: placebo s.c. (n=51), 50 μg octreotide s.c. (n=51), 2.5 mg bromocriptine p.o. (n=40) or a combination of both drugs (n=25).

With octreotide, in 28 patients (55%) GH levels were suppressed to less than 5 μg/L and 39 of them (76.5%) had a 50% or greater decrease of their basal GH level from 2 to 6 hours. During bromocriptine, GH values were suppressed to below 5 μg/L in 11 patients (27.5%) and reduced by 50% or more in 21 (52.5%). The combination of both drugs acutely suppressed GH levels to less than 2 μg/L in 32%, to less than 5 μg/L in 56% and by more than 50% in 84% of patients.

Octreotide produced a stronger and faster suppression of GH levels than bromocriptine in most patients. The combination of both drugs had an additive effect on the lowering of GH levels, especially between 7 and 10 hours after drug administration. These results suggest that chronic therapy with a combination of both drugs may be as effective as therapy with higher doses of either compound alone.

Albeit transient, octreotide caused a rapid near total suppression of insulin release in the morning, 15 to 45 min after administration. Postprandial glucose rise, between 2 and 3.5 hours after breakfast was significantly higher during octreotide than on the control day.

Introduction:

Medical therapy of acromegaly has been greatly improved in recent years following the availability of the long-acting somatostatin analogue, octreotide (Sandostatin, SMS 201-995). Comparative studies between the acute inhibitory effects of bromocriptine (Parlodel) and those of octreotide, on GH secretion in small numbers of acromegalic patients indicate that the majority of these patients show a significantly higher sensitivity to octreotide that to bromocriptine [1 2 3].

Chronic therapy with octreotide produces rapid clinical improvement in virtually all acromegalic patients, which is accompanied by a decease in circulating GH and IGF-I levels. However, virtually complete normalization of mean 24 hour GH and IGF-I levels is reached in only approximately 50% of patients during intermittent subcutaneous administration of 50-100 μg octreotide twice or thrice daily [2 4 5 6 7 8 9 10 11]. It has been hypothesized that the absence of complete suppression of GH levels throughout the

24 hour period is related to the pharmacokinetics of octreotide, which allows an increase of tumoral hormone secretion during the hours immediately preceding the next injection [5 6 7 8 9 12]. Indeed, studies in which octreotide was administered by 24 hour subcutaneous infusion, acromegalic patients tend to show better control of GH secretion over the 24 hour period, with concomitant normalization of circulating IGF-I levels occurring more frequently [9 10 11 12 13].

In the present study we have investigated whether octreotide and bromocriptine given in combination to acromegalic patients might result in more sustained control of tumoral growth hormone secretion. by virtue of the different pharmacokinetics of the two drugs. In addition, the acute effects of single doses of octreotide and bromocriptine were compared in a much larger group of acromegalic patients than in previous investigations [1 3]. Finally the apparent contradictory marginal changes were analyzed in view of the marked, but transient inhibitory effects of the analogue on insulin secretion.

Patients and Methods:

Fifty-one acromegalic patients (28 males and 23 females) aged 24-75 years were studied. Informed consent was obtained from each patient and the study was approved by the local ethics committee. The diagnosis of acromegaly was established clinically and confirmed by the presence of a radiologically evident pituitary tumor, increased levels of IGF-I and elevated circulating GH levels which were not suppressed below 5 μg/L following an oral administration of 100 g of glucose. Forty patients were untreated, whereas 10 had previously undergone transsphenoidal surgery and external pituitary irradiation with 4500 rads (patient 5,13,14,16,17,18,20,30,36 and 40). Hyperprolactinemia was present in patients 14 and 17. Immunocytochemical investigation of the pituitary tumor tissue removed at operation showed that both patients had a mixed GH/PRL secreting tumor. One patient (no. 22) had previously undergone surgery alone.

Fifty-one patients received placebo, given subcutaneously, at 08.15 h, on the first day, and 50 μg octreotide subcutaneously, at 08.15 h, on the second day. Forty of these patients received 2.5 mg bromocriptine orally, 2 days later, at 08.00 h. Twenty five of these patients received a combination of 2.5 mg bromocriptine taken orally, at 08.00 h, and 50 μg octreotide administered subcutaneously, at 08.15 h, at least 2 days later. All four treatment regimes were administered in the hospital. The patients had a standardized breakfast, lunch and evening meal at 08.30 h, 13.00 h and 17.30 h. The meals consisted of at least 75 g carbohydrate, 50 g fat and 25 g protein.

Serial blood samples were collected through an indwelling polyethylene catheter

placed in an antecubital vein. Blood for GH determinations was withdrawn at hourly intervals throughout a 24 hour period during which placebo or octreotide were administered. Insulin and glucose levels were also measured in blood specimens obtained between 08.00 h and 11.30 h and 17.30 h and 21.00 h. Only on the bromocriptine day, plasma GH was determined hourly between 08.00 h and 20.00 h. Plasma GH and insulin were determined by specific RIA's using commercial kits supplied by Eurodiagnostics (Apeldoorn, the Netherlands). Blood glucose levels were measured by a glucose oxidase method. Plasma PRL levels were measured with a specific RIA kit supplied by Eurodiagnostics (Apeldoorn, the Netherlands). Upper limits of normal were 12 μg/L in men and 15 μg/L in women.

The World Health Organization (WHO) criteria were used to assess patients glucose tolerance. Statistical evaluation was performed by means of a non-parametric Wilcoxon signed rank test (one-sided) of the area under the time/GH-response curve and a one sample t-test. Results are expressed as mean ± S.E.

Results:

The mean of 5 plasma GH levels, taken hourly between 10.00 h and 14.00 h were measured in the 51 acromegalic patients who received placebo and octreotide. Serum GH values were suppressed to less than 2 μg/L between 2 and 6 hours after octreotide administration in 12 (24%) and to less than 5 μg/L in 28 of these patients (55%), while GH levels decreased by at least 50% in 39 of them (76.5%).

Plasma GH values were suppressed to less than 2 μg/L between 2 and 6 hours after bromocriptine in 4 (10%) and less than 5 μg/L in 11 out of 40 (28%), while GH levels were reduced by 50% or more in 21 patients (53%). The combination of both drugs in 25 patients, suppressed plasma GH levels to less than 2 μg/L in 8 patients (32%), to less than 5 μg/L in 14 (56%) and by more than 50% in 21 patients (84%). Octreotide alone suppressed GH levels in the same 25 patients, 2 to 6 hours after administration to 2 μg/L or less in 5 patients (20%), to 5 μg/L or less in 12 (48%) and to less than 50% of control in 19 of them (76%). Bromocriptine failed to suppress GH levels to less than 2 μg/L in any of these patients, while suppressing GH levels to less than 5 μg/L in 8 patients (32%) and to less than 50% of control in 14 of them (56%). The mean of the absolute decrease of circulating GH levels in these 25 patients throughout the day are shown in Fig. 1. Octreotide rapidly suppressed mean GH values within 45 minutes after its administration by 50% or more. The lowest GH values were reached between 3 and 5 hours after octreotide administration, after which time its

effects on GH slowly waned, although a 50% reduction could still be observed at 15.00 h. In contrast, bromocriptine took 2 hours reduce GH levels by more than 50%, but the effects of the drug abated more slowly than those of octreotide. Overall, octreotide was more effective in suppressing GH values than bromocriptine ($p<0.05$). The combination of both drugs suppressed GH levels significantly better than octreotide and bromocriptine separately (area under the curves between 08.00 and 20.00 h; $p<0.05$).

Analysis of the course of the GH curves in Fig.1 shows that the "additive" inhibitory effects of both drugs, in comparison to their separate effects, is at its maximum between 15.00 h and 18.00 h. The additive effect of bromocriptine on octreotide-induced inhibition of GH secretion is highly significant during that period ($p<0.01$).

The individual data from the 25 patients who received all 4 regimes is shown in Fig. 2A and B. The areas under the GH curves between 09.00 h and 16.00 h (from 1 to 8 hours after drug administration) are shown as a percentage of the AUC of the GH levels recorded on the control day. Octreotide was more effective than bromocriptine in 19 of these patients (76%), while bromocriptine was more effective than octreotide in 3 patients (12%) (patients 4, 14 and 46, see Fig. 2A and 2B). The combination of both drugs was more effective than both compounds separately in 16 patients (64%).

Sixteen of the 51 acromegalic patients had hyperprolactinemia (patient no 3,4,11,12,14,17,24,25,29,32,37,38,40,42,46 and 49). Further analysis showed that there was no relationship between circulating PRL levels and the sensitivity of GH to octreotide. However, in acromegalic patients with increasing circulating PRL levels, GH secretion was more sensitive to one single dose of bromocriptine than in normoprolactinemic acromegalics: the mean GH levels between 2 and 6 hours after 2.5 mg bromocriptine were significantly lower in the hyperprolactinemic than in the normoprolactinemic patients ($p<0.05$ rank correlation).

The effects of octreotide on plasma glucose concentrations in the 51 patients are shown in Fig.3. The postprandial increment in glucose between 2 and 3.5 hours after breakfast was significantly higher (AUC between 10.00 h and 13.00 h; $p<0.05$) after octreotide than on the control day. However, glucose levels had returned to control values at 17.30 h. Moreover, a significantly lower increase in post-dinner glucose concentrations was observed after octreotide administration at 08.15 h than on the placebo day (AUC 18.00-21.00 h; $p<0.05$).

Octreotide caused a rapid and transient near total suppression of insulin release in the morning, 15 to 45 minutes after its administration. However, this suppression was short-lived, since post-prandial peak insulin levels reached 3 hours after octreotide administration, i.e. at 11.30 h, were similar to those recorded at 10.00 h on placebo.

Insulin secretion was significantly higher after placebo administration than after octreotide (AUC between 08.30 h and 11.30 h; $p < 0.05$). The lower blood glucose levels seen following dinner after octreotide administration, early in the morning, were associated with lower insulin levels than on the control day. With regard to carbohydrate tolerance, 43 of the 51 patients showed on the placebo day normal glucose tolerance after breakfast, 5 patients showed impaired glucose tolerance, and 3 patients had glucose levels suggestive of frank diabetes mellitus. After octreotide, post-prandial glucose tolerance remained normal in 23 patients and became abnormal in 25 patients, while the 3 diabetic patients remained diabetic in the morning. However, in the evening 43 patients showed normal glucose tolerance and 6 had impaired glucose tolerance, while 2 remained diabetic after octreotide given in the morning. The highest post-prandial glucose levels observed were 21.4 and 19.9 mmol/l on the placebo day and 18.9 and 15.2 mmol/l after octreotide administration (in the same patients).

Discussion:

In this study we confirm other previous observations that GH secretion in acromegaly is significantly better suppressed by octreotide than by bromocriptine [1 2 3 8]. Only exceptionally (in this study 3 of 40 patients) does a single administration of bromocriptine suppress GH secretion more markedly than octreotide.
Although an oral dose of 2.5 mg bromocriptine and a subcutaneous dose of 50 μg of octreotide are not necessarily optimal therapeutically equivalent doses, previous studies have shown that both the acute reaction of GH to a single dose of 2.5 mg bromocriptine [14], as well as that to a single subcutaneous injection of 50 μg Sandostatin [15] predicts the ultimate effect of chronic therapy with these drugs on circulating GH and IGF-I levels.

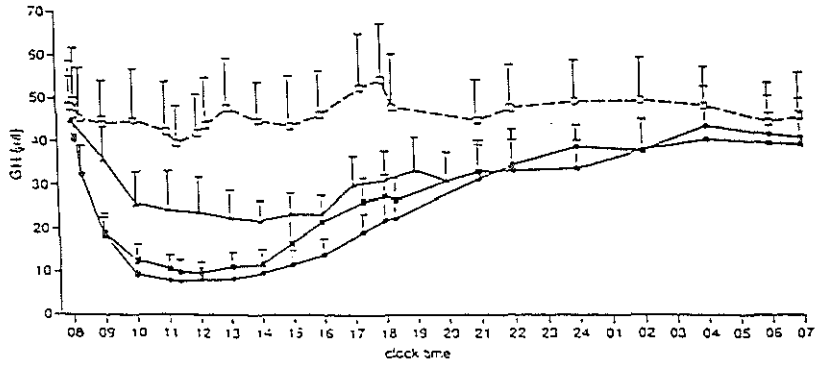
However, the most important new observation of our study is that there is probably a difference in the inhibitory effects of the two compounds on tumoral GH secretion. Octreotide caused a rapid decrease in GH levels, although its effects were slightly shorter-lived in comparison with the longer, but delayed inhibitory effect of bromocriptine. Interestingly, a combination of the two drugs inhibits GH in an additive manner not only in the first hours but also particularly later on, between 7 and 10 hours after drug administration. As mentioned in the introduction, the therapeutic effects of 2 or 3 subcutaneous administrations of octreotide over a 24 hour period are reduced by the commonly occurring tendency of GH levels to increase towards the next injection of octreotide [4 5 6 7 9 10 11 13]. It has been hypothesized that these artificially determined GH

"peaks" often cause incomplete suppression of circulating IGF-I levels, while complete suppression is more often observed during 24 hour continuous subcutaneous infusion of octreotide [8 11 12 13]. It is therefore suggested that therapeutic regimens including combination of two or three subcutaneous administrations of lower doses of octreotide with oral administration of a low dose of bromocriptine, might optimize drug treatment in a number of acromegalic patients.

The observation that the mean GH levels 2 to 6 hours after 2.5 mg bromocriptine were significantly lower in the hyperprolactinemic patients than in the normoprolactinemic patients is in accordance with previous studies in different groups of acromegalic patients [16 17 18].

Our studies on glucose tolerance during the first hours after octreotide administration, as well as after 10-12 hours (after dinner), show the considerable importance of insulin resistance associated with increased GH levels in acromegaly. The sudden, nearly complete suppression of insulin levels during the first hour after octreotide administration causes a significant impairment of glucose tolerance after breakfast. However, the lower post-prandial glucose and insulin levels after dinner (10 hours after octreotide administration) indicate that the long-acting inhibitory effects on circulating GH levels, after a single administration of 50 μg octreotide, results almost immediately in a highly significant decrease in insulin resistance. It is these seemingly contradictory effects that form the basis for the limited deterioration of glucose tolerance in a minority of acromegalic patients, as observed during long-term therapy with octreotide [2 4 8 9 12].

Fig.1

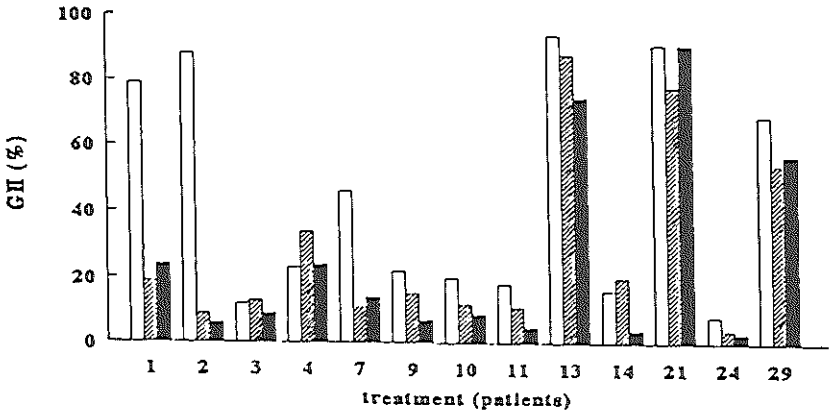


Absolute mean changes of circulating GH levels throughout the day in 25 patients who received all 4 regimens. □ = placebo;

▲ = octreotide; ■ = bromocriptine;

● = octreotide and bromocriptine.

Fig. 2A

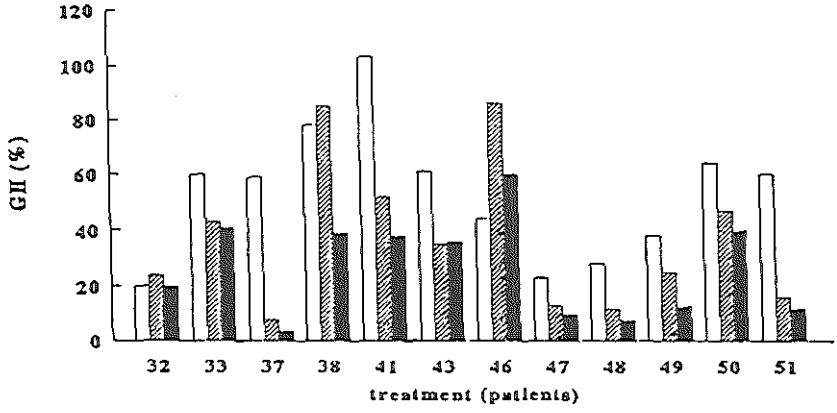


Areas under the GH curve between 09.00 h and 16.00 h (from 1 to 8 hours after drug administration) are shown as a percentage of the AUC of the GH levels recorded on the placebo day in 25 acromegalic patients who received all 3 regimens.

□ = bromocriptine; ▨ = octreotide;

■ = octreotide and bromocriptine.

Fig. 2B

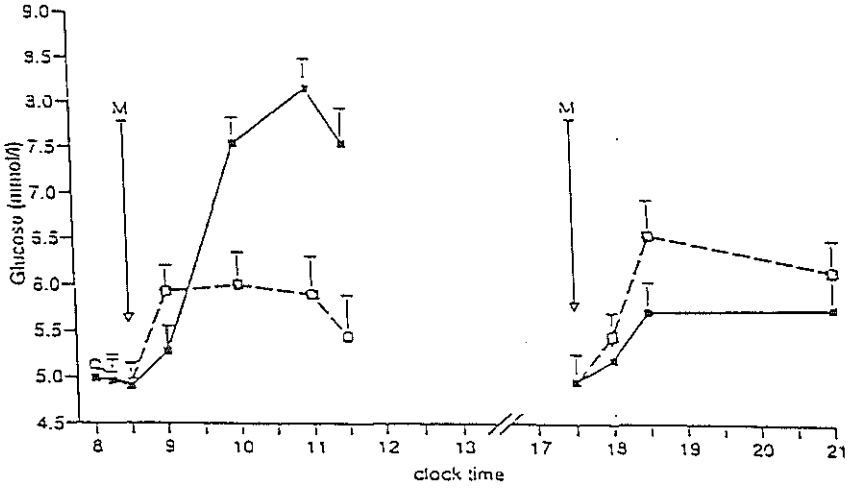


Areas under the GH curve between 09.00 h and 16.00 h (from 1 to 8 hours after drug administration) are shown as a percentage of the AUC of the GH levels recorded on the placebo day in 25 acromegalic patients who received all 3 regimens.

□ = bromocriptine; ▨ = octreotide;

■ = octreotide and bromocriptine.

Fig. 3



Mean blood glucose levels in 51 patients who received placebo and octreotide. □ = placebo; ■ = octreotide.

References:

1. Chiodini PG, Cozzi R, Dallabonzana D, et al. Medical treatment of acromegaly with SMS 201-995, a somatostatin analog: a comparison with bromocriptine. J Clin Endocrinol Metab 1987;64: 447-453.

2. Cozzi R, Dallabonzana D, Opppizi G, et al. Medical treatment of acromegaly with SMS 201-995, a new somatostatin analogue: a comparison with bromocriptine. Neuroendocrine Perspectives. (eds. E.E. Muller & R.M. MacLeod). Elsevier; vol 5. New York 1986: 161-166.

3. Lamberts SWJ, Zweens L, Verschoor L, Del Pozo E. A comparison among the growth hormone-lowering effects in acromegaly of the somatostatin analog SMS 201-995, bromocriptine, and the combination of both drugs. J Clin Endocrinol Metab 1986;63: 16-19.

4. Barkan AL, Kelch RP, Hopwood NJ, Beitins IZ. Treatment of acromegaly with the long-acting somatostatin analog SMS 201-995. J Clin Endocrinol Metab 1988;66: 16-23.

5. George SR. Sandostatin (SMS 201-995) treatment of acromegaly: acute and chronic effects. "Sandostatin in the treatment of acromegaly". Consensus Round Table, Amsterdam 1987 (ed. Lamberts SWJ.), Springer Verlag, 1987: 113-115.

6. Lamberts SWJ, Uitterlinden P, Verschoor L, Van Dongen KJ, Del Pozo E. Long-term treatment of acromegaly with the somatostatin analogue SMS 201-995. N Eng J Med 1985;313: 1576-1580.

7. Lamberts SWJ, Uitterlinden P, Del Pozo E. SMS 201-995 induces a continuous decline in circulating growth hormone and somatomedin-C levels during therapy of acromegalic patients for over two years. J Clin Endocrinol Metab 1987;65: 703-710.

8. Lamberts SWJ. The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of its analogs in the treatment of human pituitary tumors. Endocrin Reviews 1988;9: 417-436.

9. Orskov H, Weeke J, Christensen SE, et al. Observations during a Clinical Trial of Sandostatin in Acromegalic Patients. "Sandostatin in the Treatment of Acromegaly", Consensus Round Table, Amsterdam (ed. Lamberts SWJ.) Springer Verlag 1987: 83-87.

10. Sandlers LM, Burrin JM, Williams G, et al. Effective long-term treatment of acromegaly with a long-acting somatostatin analogue (SMS 201-995). Clin Endocrinol 1987;26: 85-95.

11. Tauber JP, Babin TH, Ducasse MCR, Tauber MT, Harris AG, Bayard F. Long-term treatment of acromegaly by continuous subcutaneous infusion of the long-acting somatostatin analog Sandostatin (SMS 201-995, octreotide). "Sandostatin in the treatment of acromegaly:, Consensus Round Table, Amsterdam 1987. (ed. Lamberts SWJ.), Springer Verlag 1987: 109-112.

12. Christensen SE, Weeke J, Orskov H, et al. Continuous subcutaneous pump infusion of somatostatin analogue SMS 201-995 versus subcutaneous injection schedule in acromegalic patients. Clin Endocrinol 1987;27: 297-306.

13. Ducasse MCR, Tauber JP, Tourre A, et al. Shrinking of a growth hormone-producing pituitary tumor by continuous subcutaneous infusion of the somatostatin analog SMS 201-995. J Clin Endocrinol Metab 1987;65: 1042-1046.

14. Nortier JWR, Croughs RJM, Thijssen JHH, Schwarz F. Plasma growth hormone suppressive effect of bromocriptine in acromegaly. Evaluation by plasma GH day profiles and plasma GH concentration during oral glucose tolerance tests. Clin Endocrinol 19874;20: 565-571.

15. Lamberts SWJ, Uitterlinden P, Schuijff PC, Klijn JGM. Therapy of acromegaly with Sandostatin: the predictive value of an acute test, the value of serum somatomedin-C measurements in dose adjustment and the definition of a biochemical "cure". Clin Endocrinol 1988;29: 411-420.

16. Lamberts SWJ, Liuzzi A, Chiodini PG, Verde S, Klijn JGM, Birkenhager JC. The value of prolactin levels in the prediction of the responsiveness of growth hormone secretion to bromocriptine and TRH in acromegaly. Eur J Clin Invest 1982;12: 151-155.

17. Lamberts SWJ, Klijn JGM, van Vroonhoven CGJ, Stefanko SSZ, Liuzzi A. The role of prolactin in the inhibitory action of bromocriptine on growth hormone secretion in acromegaly. Acta Endocrinol (Copenh) 1983;103: 445-450.

18. Lamberts SWJ, KlijnJGM, van Vroonhoven CCJ, Stefanko SZ. Different responses of growth hormone secretion to guanfacine, bromocriptine, and thyrotropin-releasing(GH)-containing and mixed GH/prolactin-containing pituitary adenomas. J Clin Endocrinol Metab 1985;60: 1148-1155.

CHAPTER 5.

LONG-TERM IN VITRO TREATMENT OF HUMAN GROWTH HORMONE (GH)-SECRETING PITUITARY ADENOMA CELLS WITH OCTREOTIDE CAUSES ACCUMULATION OF INTRACELLULAR GH AND GH-mRNA LEVELS

Short title: Long-term in vitro effects of octreotide on GH-adenoma cells.

L.J. Hofland, B. Velkeniers*, A.J. v.d. Lely, P.M. van Koetsveld, M. Kazemzadeh*, M. Waaijers, E.L. Hooghe-Peters*, and S.W.J. Lamberts.

Department of Medicine III, Erasmus University, Rotterdam, The Netherlands; *Department of Pharmacology, Free University Brussel, Brussels, Belgium.

Submitted for publication

Abstract

The effects of long term in vitro exposure with octreotide of human GH secreting pituitary adenoma cells were investigated on GH release, intracellular GH concentrations, and GH mRNA levels. Incubation of the adenoma cells for 4 days with 10nM octreotide induced a dose-dependent inhibition of GH release, while in parallel an increase (maximal increase varying between 24 and 517%) in the intracellular GH levels was observed in 6 of 7 adenomas. In addition bromocriptine, when effective in inhibiting GH release by the adenomas, also induced an increase in intracellular GH levels. Even after three weeks of exposure to 10nM octreotide in vitro there was a statistically significant increase in intracellular GH levels (variation between 91 and 923% increase). Withdrawal of octreotide after 6 days of incubation resulted in a lowering of intracellular GH levels to control values, showing that the octreotide-induced increase in intracellular GH is reversible. In a 96 h incubation with 10nM octreotide GH mRNA levels were increased in 2, and slightly decreased in 1 of the 3 adenomas tested. This effect was time-dependent in that there was no significant effect of 10nM octreotide on GH mRNA levels in a 24 h incubation.

We conclude that: 1) long-term in vitro exposure of GH-adenoma cells to octreotide causes an increase in intracellular GH levels in the majority of the adenomas, which is probably due to an increase in GH mRNA levels in the adenoma cells; and 2) this considerable increase in intracellular GH levels may be one of the explanations for the relatively poor effect of octreotide on tumour shrinkage in patients with GH-secreting pituitary adenomas.

Introduction

The somatostatin analogue octreotide is very successful in the treatment of growth hormone (GH) hypersecretion by human GH-secreting pituitary adenomas. Long-term treatment of acromegalic patients with octreotide results in a considerable improvement of the clinical symptoms in most acromegalic patients (Lamberts, 1988). In addition long-term treatment of acromegalic patients with a constant daily dose of 200-300 μg octreotide induces a decline in mean GH and IGF-I levels in most patients, while normalization of plasma IGF-I concentrations is eventually reached in approximately 50% of patients. Apart from its GH- suppressive action treatment of acromegalic patients with octreotide also induces slight pituitary tumour shrinkage in about half of the patients (Lamberts et al., 1985; Jackson et al., 1986; Barkan et al., 1988). However, in

most patients the maximal shrinkage is not more than by 20-50% of pretreatment tumour size. This effect of octreotide is relatively poor as compared to the tumour shrinking effects of bromocriptine as previously reported in prolactinomas (Lamberts, 1984). This latter effect may be the result of inhibition of prolactin (PRL) synthesis by an inhibitory action of bromocriptine on intracellular PRL-mRNA levels, ultimately leading to shrinkage of the individual tumour cell volume (Maurer, 1980; Maurer, 1981; Bassetti et al., 1984).

We previously demonstrated that human GH- secreting pituitary adenomas are heterogeneous, in that they contain a majority of low-secretor cells and a minority of high- secretor cells, while in some adenomas octreotide preferentially inhibits hormone release by the high-secretor cells (Hofland et al., 1989 [a]). This heterogeneity in the responsiveness to octreotide might be a basis for the above mentioned relatively poor effect of octreotide on tumour shrinkage. Another possibility is that somatostatin (analogues) do not affect GH mRNA expression to the same extent as bromocriptine does in prolactinoma cells. Data with respect to the effect of somatostatin (analogues) on GH mRNA expression are conflicting. No effect of somatostatin on GH mRNA was observed in rat pituitary (tumour) cells (Gick and Bancroft, 1987; Nambda et al., 1989), while in two other studies a reduction of GH mRNA expression was demonstrated in rat and ovine pituitary cells (Wood et al., 1987; Silverman et al., 1989). In addition, the native peptide somatostatin had, in short-term (Davis et al., 1989) or long-term (Levy and Lightman, 1988) incubation studies, no effect on GH mRNA levels in human GH-secreting adenoma cells .

In the present study we further investigated the effect of octreotide on the release and the intracellular concentrations of GH, PRL, and α-subunit, as well as the effect of octreotide on GH mRNA expression of GH- adenoma cells after long-term exposure of the cells to this stable somatostatin analogue. In addition a comparison was made with the effect of bromocriptine on GH release and intracellular GH concentrations.

Materials and methods

Patients and cell isolation

Tumour tissue from 13 acromegalic patients was obtained by transsphenoidal operation. Twelve patients were untreated while one patient (no. 7) had been treated preoperatively during 12 weeks with octreotide (3x100 μg octreotide daily). Single cell suspensions of the adenoma tissue were prepared by enzymatic dissociation with dispase as described in detail previously (Oosterom et al., 1984).

Details on patient data, tumour volume, basal GH, PRL and IGF-I levels, as well as basal GH expressed per cm^3 tumour volume are shown in Table 1. All patients had elevated plasma GH and IGF-I levels, while PRL levels were slightly elevated in 3, and considerably elevated in 2 patients.

Cell culture

Monolayer cultures: the isolated cells were plated in 48-well plates (Costar, Cambridge, MA) at a density of 10^5 cells per well per 1 ml culture medium. The cells were allowed to attach for 3 to 4 days at 37°C in a CO_2- incubator. After this period the medium was changed and 96 hour incubations without or with octreotide or bromocriptine were started. At the end of the incubation the medium was removed and centrifuged for 5 min. at 600xg. The supernatant was collected and stored at -20°C until analysis. The cells were rinsed twice with ice-cold saline solution and lysed in 0.5 ml of a 0.05% NH_3 solution during 30 min. Thereafter, 0.5 ml phosphate buffered saline solution (PBS; 0.1 M, pH 7.0), containing 0.1% bovine serum albumin (Sigma Chemical Co., St Louis, USA) was added. The cell extracts were collected and stored at -20°C until analysis.

Transwell studies: the isolated pituitary tumour cells were plated in transwell-COL membranes (Costar) at a density of 10^5 cells per well. Transwells containing the cells were placed into multiwell plates (24-well, Costar) containing 1 ml culture medium. After 24 hr incubation at 37°C in a CO_2-incubator the transwells were transferred to wells containing fresh medium (without or with octreotide). Every 3-4 days the cells were placed into fresh medium (without or with octreotide) and the incubation media were collected and stored at -20°C until determination of hormone concentrations. Intracellular hormone concentrations were determined in cell lysates as described above

for the monolayer cultures. The use of transwells for studying long-term effects of drugs was previously described by our group in clinically non-functioning human pituitary adenoma cell cultures (Kwekkeboom et al., 1990).

In situ hybridization: the isolated cells were seeded on poly-L-lysine (0.5 mg/ml) coated coverslips. After 30 min. at 37°C in a CO_2-incubator the cells had attached to the coverslips. The cells were then placed in 2 ml culture medium (without or with octreotide) in 6-well plates (Costar). After 96 hour of incubation the coverslips plus cells were rinsed twice in phosphate buffered saline (PBS) and fixed with paraformaldehyde 4% in PBS, as described previously (Hofland et al., 1991). The incubation media were collected and stored at -20°C until analysis. In situ hybridization was performed using a ^{35}S-labelled 800 base pair human GH cDNA probe as described. Controls and quantification of grain numbers in individual adenoma cells were done as described (Velkeniers et al., 1988).

Hormone determinations

Human GH and PRL concentrations in the media and cell extracts were determined by immunoradiometric assays as described previously (Hofland et al., 1989 [b]). Glycoprotein alpha-subunit concentrations were determined by a double antibody radioimmunoassay as described by Kwekkeboom et al. (1989). IGF-I levels were measured by radioimmunoassay in EDTA plasma using a commercially available kit from Medgenix Diagnostics (Brussels, Belgium).

Data analysis

All data on hormone release and intracellular hormone concentrations are expressed in mean ± SE, n=4 wells per treatment group. Data of in situ hybridization experiments are expressed as the mean of at least 300 cells counted on two different slides. All data were analyzed by analysis of variance (ANOVA) to determine overall differences between treatment groups. When significant differences were found by ANOVA, a comparison between treatment groups was made using the Newman-Keuls test (Snedecor and Cochran, 1980). $p < 0.05$ was considered to be statistically significant.

Results

Monolayer studies

Table 2 shows the effects of a 96 hour incubation with octreotide (10nM) and bromocriptine (10nM) on GH, PRL, and α-subunit release and intracellular concentrations of pituitary adenoma cells from 7 acromegalic patients, cultured as monolayers. Octreotide significantly inhibited GH release in all seven cultures with a variation from 27 to 77% inhibition. Bromocriptine significantly inhibited GH release in 4 out of 6 cultures (variation between 18 and 59% inhibition). When effective on GH release, octreotide and bromocriptine induced a significant rise in intracellular GH levels in 6 of 7 and 2 of 4 adenomas, respectively (variation of rise by octreotide 24-517 %; variation of rise by bromocriptine 92-166 %). Octreotide inhibited PRL release in 3 of 4 cultures (variation 46-83 %) but caused a small rise (28%) in intracellular PRL levels in only 1 adenoma (patient 7). Bromocriptine inhibited PRL release in 3 out of 3 adenomas (variation 43-82 %), while it had no effect on intracellular PRL concentrations. Octreotide inhibited α-subunit release in 3 out of 3 cultures and caused a rise in the intracellular α-subunit concentration in only one adenoma (+58%, patient 7). In the two cultures producing α-subunit, in which the effect of bromocriptine was investigated, bromocriptine inhibited α-subunit in only 1 adenoma (by 72 %), without having a significant effect on intracellular α-subunit concentrations. In the majority of the adenomas the total amount of hormone (hormone in medium + hormone in cells) was significantly suppressed by octreotide (GH: 4 of 7 adenomas; PRL: 3 of 4 adenomas; α-subunit: 2 of 3 adenomas; data not shown in detail; see Table 2).

Figure 1 (upper part) shows that the effect of octreotide on both GH release and the intracellular GH concentration of the cultured adenoma cells from patient 5 is dose-dependent. In addition it is evident that, despite the significant dose-dependent inhibition of octreotide on PRL (middle part) and α-subunit (lower part) release, there is no effect of octreotide on the intracellular concentrations of these hormones. In all instances total hormone (medium plus intracellular) was significantly decreased by octreotide. The data presented in fig. 1 are representative for those found in the other tumour cell cultures. Adenoma cells of the two patients with significant elevated plasma PRL levels (patient no. 5 and 7) also showed the highest PRL production in vitro.

We found no correlation between basal plasma GH levels (Table 1) and GH secretion in the medium by the seven adenomas summarized in table 2. However, when the plasma GH level was expressed per tumour volume there was a statistically significant correlation ($p < 0.05$) between plasma GH per tumour volume and the in vitro

GH release by the seven adenomas summarized in Table 2 suggesting that the parameter GH/tumour volume may be a measure for the GH releasing activity of the GH secreting adenoma.

Transwell studies

Figure 2 (upper part) shows the cumulative GH production by GH-secreting pituitary adenoma cells from 6 patients (patient no. 3, and 7-11), cultured in transwells for periods up to 3 weeks. There was a considerable variation in GH production between the six adenoma cell cultures (variation from 0.1 to 400 μg GH produced after 3 weeks of culture). However, in all cases the adenoma cell cultures remained producing GH over the full 3 week period of culture. The lower part of figure 2 shows the effect of 10 nM octreotide on GH release of the six adenomas. The effect of octreotide is expressed as the percentage of control GH release at each time point. Octreotide significantly inhibited GH release in 5 of 6 adenomas at all time-points studied. GH release by the adenoma cells from patient 8 was not significantly inhibited by 10nM octreotide. As in the monolayer cultures there was a considerable variation between the cultures in the percentage inhibition induced by octreotide. Maximal inhibition varying between 14 and 90 %. Interestingly, the three adenomas with the highest cumulative GH production also showed the highest inhibitory response of GH release to octreotide. In 3 of 6 cultures we also investigated the effect of 3 weeks of exposure to octreotide in vitro on the intracellular GH concentrations. As shown in table 3 there was a significant rise (from 2 to 10-fold) in intracellular GH concentrations in all three adenomas, even after 3 weeks of exposure to octreotide in vitro. Finally, in order to show that octreotide treatment did not select a subpopulation of tumour cells, we withdrew octreotide treatment after 1 week of exposure in vitro (fig 3; patient no. 3, time-point of octreotide withdrawal is indicated by the black arrow). Withdrawal of octreotide resulted in a rapid increase in GH production as compared to continuously exposed cells (figure 3, upper part; $P < 0.01$ vs continuously treated cells at each time-point after octreotide withdrawal) resulting in a cumulative GH production parallel to that observed by untreated control cells. In addition the increase in intracellular GH concentrations which was observed after long-term in vitro exposure was also reversible by octreotide withdrawal (figure 3, lower part).

In situ hybridization

Finally, we investigated the effect of octreotide in a 24 and 96 hour incubation on GH mRNA expression in individual adenoma cells of three acromegalic patients. Table 4 shows that after 96h of incubation with 10nM octreotide GH mRNA levels were significantly increased by 27 and 109% in the cells from patient no. 2 and 12, respectively ($p<0.01$ vs control), whereas in the cells from patient no. 13 there was a slight but significant decrease in GH mRNA levels as compared to control cells (-16%, $p<0.01$). Parallel incubation in multiwell plates of the adenoma cells of patient 2 and 13 with 10nM octreotide resulted in a slight rise (+36%, table 2), and a slight decrease (-14%, data not shown) in intracellular GH levels respectively. The effects of octreotide on GH mRNA expression were time-dependent since after 24h of incubation there was no statistically significant effect on GH mRNA levels in the two cultures in which this was studied, while octreotide inhibited GH release by these adenomas at the same time (table 4).

Discussion

In contrast to the inhibitory effect of bromocriptine on tumour volume in patients with prolactinomas, the tumour shrinkage in response to octreotide is relatively poor in patients with GH-secreting adenomas (Lamberts, 1988). Tumour shrinkage of prolactinomas induced by treatment with bromocriptine may be the result of inhibition of PRL synthesis, caused by inhibition of intracellular PRL-mRNA levels (Maurer, 1980; Maurer, 1981), ultimately leading to a decrease in the individual tumour cell volume (Bassetti et al., 1984). At present however, little is known with respect to the effects of the somatostatin analogue octreotide on GH- mRNA production in human GH- secreting pituitary adenoma cells. In two previous studies it was demonstrated that in vitro exposure of human GH-secreting adenoma cells for 6-12 days and 24 hour respectively, to the native peptide somatostatin had no effect on hGH mRNA levels (Levy and Lightman, 1988; Davis et al., 1989). In the present study we further investigated the effects of long-term in vitro exposure of human GH-secreting pituitary adenoma cells to the stable somatostatin analogue octreotide (Bauer et al., 1982) on GH release, on the intracellular GH levels and on GH mRNA levels. In addition a comparison was made with the effects of bromocriptine on intracellular GH levels and GH release. Incubation of the adenoma cells for 4 days with octreotide resulted in a significant, dose-dependent increase in intracellular GH levels, although there was a considerable

variability among the adenoma cell cultures with respect to the percentage increase. In addition bromocriptine, when effective in inhibiting GH release by the adenoma cells, also induced an increase in intracellular GH levels. Even after 3 weeks of exposure of GH-adenoma cells to 10nM octreotide there was a considerable increase in the intracellular GH levels (+91%, +388% and +923%). Interestingly, in one adenoma (no. 3) which was cultured for 96 hours and for 3 weeks without or with octreotide, we found the increase of intracellular GH concentrations to be considerable higher in the cells which had been exposed to octreotide for three weeks. This suggests a stimulatory effect of octreotide on GH synthesis in this adenoma. These data are in contrast to the effects of long-term in vitro exposure of non functioning pituitary adenoma cells to bromocriptine, in which we demonstrated a significant inhibition of intracellular concentrations of gonadotropins or α-subunit (Kwekkeboom et al., 1990). In three adenoma cell cultures the effect of octreotide on GH mRNA content of the adenoma cells was also investigated. Incubation for 24 hours with 10nM octreotide had no effect on GH mRNA levels, while GH release by the adenoma cells was inhibited by octreotide at the same time. However, after 96 hour incubation with this concentration of octreotide GH mRNA levels were increased in two, and slightly decreased in one of the three cultures. These data suggest that somatostatin (analogues) may have an effect on GH mRNA production in GH-adenoma cells, but only after long-term exposure. Our data may also provide an explanation for the "lack" of effect of somatostatin on GH mRNA expression in human GH secreting pituitary adenomas as was previously shown in two other studies (Levy and Lightman, 1988; Davis et al., 1989). First, we used instead of native somatostatin a stable somatostatin analogue, which has a considerable longer biological half-life. Secondly, we only observed effects of octreotide on GH mRNA levels after a longer incubation time in vitro.

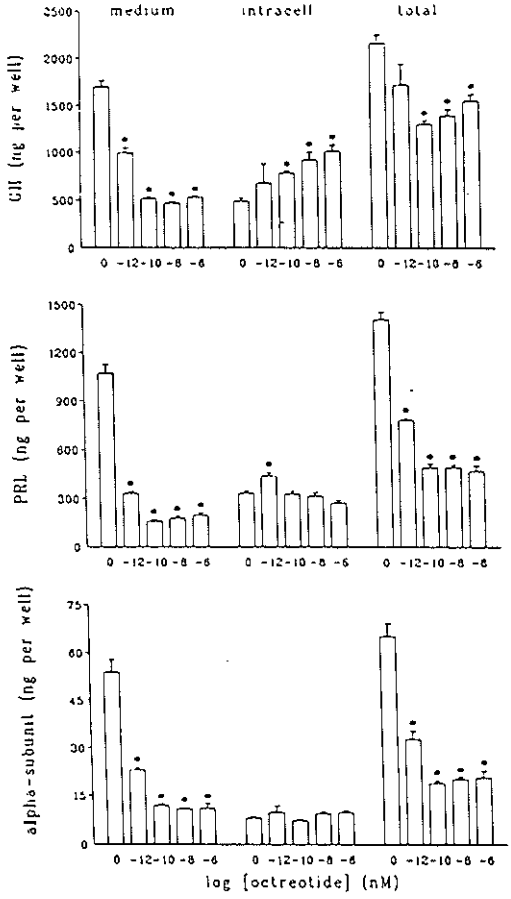
The considerable increase in intracellular GH concentrations, as well as the stimulation of GH mRNA concentrations in part of the adenomas may provide an additional explanation for the relatively poor effects of octreotide- treatment on tumour shrinkage. Previously we showed, using the reverse haemolytic plaque assay to quantify GH release by individual GH-adenoma cells, that these tumours contain a majority of low-secretor cells and a minority of high-secretor cells, with the high-secretor cells often being preferentially responsive to octreotide (Hofland et al., 1989 [a]). We have proposed that this heterogeneity in GH secretion among adenoma cells within a single adenoma might be a basis for the relatively poor effect of octreotide on tumour shrinkage (Hofland et al., 1989 [a]).

Interestingly, in the transwell studies (3 weeks of exposure with octreotide) we found that inhibition of GH release remained constant (on a percentage base), showing

no signs of desensitization to octreotide. These latter results are in agreement with in vivo studies in acromegalic patients, in which no "escape" from long-term therapy with octreotide has been reported yet (Lamberts, 1988). In our study we also found that total GH (sum of GH concentrations released into the medium and the GH concentrations in the adenoma cells) was significantly lowered by 10nM octreotide in most adenoma cell cultures. Since from the above experiments inhibition of GH synthesis seems an unlikely explanation for these observations, this inhibition may reflect an increase in GH breakdown by the lysosomes (crinophagy) (Lamberts, 1988; Asa et al., 1990). The increase in intracellular GH concentrations may be in agreement with several previous studies in which the number of secretory granules in GH- adenoma cells increased after in vitro (Peillon et al., 1976; Prysor Jones et al., 1981) exposure of human GH-adenoma cells to somatostatin. It also may provide an explanation for the rapid increase in plasma GH levels in acromegalic patients after withdrawal of octreotide therapy (Lamberts et al., 1987; Barakat and Melmed, 1989). Although we did not investigate in this study the effect of bromocriptine on GH mRNA levels, the significant increase in intracellular GH concentrations after a 96 hour exposure of the cells to 10nM bromocriptine suggests that the GH-adenoma cell responds with an increase in its GH storage pattern, irrespective of the nature of the GH release- inhibitory compound (octreotide or bromocriptine). At present we do not have an explanation for the observation that intracellular PRL and α-subunit concentrations did not increase, while the release of PRL and α-subunit was inhibited at the same time. At least in case of co-secretion of α-subunit these observations are not in line with the close relationship between basal- and secretagogue-induced GH and α-subunit release in cultured GH-adenoma cells (Hofland et al., 1989[b]), as well as in vivo (Beck-Peccoz et al., 1985; Ishibashi et al., 1987). Since GH and α-subunit might be released from the same secretory granule, as has been suggested by Beck-Peccoz et al. (1985), it is suggested from our experiments that the regulation of GH, PRL, or α-subunit synthesis, in case of co-production of these hormones in GH-adenoma cells follows a different pathway.

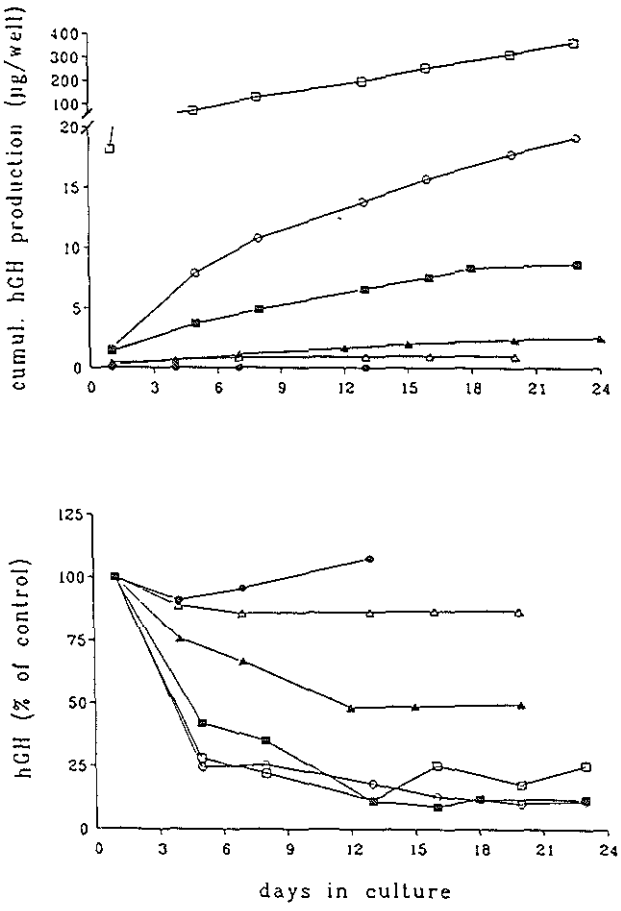
We conclude that: 1) long-term in vitro exposure of GH-adenoma cells to octreotide causes an increase in intracellular GH levels in the majority of the adenomas, which is probably due to an increase in GH mRNA levels in the adenoma cells; and 2) this increase in intracellular GH levels may be one of the explanations for the relatively poor effect of octreotide on tumour shrinkage in patients with GH-secreting pituitary adenomas.

Fig 1.



Effect of increasing concentrations of octreotide on GH, PRL, and α-subunit release, intracellular concentrations, and total hormone (hormone in medium + intracellular hormone) from the cultured GH secreting pituitary adenoma cells from patient 5. The cells were cultured for 4 days in MEM + 10% FCS, after which a 96 h incubation without or with octreotide was performed in quadruplicate. *$p<0.01$ vs control (0 nM octreotide).

Fig 2.

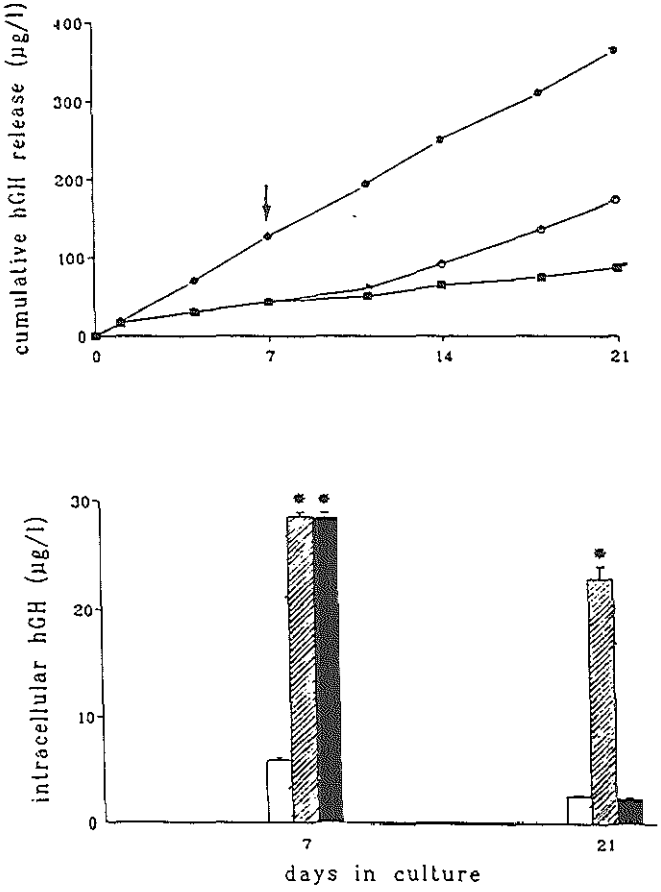


Cumulative GH production of six GH- secreting human pituitary adenoma cell suspensions (upper part), and response of GH release by the adenoma cells to 10 nM octreotide (lower part) during a 3 week exposure to the somatostatin analogue. Pituitary adenoma cells were cultured in MEM + 10% FCS in transwells as described in the materials and methods section. The effect of octreotide on GH release is expressed as the percentage of control GH release at each time point. n=4 wells per treatment group; SEM's were always less than 10%.

□ = patient No 3; ○ = No 7; ● = No 8; △ = No 9; ▲ = No 10

■ = patient No 11.

Fig 3.



Effect of withdrawal of octreotide in vitro on GH release (upper part) and on intracellular GH concentrations of cultured human GH-secreting pituitary adenoma cells from patient 3.
Upper part: (●) GH release by control cells; (■) continuous incubation with 10nM octreotide; (○) discontinuation (see arrow) of incubation with 10nM octreotide. Lower part: open bars, intracellular GH concentration of control cells; hatched bars, continuous incubation with 10nM octreotide; filled bars, discontinuation of incubation with 10nM octreotide after 6 days of exposure to octreotide. *$p<0.01$ vs. control

Table 1: data of the patients included in this study

patient no.	sex	tumour volume (cm')	basal GH (μg/L)	basal PRL (μg/L)	basal IGF-I (U/ml)	basal GH /tumour volume (μg/L per cm3)
1	M	72.160	14.4	7.0	4.6	0.2
2	M	8.280	9.7	14.2	4.2	1.2
3	M	12.000	129.0	16.2	4.1	10.8
4	M	0.576	6.3	3.8	7.7	10.9
5	M	1.200	28.5	35.6	6.9	23.8
6	M	0.576	4.4	4.0	4.4	7.6
7	F	5.632	38.6	125.0	4.0	6.9
8	F	5.700	19.3	19.9	8.0	3.4
9	M	1.056	6.5	6.6	7.8	6.2
10	M	1.000	25.0	4.2	8.6	25.0
11	F	1.716	32.4	13.7	7.4	18.9
12	M	3.150	106.0	4.4	6.1	33.7
13	F	1.716	110.0	9.4	6.5	64.1

M=male, F=female; Normal GH values ranged from 0.2-0.5 μg/L, the upper limit of normal PRL levels is 12μg/L in men and 15μg/L in women, normal IGF-I levels are values ranged from 0.6-1.7 U/ml. Tumour volume was calculated by multiplying the maximal diameter in three directions of the pituitary adenoma as seen at CT scanning.

Table 2: Effects of octreotide (10nM) and bromocriptine (10nM) on GH, PRL, and α-subunit release and intracellular concentrations of GH- secreting pituitary adenoma cells from seven acromegalic patients.

PATIENT NO.	TREATMENT	GH (NG/WELL) MEDIUM	GH (NG/WELL) INTRACELL.	PRL (NG/WELL) MEDIUM	PRL (NG/WELL) INTRACELL.	α-SUBUNIT (NG/WELL) MEDIUM	α-SUBUNIT (NG/WELL) INTRACELL.
1	CONTROL	21±1	29±2	-	-	-	-
	OCTR (10nM)	13±0[a] (59)	36±0[a] (124)	-	-	-	-
	BROMO(10nM)	18±1[a] (82)	25±1	-	-	-	-
2	CONTROL	288±6	39±1	-	-	-	-
	OCTR (10nM)	210±14[a] (73)	53±3[a] (136)	-	-	-	-
	BROMO(10nM)	267±5	44±3	-	-	-	-
3	CONTROL	24000±707	1830±56	-	-	-	-
	OCTR (10nM)	4533±233[a] (19)	11283±502[a] (617)	-	-	-	-
	BROMO(10nM)	12567±296[a] (52)	4867±262[a] (266)	-	-	-	-
4	CONTROL	269±17	667±33	51±3	39±2	-	-
	OCTR (10nM)	101±8[a] (38)	761±31	46±1	40±3	-	-
	BROMO(10nM)	176±7[a] (65)	693±22	29±1[a] (57)	33±2	-	-
5	CONTROL	1697±66	437±41	1071±54	325±18	54±4	8±0
	OCTR (10nM)	470±12[a] (28)	913±83[a] (209)	178±14[a] (17)	315±25	11±0[a] (20)	9±1
	BROMO(10nM)	693±64[a] (41)	839±126[a] (192)	193±14[a] (18)	320±22	15±1[a] (28)	9±1
6	CONTROL	4528±294	1288±63	350±23	97±3	67±3	12±0
	OCTR (10nM)	1097±42[a] (24)	3027±107[a] (235)	136±5[a] (39)	104±3	16±0[a] (24)	14±1
	BROMO(10nM)	3920±166	1600±87	117±6[a] (33)	109±2	54±4	13±1
7	CONTROL	4575±105	3952±170	3027±139	2307±54	6±0	7±0
	OCTR (10nM)	1063±11[a] (23)	6453±337[a] (163)	1627±54[a](54)	2970±26[a](128)	3±0[a] (50)	11±1[a] (158)

Incubation time was 96 hour; n=4 wells per treatment group. [a]$p<0.05$ vs control. Values in brackets are the percentages of control.

Table 3: The effect of long-term treatment in vitro with octreotide on intracellular GH concentrations of human GH-secreting pituitary adenoma cells.

PATIENT no.	INCUBATION TIME (DAYS)	TREATMENT	INTRAC. GH (NG/WELL)
3	23	CONTROL	2488 ± 174
		OCTR (10nM)	22975 ± 1159*
7	23	CONTROL	681 ± 86
		OCTR (10nM)	2642 ± 270*
9	21	CONTROL	4.4 ± 0.7
		OCTR (10nM)	8.4 ± 0.6*

values in mean ± SE; n=4 wells per group; *p<0.01 vs control

Table 4: The effect of octreotide on GH mRNA levels and GH release of cultured human GH secreting pituitary adenoma cells.

PATIENT no.	INCUBATION TIME	TREATMENT	MEAN GRAIN COUNT	GH RELEASE (ng per well)
2	24 h	CONTROL	24.5	71 ± 1
		OCTR (10nM)	21.3	49 ± 1*
	96 h	CONTROL	16.4	210 ± 2
		OCTR (10nM)	20.9*	126 ± 14*
12	24 h	CONTROL	-	-
		OCTR (10nM)	-	-
	96 h	CONTROL	60.2	968 ± 3
		OCTR (10nM)	125.8*	750 ± 40*
13	24 h	CONTROL	120.7	353 ± 22
		OCTR (10nM)	121.1	173 ± 7*
	96 h	CONTROL	123.2	560 ± 50
		OCTR (10nM)	102.9*	323 ± 9*

Mean grain count is expressed in number of silver grains per cell; at least 300 cells counted on 2 different slides. Values of GH release are means ± SE; n = 2 wells per group; *$p<0.01$ vs control; - = not done.

References

Asa SL, Felix I, Singer W, Kovacs K. (1990) Effects of somatostatin on human pituitary somatotroph adenomas: an in vitro functional and morphologic study. *Endocrine Society,* abstr 93.

Barakat S, Melmed S. (1989) Reversible shrinkage of a growth hormone-secreting pituitary adenoma by a long-acting somatostatin analogue, octreotide. *Archives of Internal Medicine,*149, 1443-1445.

Barkan AL, Lloyd RV, Chandler WF, Hatfield MK, Gebarski SS, Kelch RP, Beitins IZ. (1988) Preoperative treatment of acromegaly with the long-acting somatostatin analog SMS 201-995: shrinkage of invasive pituitary macroadenomas and inproved surgical remission rate. *Journal of Clinical Endocrinology and Metabolism,*67, 1040-1048.

Bassetti M, Spada A, Pezzo G, Giannatasio G. (1984) Bromocriptine treatment reduces the cell size in human macroprolactinomas: a morphometric study. *Journal of Clinical Endocrinology and Metabolism,*58, 268-273.

Bauer W, Briner U, Doepner W, Haller R, Huguenin R, Marbach P, Petcher TJ, Pless J. (1982) SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Science,*31, 1133-1140.

Beck-Peccoz P, Bassetti M, Spada A, Medri G, Arosio M, Giannattassio G, Faglia G. (1985) Glycoprotein hormone alpha-subunit response in patients with active acromegaly. Evidence for alpha-subunit and GH coexistence in the same tumoral cell. *Journal of Clinical Endocrinology and Metabolism,*61, 541-546.

Davis JRE, Wilson EM, Vidal ME, Johnson AP, Lynch SS, Sheppard MC. (1989) Regulation of growth hormone secretion and messenger ribonucleic acid accumulation in human somatotropinoma cells in vitro. *Journal of Clinical Endocrinology and Metabolism,*69, 704-708.

Gick G, Bancroft FC. (1987) Glucocorticoid stimulation of growth hormone messenger ribonucleic acid levels in GH3 cells is inhibited by calcium but not by somatostatin. *Endocrinology,*120, 1986-1990.

Hofland LJ, van Koetsveld PM, van Vroonhoven CCJ, Stefanko SZ, Lamberts SWJ. (1989 [a]) Heterogeneity of GH release by individual pituitary adenoma cells from acromegalic patients as determined by the reverse haemolytic plaque assay: Effects of SMS 201-995, growth hormone releasing hormone (GHRH), and thyrotropin-releasing hormone (TRH). *Journal of Clinical Endocrinology and Metabolism,*68, 613-620.

Hofland LJ, van Koetsveld PM, Verleun TM, Lamberts SWJ. (1989 [b]) Glycoprotein hormone alpha-subunit and prolactin release by cultured pituitary adenoma cells from acromegalic patients: correlation with GH release. *Clinical Endocrinology,*30, 601-611.

Hofland LJ, Velkeniers B, van Koetsveld PM, Hooghe-Peters EL, Lamberts SWJ. (1991) The relationship between growth hormone (GH) messenger ribonucleic acid levels and hormone release from individual cells derived from human GH-secreting pituitary adenomas. *Clinical Endocrinology*,34, 5-11.

Ishibashi M, Yamaji T, Takaku F, Teramoto A, Fukushima T. (1987) Secretion of glycoprotein hormone alpha-subunit by pituitary tumors. *Journal of Clinical Endocrinology and Metabolism*,64, 1187-1193.

Jackson I, Barnard LB, Lamberton P. (1986) Role of a long-acting somatostatin analogue (SMS 201-995) in the treatment of acromegaly. *American Journal of Medicine*,94, 94-99.

Kwekkeboom DJ, de Jong FH, Lamberts SWJ. (1989) Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas in vivo and in vitro: relation to sex and effects of TRH, GnRH and bromocriptine. *Journal of Clinical Endocrinology and Metabolism*,68, 1128-1135.

Kwekkeboom DJ, Hofland LJ, van Koetsveld PM, Singh R, van den Berge JH, Lamberts SWJ. (1990) Bromocriptine increasingly suppresses the in vitro gonadotropin and α-subunit release from pituitary adenomas during long term culture. *Journal of Clinical Endocrinology and Metabolism*,71, 718-724.

Lamberts SWJ. (1984) Antimitotic actions of dopaminergic drugs on human pituitary tumors. *Neuroendocrine Perspectives*,3, 317-343.

Lamberts SWJ, Uitterlinden P, Verschoor L, van Dongen KJ, del Pozo E. (1985) Long-term treatment of acromegaly with the somatostatin analogue SMS 201-995. *New England Journal of Medicine*,313, 1576-1580.

Lamberts SWJ, Uitterlinden P, del Pozo E. (1987) SMS 201-995 induces a continuous decline in circulating growth hormone and somatomedin-C levels during therapy of acromegalic patients for over two years. *Journal of Clinical Endocrinology and Metabolism*,65, 703-710.

Lamberts SWJ. (1988) The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of its analogs in the treatment of human pituitary tumors. *Endocrine Reviews*,9, 417-436.

Levy A, Lightman SL. (1988) Quantitative in-situ hybridization histochemistry studies on growth hormone (GH) gene expression in acromegalic somatotrophs: effects of somatostatin, GH-releasing factor and cortisol. *Journal of Molecular Endocrinology*,1, 19-26.

Maurer RA. (1980) Dopaminergic inhibition of prolactin synthesis and prolactin messenger RNA accumulation in cultured pituitary cells. *Journal of Biological Chemistry*,255, 8092-8097.

Maurer RA. (1981) Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP. *Nature*,194, 94-97.

Nambda H, Morita S, Melmed S. (1989) Insulin-like growth factor-I action on growth hormone secretion and messenger ribonucleic acid levels: interaction with somatostatin. *Endocrinology*,124, 1794-1799.

Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SWJ. (1984) Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. *Journal of Endocrinological Investigation,*7, 307-311.

Peillon F, Garnier PE, Brandi A, Rivaille P, Chaussain JL. (1976) Effect in vitro du SRIF (somatostatin) sur les adenomas somatotropes humains. Ultrastructure et secretion etudiees a l'aide de ^{3}H-Leucine. *Annals d'Endocrinologique,*37, 271-272.

Prysor Jones RA, Kennedy SJ, O'Sullivan JP, Jenkens JS. (1981) Effect of bromocriptine, somatostatin and oestradiol-17 on hormone secretion and ultrastructure of human pituitary tumours in vitro. *Acta Endocrinologica,*98, 14-23.

Silverman BL, Bettendorf M, Kaplan SL, Grumbach MM, Miller WL. (1989) Regulation of growth hormone (GH) secretion by GH-releasing factor, somatostatin, and insulin-like growth factor I in ovine fetal and neonatal pituitary cells in vitro. *Endocrinology,*124, 84-89.

Snedecor GW, Cochran WG. (1980) *Statistical Methods.* 7th ed. Ames, Iowa State university Press p. 235.

Velkeniers B, Hooghe-Peters EL, Hooghe R, Belayew A, Smets G, Claeys A, Robberecht P, Vanhaelst L. (1988) Prolactin cell subpopulations separated on discontinuous percoll gradient: an immunocytochemical, biochemical, and physiological characterization. *Endocrinology,*123, 1619-1630.

Wood DF, Docherty K, Ramsden DB, Sheppard. (1987) A comparison of the effects of bromocriptine and somatostatin on growth hormone gene expression in the rat anterior pituitary gland in vitro. *Mol Cell Endocrinology,*52, 257-261.

CHAPTER 6

PREOPERATIVE TREATMENT OF ACROMEGALY WITH THE SOMATOSTATIN ANALOG OCTREOTIDE

Introduction:

When postoperative GH levels of less than 5 μg/l are used as a criterium, only 60 % of the patients with acromegaly are cured after transsphenoidal resection of the pituitary adenoma. Only 30% of the patients with macroadenoma reach GH levels < 5 μg/l after surgery and in the case of tumors with clear extrasellar extension only very few patients are "cured" after operation [1] [2] [3]. These findings indicate rather poor results of surgery, especially when one realizes that about 50% of patients with post-operative GH levels of less than 5 μg/l do still have elevated circulating IGF-I concentrations [4]. One has to conclude therefore that a real "cure" of acromegaly after surgery in patients with tumors bigger than 1 cm and/or with extrasellar extension might be rare.

Fahlbusch and coworkers claimed that the outcome of transsphenoidal operation for macroprolactinomas can be improved by treating these patients preoperativily with dopaminergic drugs [5]. It has been shown previously that treatment with the somatostatin analog octreotide reduces tumor volume in about 50% of acromegalic patients [6] [7]. Also total resolution of the adenoma has been reported in exceptional cases [8]. The separation during operation of GH-producing tumor tissue from the surrounding "normal" pituitary gland might be facilitated by treating patients preoperativily with octreotide [9]. Barkan and coworkers found an improved surgical remission rate in 10 patients treated preoperativily with octreotide for 3-30 weeks [7].

To investigate whether these findings can be confirmed in a larger series of patients, a prospective multicentre open label randomized comparative study is currently performed by the Sandoz Company (Basel, Switzerland) in 100 previously untreated acromegalic patients. These 100 patients are randomly allocated to receive either transsphenoidal surgery immediately, or octreotide, administered subcutaneously 100 μg t.i.d. for a period of 4 months prior to surgery. Only patients with adenomas exceeding 10 mm in diameter on MRI/CT scan can enter the study. In all patients standard baseline laboratory assessment is performed. Also baseline and stimulated hormone levels of all the pituitary target organ axes are determined. Evaluation of serum IGF-I levels and 24 hour GH concentrations is done as well as an oral glucose tolerance test. After four months of octreotide therapy the size of the adenoma is determined again in order to measure potential tumor shrinkage. All measurements are repeated within 7 days following transsphenoidal surgery, as well as 2, 4 and 6 months after surgery.

In this study we participated by entering 20 patients. Ten of them were allocated to receive octreotide and the other ten were operated upon directly without pretreatment. This multicentre trial is currently (december 1991) not finished yet. No final results of the study are available therefore and final conclusions can not yet be drawn. However, we examined the results of the 20 patients we investigated according to the rules of the protocol. Statistical analysis was done, using a paired Student-t test. It has to be emphasized that our results are preliminary and that we mention here only part of the data accumulated in these 20 patients.

Results and discussion:

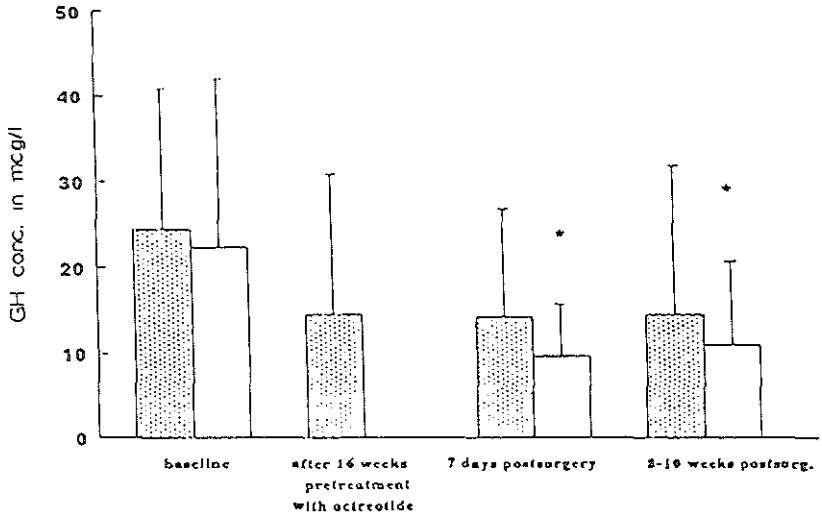
Pituitary tumor volume was measured by multiplying height, width and depth of the tumor image on CT/MRI scan, before and 16 weeks after octreotide therapy. In three patients a shrinkage by 8, 21 and 67% of pretreatment volume was observed while tumors of the five other evaluable patients had not changed in size during octreotide administration. Mean GH levels (24 samples, taken hourly in 24 hours) were reduced after 16 weeks of octreotide therapy by 51% from 24.4 ± 16.5 to 14.6 ± 16.2 μg/l (mean ± SEM; $p<0.05$; Fig. 1). In these patients IGF-I levels had also decreased significantly from 7.5 ± 1.4 mU/l before therapy to 3.3 ± 1.5 mU/l after 16 weeks of octreotide therapy ($p<0.05$). Postoperatively, GH levels in these pretreated patients amounted to 14.3 ± 12.5 μg/l ($p<0.05$ vs pretreatment) while IGF-I levels were 4.1 ± 1.6 mU/l ($p<0.05$ vs pretreatment values).
In the control (non octreotide pretreatment) group postoperative GH levels had decreased from 22.3 ± 19.8 to 9.7 ± 6.0 μg/l ($p<0.05$), while IGF-I levels had remained unchanged (7.2 ± 1.6 vs 7.9 ± 2.4 mU/l; p: N.S.; see Fig. 1). Eight to ten weeks after surgery however, the initially observed postoperative in IGF-I levels in the octreotide pretreated group was not significant any more. The reduction in serum GH levels after surgery which occurred both in the control as well as in the octreotide pretreatment group was still present after 10 weeks postsurgery, however.

These findings suggest that pretreatment of acromegalic patients with octreotide before surgery does not improve the outcome of surgery when postoperative GH and IGF-I levels are used as criterium. However, we want to emphasize again that final conclusions can only be drawn when the results of all 100 patients who entered the study have been analyzed.

We conclude that the significant reduction in IGF-I levels initially observed after surgery in the octreotide-pretreated patient group must have been the result of the

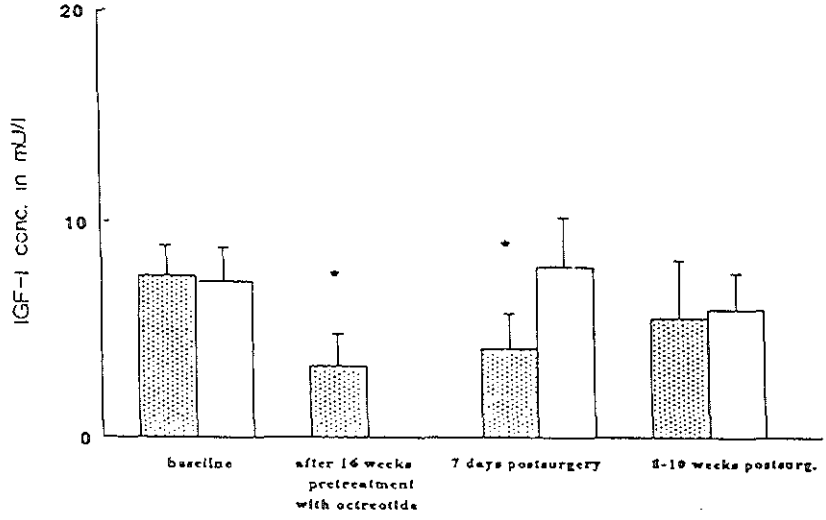
administration of octreotide itself, which had been continued until the day of the transsphenoidal resection.

Fig.1



Mean GH concentrations ± SEM in 20 acromegalic patients. Ten patients received octreotide preoperatively for a period of 16 weeks (dotted areas), while ten patients were treated with surgery without pretreatment (white areas). * reflects a significance of $p < 0.05$.

Fig.2



Mean IGF-I concentrations ± SEM in 20 acromegalic patients. Ten patients received octreotide preoperatively for a period of 16 weeks (dotted areas), while ten patients were treated with surgery without pretreatment (white areas). * reflects a significance of $p < 0.05$. Note that octreotide therapy caused a significant decrease of IGF-I levels not only 16 weeks after start of treatment but also in IGF-I levels seven days after surgery. This significant decrease has disappeared 8-10 weeks after surgery.

References:

1. Ross DA, Wilson CB. Results of transsphenoidal microsurgery for growth hormone-secreting pituitary adenoma in s series of 214 patients. J Neurosurg 1988;68: 854-867.

2. Laws ER, Carpenter SM, Scheithauer BW, Randall RV. Long-term results of transsphenoidal surgery for the management of acromegaly. In: Robbins R, Melmed S, (eds). Acromegaly: a century of scientific and clinical progress. Plenum Press, New York. 1987: 241-248.

3. Quabbe HJ. Treatment of acromegaly by transsphenoidal operation. 90-yttrium implantation and bromocriptine: results in 230 patients. Clin Endocrinol (Oxf) 1982;16: 107-119.

4. Barkan AL. Acromegaly, diagnosis and therapy. Endocrinol Metab Clin 1989;18: 277-310.

5. Fahlbusch R, Buchfelder M, Schrell U. Short-term preoperative treatment of macroprolactinomas by dopamine agonists. J Neurosurg 1987;67: 807-810.

6. Lamberts SWJ, Uitterlinden P, Verschoor L, van Dongen KJ, del Pozo EE. Long-term treatment of acromegaly with the somatostatin analog SMS 201-995. N Eng J Med 1985;313: 1576-1580.

7. Barkan AL, Lloyd RV, Chandler WF, Hatfield MK, Gebarski SS, Kelch RP, Beitins IZ. Preoperative treatment of acromegaly with long-acting somatostatin analog SMS 201-995: shrinkage of invasive pituitary macroadenomas and improved surgical remission rate. J Clin Endocrinol Metab 1988;67: 1040-1048.

8. Barakat S, Melmed S. Reversible shrinkage of a growth hormone-secreting pituitary adenoma by a long-acting somatostatin analogue, octreotide. Arch Intern Med 1989;149: 1443-1445.

9. Spinas GA, Zapf J, Landolt AM, Stuckman G, Froesch ER. Pre-operative treatment of 5 acromegalics with a somatostatin analogue: endocrine and clinical observations. Acta Endocrinol (Copenh) 114;1987: 249-256.

CHAPTER 7.

THE EFFICACY AND TOLERABILITY OF CV 205-502 (A NON-ERGOT DOPAMINERGIC DRUG) IN MACROPROLACTINOMA PATIENTS AND IN PROLACTINOMA PATIENTS INTOLERANT TO BROMOCRIPTINE.

A.J. van der Lely, J. Brownell and S.W.J. Lamberts, Department of Medicine, Erasmus University, Rotterdam, The Netherlands, and Department of Neuroendocrinology, Clinical Research Sandoz Pharma, Basle, Switzerland.

This manuscript was published in the Journal of Clinical Endocrinology and Metabolism 1991;72: 1136-1141.

ABSTRACT.

We studied the effect of CV 205-502 in 12 patients with macroprolactinomas and in 8 patients with PRL secreting tumors, who were selected because of previous repeatedly shown intolerance to bromocriptine in even small doses. We also investigated serum insulin-like growth factor-I (IGF-I) levels before and during CV 205-502 therapy. In 12 macroprolactinoma patients followed for 1 year, 0.075-0.450 mg CV 205-502 lowered PRL levels by 91.2 ± 5.4 %. Only 3 of the patients had transient side-effects of nausea, dizziness or fatigue. In eight patients with PRL-secreting tumors who were bromocriptine intolerant, CV 205-502 (0.075-0.300 mg daily) lowered PRL levels by 80.2 ± 6.3 %. Four of these patients showed transient side effects (nausea, fatigue and/or tachycardia). None of the patients discontinued therapy.

There was a close correlation between pretreatment circulating PRL levels and tumor size, expressed in cubic millimeters. The decrease in pituitary tumor size after 52 weeks of CV 205-502 therapy (-74 ± 6%) was also correlated with the decrease in PRL levels ($p<0.01$). In four patients with hypopituitarism, lowered IGF-I levels did not change during CV 205-502 therapy. However, in seven previously untreated patients with a macroprolactinoma and normal anterior pituitary function IGF-I levels increased during CV 205-502 therapy by 113-167 % ($p<0.05$).

CV 205-502 is a potent dopaminergic drug, which effectively controls PRL secretion and induces tumor shrinkage. At the doses used in our study, it causes only mild and transient side-effects in a minority of patients and can also be used to treat hyperprolactinemic patients who have shown intolerance to bromocriptine therapy.

INTRODUCTION

CV 205-502, an octahydrobenzo-[g]-quinoline, is a non-ergot dopamine agonist which recently became available for therapy of hyperprolactinemia (1,2). It is a potent D-2 receptor agonist with weak D-1 receptor activity. In studies using single oral doses of 0.06 mg, CV 205-502 suppressed normal PRL secretion from within two hours of intake continuously for 36 hours (3).In several studies CV 205-502, given orally, was on a weight basis far more potent than bromocriptine in suppressing normal and pathological PRL secretion (4,5).

We studied the effect of CV 205-502 in 12 macro- prolactinoma patients and in eight patients with PRL secreting tumors who were previously repeatedly shown to be intolerant to bromocriptine. We also investigated circulating IGF-I concentrations before and during CV 205-502 therapy in 11 of the prolactinoma patients who had not been previously treated with dopaminergic drugs.

MATERIALS AND METHODS

Written informed consent was obtained from each patient prior to entering the study, which was approved by the Ethics Committee of the University Hospital of Rotterdam. Criteria for exclusion were pregnancy, radiation therapy for a pituitary tumor within the previous five years, mixed cell tumors with clinical or biochemical evidence of acromegaly or Cushing's disease, a history of chronic liver or kidney disease and clinically relevant hyper- or hypotension. The study was supported by Sandoz (Basel; Switzerland). No financial gain was involved, however, for patients or investigator.

Fourteen Patients with a macroprolactinoma (eight men and six women; age 23-61 years) entered the study. There were two dropouts from this group, one after 6 months of therapy because of a squamous cell carcinoma of the oropharynx and one after 9 months for personal reasons. A second group of nine prolactinoma patients (three macro- and six microprolactinomas) were investigated who had been referred to our hospital because of intolerance to bromocriptine. In all patients bromocriptine therapy had repeatedly failed because of side-effects, despite all usual precautions including low initial doses starting at 1.25 mg orally during meals twice daily, followed by gradual increase of the dose to 2.5 mg b.i.d. over a period exceeding 14 days. Such trials to build up the dose of bromocriptine had failed in all 9 patients at least twice, both in the referring hospital as well as in ours. There was one dropout after eleven months of CV

205-502 therapy because of carcinoma of the bladder. The three patients who dropped out of the study, had not had side-effects of CV 205-502 therapy.

All patients were followed for 12 months. The patients were advised to take CV 205-502 at bedtime together with a small snack. During the first three days 0.025 mg was administered, while the dose was doubled to 0.050 mg on the evening of days 4-6. Thereafter 0.075 mg CV 205-502 was given for the subsequent 24 days. In principle the dose had to be further increased by 0.075 mg every 2-4 weeks until normalization of PRL levels was reached. However, when galactorrhea, amenorrhea and sexual dysfunction had disappeared before PRL levels had normalized, the dose of CV 205-502 was not further increased in a number of patients. Patients who had been treated with bromocriptine stopped the use of this drug at least one month before the start of the study. Each subject was monitored for routine liver and kidney function, serum electrolytes and hematologic parameters at the baseline and after 3, 6 and 12 months of treatment. Throughout the study measurements of vital signs, including supine and standing blood pressure and pulse rates were collected at each visit to the out-patient clinic, i.e. at weeks 2,4,6, 8 and 12 and at months 4,5,6,9 and 12. In each patient the function of the pituitary-thyroid and pituitary-adrenal axis were measured before the start of CV 205-502 therapy. If hypothyroidism and/or adrenal insufficiency were present supplementation therapy was started prior to the study. Serum PRL and IGF-I levels were measured at each clinic visit.

PRL levels were measured by IRMA,(Medgenix Diagnostics; Brussels, Belgium, inter-assay variation 6.8%, intra-assay variation 6.4%).Normal serum PRL levels are 15 μg/l or less. IGF-I levels were measured by RIA,(Medgenix Diagnostics; Brussels,Belgium, inter-assay variation 9.0%, intra-assay variation 5.2%.Normal serum IGF-I levels vary between 0.5 and 2.2 U/ml.

Pituitary tumor size was evaluated before and after 1 yr of CV 205-502 therapy by CT-scanning using a Philips Tomoscan 310 (Phillips Electronic Instruments, Mahway, NJ). Special care was given to obtain similar pictures at both scannings by making slices throughout the tumor at a distance of two mm. Tumor volume was calculated in mm3 by multiplying the maximal tumor diameter measured in three directions.

Statistical analysis of the results was performed using analysis of variance for comparison of the PRL and IGF-I levels before and at several intervals after the start of the study. The results were considered to be statistically significant at $p<0.05$.

RESULTS

Both in the patients with macroprolactinomas as well as in those who were previously intolerant to bromocriptine treatment with CV 205-502 caused a significant reduction in serum PRL levels. Table 1 shows the PRL levels before and after 12 and 52 weeks of treatment with CV 205-502 in the twelve patients with macroprolactinomas. After 52 weeks of therapy, CV 205-502 (0.075-0.450 mg/day) had decreased PRL levels by 34-100% (mean 91.2 ± 2.9 %; mean ± SEM). Only 3 of these patients had transient side-effects during the first 2 weeks of therapy. (nausea,dizziness or fatigue). No differences in the percentage decrease in circulating PRL levels were observed between 12 and 52 weeks of therapy.

Table 1 shows the results of CV 205-502 therapy on PRL levels in the eight bromocriptine-intolerant patients. CV 205-502 (0.075-0.300 mg/day) lowered prolactin levels by 58-98% (mean 80.2 ± 6.3 %). Four of these patients showed transient side-effects including nausea, tiredness and/or tachycardia, but none of the patients discontinued CV 205-502 therapy.

In 8 of the 20 prolactinoma patients who completed the study the slowly increasing dose schedule of CV 205-502 already resulted in normalization of circulating PRL levels after 3 months. After 1 yr of CV 205-502 therapy PRL levels had normalized in 13 patients. In a number of patients in whom galactorrhea, amenorrhea and/or sexual dysfunction had normalized before circulating PRL levels had normalized the dose of CV 205-502 was not further increased. However, such an increase in dose had not been omitted because of the occurrence of side-effects of the drug. No changes in physical, electrocardographic or hematological profiles or serum chemistries occurred. A slight postural hypotension was noted at the clinic visits throughout CV 205-502 therapy in almost all patients. However, this decrease in upright systolic and diastolic blood pressure by a mean of 5 mmHg did not result in clinical symptomatology.

Table 2 shows the effects of CV 205-502 therapy on galactorrhea, menstrual cycles, libido and/or potency as well as on headache and on the general condition. Eight of the 12 female patients studied showed normalization of the menstrual cycle. The four remaining patients, who also had pituitary insufficiency needing replacement therapy with thyroid and adrenal hormones, remained amenorrheic during therapy. Galactorrhea, which was present before the start of therapy in four patients, disappeared in all of them. All but four patients showed a normalization of libido and/or potency and all patients reported an improvement in the sense of well-being during CV 205-502 therapy. This was accompanied in most instances by a disappearance of headaches.

Eleven of the patients had not been treated with dopamine agonists during the

previous 12 months and eight of them had never been treated at all. Four patients with hypopituitarism had lowered IGF-I levels before the start of therapy. During CV 205-502 treatment no changes in IGF-I levels were observed in these patients (data not shown). The other seven patients had normal pituitary thyroid and -adrenal function before therapy. Pretreatment IGF-I levels were normal in four and lowered in three patients. IGF-I levels increased during CV 205-502 therapy by 113-167 % ($p < 0.05$ vs pretreatment levels). The maximal increment in IGF-I levels occurred several weeks after the (sub)maximal suppression of PRL levels.

Two patients had visual disturbances which improved during therapy. The effect of CV 205-502 on tumor size was determined in the 13 patients with a tumor which was measurable at CT scan examination. Tumor size reduction after 1 yr varied between 37 and 99 % (74 ± 6 %, mean ± SEM), and a relationship between the pretreatment PRL levels and tumor size was seen ($r = 0.80$; $p < 0.01$). There was also a relationship between the degree of suppression of PRL levels and the degree of tumor shrinkage after one year of CV 205-502 therapy ($r = 0.72$; $p < 0.05$). Table 3 shows the changes in tumor size during CV 205-502 treatment.

DISCUSSION

Ergot-derivatves with dopamine agonist activity, like bromocriptine, have been proven to be effective in the treatment of hyperprolactinemia (6,7,8). Bromocriptine has a relatively short duration of action necessitating a b.i.d. administration in most patients. Side-effects such as nausea, vomiting, dizziness and weakness result in intolerance and have been reported to cause discontinuation drug therapy in up to 6% of hyperprolactinemic patients (9). Therefore, there is a need for new dopaminergic drugs with a longer duration of action and fewer side-effects. CV 205-502 has been shown to be effective in suppressing PRL secretion in hyperprolactinemic patients with and without tumor (10,11). The results of the present study confirm the drug's high efficacy following single once daily administration, and show that the compound can also be used successfully in patients with hyperprolactinemia who were previously repeatedly shown to be intolerant to bromocriptine. These preliminary data support the results of a double-blind controlled study which compared the efficacy and tolerability of CV 205-502 with that of bromocriptine and found that indeed the new drug caused fewer side-effects than bromocriptine in hyperprolactinemic women (24). Rasmussen et al (10) showed in 24 non-tumoral hyperprolactinemic women, that CV 205-502 in doses up to 0.150 mg daily for six months decreased PRL levels by a mean of 90%. PRL levels became normal in 71% of the patients. In another study Vance et al (11) reported that CV 205-502 therapy in doses which did not exceed 0.09 mg daily reduced PRL levels in 26 hyperprolactinemic patients by 66%. PRL levels became normal in 54% of these patients. In a later study by Vance et al (12) reported that CV 205-502 in doses up to 0.400 mg daily reduced PRL levels in macroprolactinoma patients by 98%. PRL levels became normal in 58% of patients and tumor size was reduced by an average of 19% (range 6% to 67%). In the present study we used 0.075-0.450 mg of CV 205-502 daily. Twelve of the 20 patients in our study had a macroprolactinoma. After 1 yr of therapy PRL levels had decreased by 34-100 % (mean decrease - 86%). In 13 of the 20 patients PRL levels reached normal values (65%). In parallel menstrual cycles and sexual dysfunction had normalized in most patients. Also galactorrhea, which was only observed in a rather low percentage of our patients disappeared during CV 205-502 in all patients. The lower incidence of galactorrhea in our patients, in comparison with previous reports (6,7,11) cannot be readily explained. These observations suggest that the optimal dose of CV 205-502 for each patient has to be titrated individually. None of our patients discontinued therapy of CV 205-502 because of side-effects. One of the reasons that CV 205-502 was tolerated so well might be related to the fact that CV 205-502 was administered in the evening with a snack just before bedtime. In addition, the dose of

CV 205-502 was increased very slowly over a period of days-weeks, while a very low starting dose was given. However, improved tolerability may be an intrinsic characteristic of CV 205-502. Although the incidence of side-effects of CV 205-502 reported in hyperprolactinemic patients varies considerably, good tolerability was observed both in two double-blind placebo-controlled studies (5,10) and in a later double-blind trial of CV 205-502 versus bromocriptine (24). In these trials patients participated who had history of intolerance to bromocriptine, but had few or no problems tolerating CV 205-502. In other studies by Vance et al (11,12), however, a rather high incidence of side-effects during CV 205-502 treatment was reported (39% side-effects including drop-out because of side-effects in 2 of 26 patients).

In four patients who had hypopituitarism, the lowered IGF-I levels did not change during CV 205-502 therapy. In 7 patients with normal anterior pituitary function IGF-I levels increased by 113-167 % during CV 205-502 therapy. At present it is not known whether this increase in IGF-I levels is due to tumor shrinkage, causing restoration of the hypothalamic-pituitary connection and subsequent normalization of GH and IGF-I secretion (13,14), or whether this increase in IGF-I levels is related to an increase in circulating sex steroid concentrations during CV 205-502 therapy which followed the (near)normalization of PRL levels in most patients (15-20).

In the present study a positive correlation was demonstrated between tumor size and PRL levels in the macroprolactinoma patients, as has been observed by other investigators. (21,22,23). We also found a positive correlation between the degree of tumor size reduction during CV 205-502 therapy and the percent fall in PRL levels. Such a relationship was not found in a larger group of bromocriptine-treated prolactinoma patients reported by Molitch et al. (23).

We conclude that CV 205-502, a potent non-ergot dopaminergic drug, effectively suppresses prolactin secretion and shrinks tumor size in the majority of prolactinoma patients. It causes only mild and transient side effects in a minority of patients at doses necessary to control symptomatology and/or PRL levels. Therefore it is a valuable alternative treatment both for macroprolactinoma patients as well as for patients with PRL-secreting tumors who have shown severe intolerance to bromocriptine therapy.

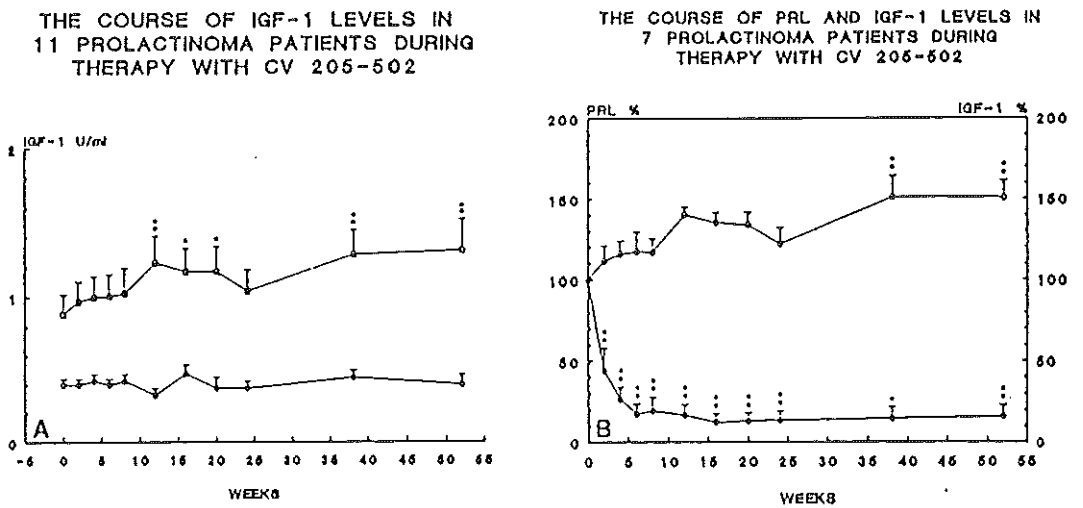


FIG. 1. The course of PRL and IGF-1 levels in prolactinoma patients during therapy with CV 205-502. *, $P < 0.005$. A, Patients with intact pituitary function (○); patients with hypopituitarism (●). B, IGF-1 levels of patients with intact pituitary function (○); their PRL levels (●).

TABLE 1. The decrease in PRL levels after 12 and 52 weeks of therapy with CV 205-502

Patient no.	Sex (M/F)	Age (yr)	PRL (week 0; μg/L)	CV 205-502 (week 12, μg/day)	PRL (week 12; μg/L)	% Decrease	CV 205-502 (week 52; μg/day)	PRL (week 52; μg/L)	% Decrease
12 patients with a macroprolactinoma									
1	M	31	3151	225	21	98.2	300	4	99.7
2	F	23	275	75	4	98.4	75	2	99.2
3	F	44	160	150	31	80.6	150	9	94.7
4	M	23	1212	225	109	92.1	300	212	82.9
5	M	42	344	225	22	93.6	225	36	89.6
6	F	31	4875	300	1241	74.5	450	198	95.9
7	F	34	174	225	46	73.6	300	115	33.7
8	F	28	224	75	3	98.8	75	2	99.0
9	M	61	5185	225	36	99.3	225	6	99.9
10	M	42	1258	150	2	99.8	150	2	99.9
11	M	40	4720	150	1	100.0	150	1	100.0
12	M	29	674	225	15	97.8	300	2	99.7
Mean decrease %						92.2 ± 2.9			91.2 ± 5.4
8 patients with intolerance to bromocriptine									
13	F	41	74	75	2	97.6	75	3	95.6
14	F	43	232	150	36	84.7	300	48	79.3
15	M	45	150	150	5	96.7	150	3	98.3
16	F	40	35	150	13	63.8	150	15	57.7
17	F	29	56	150	13	77.3	150	22	61.3
18	F	41	71	225	6	91.9	225	1	98.3
19	F	26	94	225	37	60.5	300	37	61.0
20	F	30	134	150	30	77.5	225	14	89.7
Mean decrease %						81.2 ± 5.0			80.2 ± 6.3

TABLE 2. The effects of treatment with CV 205-502 for 52 weeks on the signs and symptoms present at the beginning of the study

Patient no.	Sex (M/F)	Normal menstrual cycles after 52 weeks	Disappearance of headache and/or improvement in general condition	Normalization of libido or potency	Disappearance of galactorhea (if was present)
Macroprolactinoma patients					
1	M		Y	Y	
2	F	Y	Y	Y	Y
3	F	N	Y	N	
4	M		Y	Y	
5	M		Y	Y	
6	F	N	Y	Y	
7	F	N	Y	Y	
8	F	Y	Y	Y	
9	M		Y	Y	
10	M		Y	Y	
11	M		Y	Y	
12	M		Y	N	
Patients previously intolerant to bromocriptine					
13	F	Y	Y	Y	Y
14	F	Y	Y	Y	Y
15	M		Y	N	
16	F	Y	Y	Y	Y
17	F	Y	Y	Y	
18	F	N	Y	N	
19	F	Y	Y	Y	
20	F	Y	Y	Y	

Y, yes; N, no.

TABLE 3. Reduction in tumor size after 52 weeks of therapy with CV205-502

Patient no.	PRL (week 0; μg/L)	Vol (week 0; mm^3)	PRL (week 52; μg/L)	Vol (week 52; mm^3)	% Decrease in tumor size	% Decrease in PRL
1	1151	6732	4	126	98	99
2	275	2730	2	36	99	99
3	159	17328	9	432	98	95
4	1241	7920	212	2618	67	83
5	343	14212	36	7020	51	89
6	4875	20160	198	2880	86	96
7	173	2730	116	1716	37	34
8	224	3808	2	392	90	99
9	5158	66880	6	32760	51	99
10	1257	20064	2	5760	71	99
15	150	1232	3	96	92	98
18	56	1232	1	288	77	98
19	71	432	37	245	43	61
Mean decrease (%)					74 ± 6	88 ± 5

Correlation between pretreatment tumor size and PRL levels: $r = 0.80$; $P < 0.01$. Correlation between percent decrease in tumor size and percent decrease in PRL levels; $r = 0.72$; $P < 0.05$.

REFERENCES

1 Venetikou MS, Burrin JM, Woods CA, Yeo TH, Brownell J, Adams EF. Effects of two novel dopaminergic drugs,CV 205-502 and ECP 201-403 on prolactin and growth hormone secretion by human pituitary tumours in vitro. Acta Endocrinol. 1987;116:287-292.

2 Closse A, Camps M, Wanner A, Palacios JM. In vivo labeling of brain dopamine D2 receptors using the high-affinity specific D2 agonist [3H] CV 205-502. Brain Res. 1988;440: 123-132.

3 Gaillard RC, Nordmann R, Petcher TJ, Brownell J. A novel octahydrobenzo[g]quinoline, CV 205-502, with potent dopamine agonist properties. Endocrinology '85 (1986) p. 305-308. G.M. Molinatti and L. Martini (Eds) Elsevier Science Publishers B.V. Amsterdam.

4 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: 329-335.

5 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: 321-326.

6 Bergh T, Nillius SJ, Wide L. Hyperprolactinaemic amenorrhoea: results of treatment with bromocriptine. Acta Endocrinol. 1987;88(suppl), 147-164.

7 Wass JAH, Williams J, Charlesworth M et al. Bromocriptine in management of large pituitary tumors. Brit Med J. 1982;284: 1908-1911.

8 Thorner MO, Martin WH, Rogol AD et al. Rapid regression of pituitary prolactinomas during bromocriptine treatment. J Clin Endocrinol Metab. 1980;51: 438-445.

9 Parkes D. Drug therapy. Bromocriptine. N Eng J Med. 1978;301: 873-878.

10 Rasmussen C, Bergh T, Wide L, Brownell J. Long-term treatment with a new non-ergot long-acting dopamine agonist, CV 205-502, in women with hyperprolactinaemia. Clin Endocrinol. 1988;29: 271-279.

11 Vance ML, Cragun J, Reimnitz C et al. CV 205-502 treatment of hyperprolactinaemia. J Clin Endocrinol Metab 1989,68: 336-339.

12 Vance ML, Lipper M, Klibanski A et al. Treatment of prolactin-secreting pituitary macroadenomas with the long-acting non-ergot dopamine agonist CV 205-502. Ann Int Med 1990,112: 668-673.

13 Faglia G, Travaglini P, Beck-Peccoz P, Ambrosi B, Moriondo P, Elli R, Rondena M. Anterior

pituitary hormones, other than those hypersecreted, in patients with functioning microadenomas. Pituitary Microadenomas, Proceedings of the Serono Symposia. volume 29, 1980:277. Faglia G., Giovanelli M., Macleod R. (Eds), Academic Press, London.

14 Jaquet P, Grisoli F, Guibout M, Lissitzky J, Carayon P. Prolactin secreting tumors. J Clin Endocrinol Metab. 1978;46: 459-466.

15 Caufriez A, Tadjerouni A, Bosson D, Cantraine F, Robijn C, Copinschi G. Modulation of immunoreactive somatomedin-C levels by sex steroids. Acta Endocrinol. 1986;112: 284-289.

16 Copeland KC, Johnson DM, Kuehl TJ, Castracane VD. Estrogen stimulates growth hormone and somatomedin-C in castrated and intact female baboons. J Clin Endocrinol Metab. 1984;58: 698-703.

17 Clemmons DR, Underwood LE, Ridgway EC, Kliman B, Kjellberg RN, van Wijk JJ. Estradiol treatment of acromegaly. Am J Med. 1980;69: 571-575.

18 Copeland KC. Effects of acute high dose and chronic low dose estrogen on plasma somatomedin-C and growth hormone in patients with Turner's syndrome. J Clin Endocrinol Metab. 1988;66: 1278-1282.

19 Craft WH, Underwood LE. Effect of androgens on plasma somatomedin-C/insulin-like growth factor-I responses to growth hormone. Clin Endocrinol. 1984;20: 549-554.

20 Loche S, Corda R, Lampis A, Puggioni R, Cella SG, Muller EE, Pintor C. The effect of oxandrolone on the growth hormone response to growth hormone releasing hormone in children with constitutional growth delay. Clin Endocrinol. 1980;25: 195-200.

21 Child DF, Nader S, Mashiter K, Kjeld M, Banks L, Russell Fraser T. Prolactin studies in 'functionless' pituitary tumours. Brit Med J. 1975;1: 604-606.

22 Klijn JGM, Lamberts SWJ, de Jong FH, Docter R, van Dongen KJ, Birkenhager JC. The importance of pituitary tumour size in patients with hyperprolactinaemia in relation to hormonal variables and extrasellar extension of tumour. Clin Endocrinol. 1980;12: 341-355.

23 Molitch ME, Elton RL, Blackwell RE et al.Bromocriptine as primary therapy for prolactin-secreting macroadenomas: results of a prospective multicenter study. J Clin Endocrinol Metab. 1985,60: 689-705.

24 Homburg R, West C, Brownell J, Jacobs HS. A double-blind study comparing a new non-ergot, long-acting dopamine agonist, CV 205-502, with bromocriptine in women with hyperprolactinemia. Clin Endocrinol. 1990;32:565-571.

CHAPTER 8.

NASOPHARYNGEAL PRESENTATION OF PITUITARY TUMORS. DIFFERENTIAL DIAGNOSIS AND TREATMENT

Aart J. v d Lely, Paul P.M. Knegt, Stanley Z. Stefanko, Hervé L.J. Tanghe, Ram Singh and Steven W.J. Lamberts.

Department of Medicine (A.L., S.L.), Otorhinology (P.K.), Pathology (S.S), Neuroradiology (H.T.) and Neurosurgery (R.S.), Erasmus University, Rotterdam,The Netherlands.

This article has been accepted for publication in the Journal of Clinical Endocrinology and Metabolism.

ABSTRACT

The intranasal presentation of pituitary tumors is rare. We describe six patients with supposedly intranasal carcinomas, treated with surgery, local chemotherapy and/or radiotherapy. Because of the favourable clinical course, immuno-histochemical re-examination was done and showed a macroprolactinoma in four and a function-less chromophobe adenoma in two patients. Interestingly, anterior pituitary function was normal in four and only slightly abnormal in two. In two patients the radiological appearance of the sellar region was completely normal. Routine immuno-histochemistry would have prevented excessive therapy. Dopamine agonist therapy was effective in the four macroprolactinoma patients, but the localization of the tumor seems to increase the risk of liquorrhea and/or meningitis.

INTRODUCTION

Malignant tumors of the paranasal sinuses account for only 3% of all tumors of the upper respiratory tract (1,2). Squamous cell and undifferentiated carcinoma are the most common (60%). Esthesioneuroblastomas are very rare paranasal tumors (1). In the present study we describe four patients with macroprolactinomas and two patients with chromophobe pituitary tumors who primarily presented with complaints of nasal origin. In the clinical history no anterior pituitary insufficiency was noticed, while CT scanning of the sellar region was normal in two.

MATERIALS AND METHODS

The metyrapone test (4.5 g/24h), as well as the TRH (200 μg) and LHRH (100 μg) test have previously described in detail (3,4,5). Circulating hormone levels were measured with commercial RIA kits. (T_4,TSH :Behring,Marburg,Germany. Estradiol, testosterone,deoxycortisol: Diagnostic Products Corporation,Los Angeles,USA. LH,TSH,PRL,GH: Medgenix, Brussels,Belgium). Cross-reactions, inter- and intra assay variations of these determinations have been previously described (6). Immuno-histological examination was done with antisera against PRL, GH, LH-ß, FSH-ß and α-subunits.

CASE REPORTS

PATIENT 1. This 45 yr old male was treated with radiotherapy, because of a supposedly undifferentiated carcinoma, causing destruction of the paranasal sinuses and base of the skull. Because of the excellent condition and the unexpected long survival after two years,the biopsy was re-examined. Immuno-histochemical re-examination with anti-PRL showed a PRL secreting pituitary tumor. Indeed, serum PRL levels were greatly elevated (Table 1). Endocrine evaluation only showed a slightly impaired reaction to metyrapone (Table I). Treatment with bromocriptine (7.5 mg daily) was started. After six months a life threatening meningitis occurred.

PATIENT 2. This 61 yr old woman was treated with surgery and radiotherapy, because of a supposedly undifferentiated paranasal carcinoma, causing destruction of the paranasal sinuses and the sellar region. One year after treatment the diagnosis was reconsidered because of her excellent clinical condition and the absence of tumor progression. Very high circulating PRL levels were found (4,200 μg/l). LH, FSH and estradiol levels were lowered (Table 1). Again immuno-histochemical re-examination showed the presence of PRL-containing cells. She was treated successfully for 8 years with bromocriptine (5 mg daily).

PATIENT 3. This 61 yr old male was admitted because of a supposedly esthesioneuroblastoma, causing destruction of the sphenoid, the base of the skull and the sellar region. Immuno-histochemical re-examination showed a macroprolactinoma. At that time the patient was in good clinical condition. Circulating PRL were very high (5,200 μg/l). Endocrine evaluation showed no abnormalities. Treatment with the dopamine agonist CV 205-502 (225 μg/day) resulted in near complete normalization of circulating PRL levels (33 μg/l). Tumor size decreased after one year of therapy by 50 %. During therapy shortly liquorrhea was noticed, which disappeared without signs or symptoms of meningitis.

PATIENT 4. This 63 yr old male was investigated because of headaches. Examination by the otolaryngologist revealed a large tumor in the nasopharyngeal area, with destruction of the sellar region, (Fig. 1). Microscopical and immuno-histochemical examination showed a macroprolactinoma. Also serum PRL levels (10,700 μg/l) were very high. Although there was destruction of the sellar region, the reactions to TRH,

LHRH and metyrapone were normal (Table 1). This patient was also treated successfully with CV 205-502 (300 μg/day).

PATIENT 5. This 50 yr old male was admitted elsewhere, because of a supposedly undifferentiated carcinoma of the nasal cavity. He was treated by surgery, radiotherapy and application of chemotherapeutics to the tumor surface. CT-scan of the skull did not show abnormalities in the sellar region. Because of the good clinical condition, immuno-histochemical re-evaluation showed the tumor to be a chromophobe pituitary adenoma, because of the positivity for neuron-specific enolase, chromogranin and FSH. Endocrine evaluation showed no abnormalities (Table I). At present only remnants of the tumor can be detected at CT-scan examination.

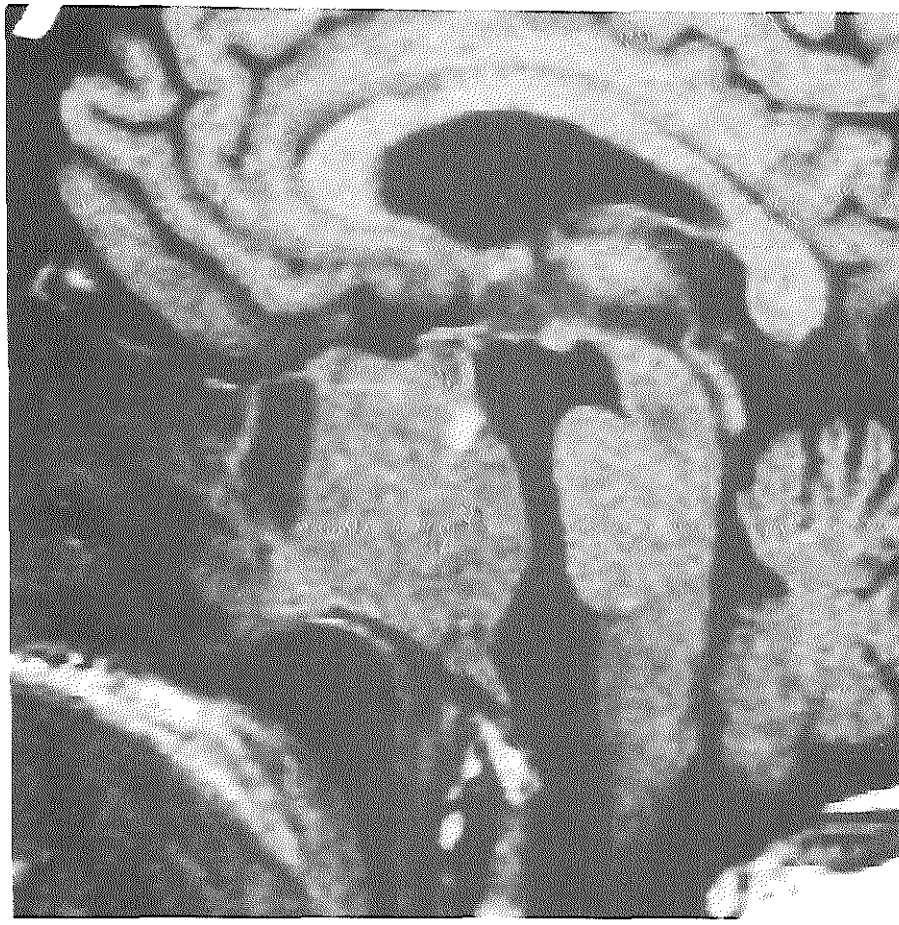
PATIENT 6. This 44 yr-old woman was treated the same way as patient 5, because of a supposedly esthesioneuroblastoma. The sellar region was normal at CT scan analysis. After one and a half year the initial diagnosis was reconsidered because of the persistent fatigue and headaches. Again, because of the same immuno-histochemical characters, the diagnosis of a chromophobe pituitary adenoma was made. Endocrine evaluation showed no abnormalities. During the use of a contraceptive drug, estradiol levels were low and no LHRH test was done (Table I). This patient is currently symptom-free and regular menstrual cycles have resumed one year after stopping contraceptive drugs.

DISCUSSION

In previous studies we observed in 62 patients that if tumor size was greater than 3 cm^2 (lateral area) the occurrence of disturbances in hormone secretion in reaction to TRH, LHRH and metyrapone rapidly increased (7). In the present study we report on six patients with very large chromophobe pituitary tumors (all exceeding 3 cm^2) in whom no or only discrete abnormalities of anterior pituitary function and/or reserve were found. In the two patients with chromophobe "non-functioning" pituitary tumors, in contrast to the macroprolactinoma patients, the pituitary fossa was normal at radiological examination. Interestingly all patients primarily presented themselves at the otorhinologist. The localization, as well as the (virtually) normal anterior pituitary function, was the reason that the primary diagnosis of a pituitary tumor was delayed in most of them. The original diagnoses were revised only after additional immuno-histochemical investigation. A revision was done, however because of a good clinical

condition in the follow-up. Medical therapy with dopamine agonists of large invasive pituitary prolactinomas is successful. Both control of hormonal hypersecretion as well as tumor shrinkage have been reported in most patients (8,9). Sometimes however, shrinkage of the tumor can result in liquorrhea. The central necrosis of the tumor during dopamine agonist therapy as observed in patient 1 and 6 and liquorrhea (patients 1 and 5) pose extra risks for the development of local infections and/or meningitis. In patients with "non-functioning" pituitary tumors extending into the pharyngeal pituitary area, surgery, followed by radiotherapy seems the therapy of choice. Previously, evidence for the existence of a pharyngeal pituitary, which persists into adulthood and which might be the origin of pituitary tumor formation in some patients was provided (10-15). The data of the six patients described might favour a primarily pharyngeal origin in some of them. Especially the existence of intact anterior pituitary function in four and the radiologically normal aspect of the pituitary fossa in two patients would support such a concept. A majority of our patients, however, seem to have primary pituitary tumors with inferior extension into the nose and paranasal sinuses. Pituitary tumors, which primarily infiltrate the paranasal sinuses and/or pharyngeal cavity seem to be rare disorders. However, one must be aware of their existence. Overtreatment, as well as the psychological burden of a diagnosis with a bad prognosis, can be prevented in most cases by the use of immuno-histochemical investigation and/or the measurement of circulating PRL levels. The right diagnosis at clinical presentation might limit the need of extensive therapy in most patients to the use of dopamine agonists and/or radiotherapy.

Fig. 1



NMR scan showing a large macroprolactinoma of the nasopharyngeal area with destruction of the sellar region in a 63 yr old male patient (patient 4).

TABLE I

HORMONE LEVELS IN SIX PATIENTS WITH A LARGE PITUITARY TUMOR WITH INVOLVEMENT OF THE PHARYNX OR PARANASAL SINUSSES

PAT. No	SEX M/F	AGE Y	PRL a) mcg/l	T 4 b) nmol/l	TSH c) mU/l	LH/FSH e) U/l	Testost d) nmol/l	E 2 f) pmol/l	METYRAPONE TEST g)
1	M	45	75000	108	0.81	2.4 / 7.3	13.0	--	292
2	F	61	4200	132	1.03	6.9 / 0.7	--	23	416
3	M	61	5200	81	0.52	4.3 / 5.2	14.7	--	385
4	M	63	10700	70	0.80	2.6 / 4.6	13.0	--	--
5	M	50	3.8	85	1.35	1.1 / 5.4	20.9	--	468
6	F	44	6.5	186	1.88	1.2 / 1.0 *	--	< 20	423

* : This patient used a contraconceptive drug, at the time the bloodsamples were taken

a) : Prolactin, N < 15 mcg/l

b) : N = 64–132 nmol/l

c) : N = 0.2–4.2 mU/l

d) : Testosterone, N = 10 – 30 nmol/l

e) : N = 1 –10 U/l

f) : Estradiol, N = 40 – 800 pmol/l

g) : De oxycortisol after 6 x 750 mg of Metyrapone orally, N > 350 nmol/l

REFERENCES:

1 Balleyer J. Diseases of the nose, throat, ear, head and neck. 13th edition. Lea & Febiger .Philadelphia 1985.

2 Knegt PP, De Jong PC, Van Andel JG et al. Carcinoma of the paranasal sinuses. Cancer 1985;56:57-62.

3 Liddle GW, Estep HL. Clinical application of a new test of pituitary reserve. J Clin Endocrinol.1959;19:875-9.

4 Snyder PJ, Jacobs LS, Rabello MM et al. Diagnostic value of thyrotrophin-releasing hormone in pituitary and hypothalamic diseases. Ann Int Med. 1974;8:751-4.

5 Jackson IMD. Diagnostic tests for the evaluation of pituitary tumors. In: The Pituitary Adenoma (eds. K.D. Post, I.M.D. Jackson, S. Reichlin), Plenum Publishing Corporation, New York, pp 219-238, 1980.

6 Kwekkeboom DJ, De Jong FH, Lamberts SWJ. Confounding factors in the interpretation of gonadotropin and gonadotropin-subunit release from cultured human pituitary adenomas. J Steroid Biochem. 1989;33:777-82.

7 Klijn JGM, Lamberts SWJ, De Jong FH et al. The inmportance of pituitary tumour size in patients with hyperprolactinaemia in relation to hormonal variables and extrasellar extension of the tumour. Clin Endocrinol. 1980; 12: 341-55.

8 Parkes D. Drug Therapy, Bromocriptine. N Eng J Med. 1978;301:873-8.

9 Wass JAH, Williams J, Charlesworth M. Bromocriptine in management of large pituitary tumors. Brit Med J. 1982; 284:1908-11.

10 Ciocca D, Puy L, Stati A. Identification of seven hormone-producing cell types in the human pharyngeal hypophysis. J Clin Endocrinol Metab. 1985;60:212-6.

11 Puy L, Ciocca D. Human pharyngeal and sellar pituitary glands: differences and similarities revealed by an immunocytochemical study. J Endocrinol. 1986;108:231-8.

12 Boyd W. A case of tumor of the pituitary body. Lancet 1910;11:1129-31.

13 Bailey O, Cutter E. Malignant adenomas of the chromophobe cells of the pituitary body. Arch Pathol. 1940;29:368-72.

14 Shea J. Nasal tumors of pituitary origin (report of a case). Trans Amer Laryngol Rhinol Otol. 1941;47:201-3.

15 Kay S, Lees J, Stout A. Pituitary chromophobe tumors of the nasal cavity. Cancer 1950;3:695-701.

CHAPTER 9

RAPID REVERSAL OF ACUTE PSYCHOSIS IN PATIENTS WITH SEVERE CUSHING'S SYNDROME

van der Lely AJ[1], Foeken K[2], van der Mast RC[2], Lamberts SWJ[1].

1 Department of Medicine, Erasmus University Hospital, Rotterdam, the Netherlands.

2 Department of Psychiatry, Erasmus University Hospital, Rotterdam, the Netherlands.

The first part of this chapter has been published in a shorter version in the Annals of Internal Medicine 1991;114: 143-144.

Summary:

Therapy with the progesterone and cortisol receptor antagonist RU 486 (mifeprestone) has been previously reported to result in clinical and biochemical improvement in patients with Cushing's syndrome. In our study, RU 486 rapidly reverses symptoms of acute psychosis in two patients with cortisol-secreting adrenal carcinomas, for whom no other therapy was available.

Introduction:

The properties of RU 38486 (mifepristone ; 17-ß-hydroxy-11ß-(41-dimethyl-aminophenyl)17-α-(1-propynyl)oestra-4,9-dien-3-one), a steroid with powerful blocking activity at the progesterone and the glucocorticoid receptor, have been studied both in vitro and in vivo [1 2 3]. In normal humans, RU 486 blocks glucocorticoid negative feedback at the hypothalamic-pituitary level, inducing a compensatory increase in plasma adrenocorticotropin (ACTH) and cortisol levels [4 5]. In previous studies in patients with Cushing's syndrome, caused by ectopic ACTH production or adrenocortical cancer, RU 486 in doses from 5 to 22 mg/kg body weight daily, showed a clear clinical improvement [4 6 7]. In our study, RU 486 had a rapid beneficial effect in reversing acute psychosis and preventing further psychiatric symptoms in two patients with inoperable end-stage cortisol-secreting adrenal cancers.

Case Reports:

Patient 1, a 43-year-old man had inoperable left-sided adrenal cancer with extensive metastases to the liver and lungs. Very high circulating cortisol (Table 1) and undetectable ACTH levels confirmed the clinical diagnosis of Cushing's syndrome. After receiving therapy with o',p'-DDD (1,1-dichlorodiphenyl-dichloroethane; mitotane) (12 gram/day; body weight 74 kg) his mental state deteriorated acutely. Different psychiatric states were observed during a period of 8 to 12 hours; these included consecutive severe clouding of consciousness, mutism, and psychosis with nihilistic delusions. His behaviour was unpredictable and he was considered to be at high risk for suicide. Psychiatric symptoms improved within 12 h after

the administration of RU 486 (800 mg) and all mental abnormalities disappeared within 24 hours. Therapy with RU 486 800 mg daily was continued, but after 5 days hypoglycemic episodes occurred and eosinophilia reappeared. The daily dose of RU 486 was lowered to 400 mg daily without side-effects. The patient died 2 weeks later from renal insufficiency, caused by tumor obstruction of the inferior vena cava. Plasma cortisol levels remained elevated and unchanged until death, but no psychiatric symptoms reoccurred. Other signs and symptoms of Cushing's syndrome had started to subside.

Patient 2, a 32-year-old woman, also developed Cushing's syndrome because of an inoperable left-sided adrenal cancer with metastases to liver and lungs. Her circulating cortisol level remained elevated (Table 1) and her ACTH level undetectable after 7 weeks of therapy with o',p'-DDD (8 gram/day; body weight 52 kg). She was admitted with rapidly developing signs and symptoms of paranoid psychosis, including depression, agitation, and hallucinations. Treatment with RU 486, 400 mg daily resulted in improvement within 24 hours with the complete disappearance of psychiatric symptoms within 3 days. These symptoms did not recur during the last 2 months of her life. Cortisol levels remained unchanged and no evidence of adrenal insufficiency (hypoglycemia or eosinophilia) was observed. Other signs and symptoms of Cushing's syndrome decreased during this period.

Discussion:

In the 1970s the role of intracellular receptors in the action of steroid hormones was clarified [8]. By creating a compound that would bind to the progesterone receptors of target cells in the endometrium, but would not elicit intracellular changes, necessary for the nidation of a fertilized ovum, one would have a drug with strong contragestional activity. Synthesized in 1980 as a pure antagonist of progesterone, RU 486 was found to have a high affinity for progesterone receptors (without agonist activity) and also an anti glucocorticoid receptor action [1 2]. By 1982 it was tested in humans for its ability to interrupt either the luteal phase of the menstrual cycle or early pregnancy [9 10 11]. The drug is also used in the chronic second-line treatment of metastatic breast cancer in postmenopausal women [12 13]. A single 400 mg dose of RU 486 induces a glucocorticoid-blocking response that lasts at least 34 h. Their is a dose-dependent competition between RU 486 and dexamethasone [14].

A wide range of mental abnormalities occur in patients with Cushing's syndrome.

Depression, often of psychotic nature, is the most frequent symptom and is accompanied by a high risk for suicide [15]. These symptoms of psychosis are difficult to treat and in most instances do not respond to anti psychotic drugs [15].

It has been previously reported that RU 486 results in clinical and biochemical improvement in patients with Cushing's syndrome [6 7]. We found that RU 486 was probably useful in rapidly reversing the severe acute psychiatric symptoms in two patients with end-stage metastatic adrenal cancers. We chose a dose of 400 to 800 mg/day of RU 486 on empirical grounds. There is currently no way to distinguish between adequate or excessive blockade of glucocorticoid action, making the recognition of adrenal insufficiency in these patients difficult [16].

TABLE 1. Serum Cortisol Levels in the Two Study Patients.

Patient	Serum Cortisol*, nmol/l		
	Before treatment	During o'p'-DDD Therapy#	During Therapy with o'p'-DDD and RU 486
	nmol/l		
1 @	2000-2500	1800-2200	1800-2200
2 $	1000-1500	800-1100	800-1100

* Normal level at 08.00 hours is less than 450 nmol/l.

\# o',p'-dichlorodiphenyldichloroethane (Mitotane).

@ Male; age 43 years.

$ Female; age 32 years.

References:

1. Philibert D, Deraedt R, Teutsch G. RU 38486. A potent antiglucocorticoid in vivo. Int Congr of Pharmacol Tokyo 1981;668: abstract 1463.

2. Proulx-Terland L, Cote J, Philibert D, Deraedt R. Potent antiglucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat. J Steroid Biochem 1982;17: xvii.

3. Lamberts SWJ, Bons EG, Uitterlinden P. Studies on the glucocorticoid receptor blocking action of RU 38486 in cultured ACTH-secreting human pituitary tumor cells and normal rat pituitary cells. Acta Endocrinol (Copenh) 1985;109: 64-69.

4. Bertagna X, Bertagna C, Luton JP, Husson JM, Girard F. The new steroid analogue RU 486 inhibits glucocorticoid action in man. J Clin Endocrinol Metab 1984;59: 25-31.

5. Gaillard RD, Poffet D, Diondel AM, Saurat JH. RU 486 inhibits peripheral effects of glucocorticoids in humans. J Clin Endocrinol Metab 1985;61: 1009-1014.

6. Nieman LK, Chrousos GP, Nisula BC et al. Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU 486. J Clin Endocrinol Metab 1985;61: 536-540.

7. Nieman LK, Udelsman R, Loriaux DL, Chrousos CP. Antiglucocorticoids: basic and clinical studies. In: D'Agata R, Chrousos GP, (eds) Recent Advances in adrenal regulation and function. New York, Raven Press 1987: 235-258.

8. O'Malley BW. Hormonal control of gene expression in reproductive tissues. In: Greep RO, Koblinsky MA. (eds). Frontiers in reproduction and fertility control: a review of the reproductive sciences and contraceptive development. Cambridge, Mass. MIT Press, 1977: 236-244.

9. Herrmann W, Wyss R, Riondel A. Effet d'un steroide antiprogesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut. C R Seances Acad Sci [III] 1982;294: 933-938.

10. Ulmann A. The antiprogestins: a recent advance in fertility regulation. J Steroid Biochem 1987;27: 1009-1012.

11. Couzinet B, Le Strat N, Ulmann A, Baulieu EE, Schaison G. Termination of early pregnancy by the progesterone antagonist RU 486 (Mifeprestone). N Eng J Med 1986;315: 1565-1569.

12. Bardon S, Vignon F, Chalbos D, Rochefort H. RU 486, a progestin and glucocorticoid antagonist, inhibits the growth of breast cancer cells via the progesterone receptor. J Clin Endocrinol Metab 1985;50: 692-697.

13. Klijn JG, de Jong FH, Bakker GH, Lamberts SWJ, Rodenburg CJ, Alexieva-Figusch J. Antiprogestins, a new form of endocrine therapy for human breast cancer. Cancer Res 1989;49: 2851-2856.

14. Raux-Demay MC, Pierret T, Bouvier d'Yvoire M, Bertagna X, Girard F. Transient inhibition of RU 486 antiglucocorticoid action by dexamethasone. J Clin Endocrinol Metab 1990;70: 230-233.

15. Haskett R. Diagnostic categorization of psychiatric disturbances in Cushing's syndrome. Am J Psychiatry 1985;142: 911-916.

16. Laue L, Callucci W, Loriaux DL, Udelman R, Chrousos GP. The antiglucocorticoid and antiprogestin steroid RU 486: its glucocorticoid agonist effect is inadequate to prevent adrenal insufficiency in primates. J Clin Endocrinol Metab 1988;67: 602-606.

After the two patients described above we treated two additional patients with Cushing's syndrome with RU 486 (mifepristone). In the following the case histories of these patients are presented, after which we tried to define what the place of mifepristone is in the medical treatment of such patients.

Case 3:

This 61 yr old female was transferred to our hospital from a hospital elsewhere because of suspected Cushing's syndrome. She had been treated for a non-insulin dependent diabetes mellitus for 13 years with a diet and oral antidiabetic drugs. During the last three years, insulin had been needed to control serum glucose levels. About six weeks before admission in our hospital, she began to complain about swelling of the face, abdomen and both feet. Also she developed polydipsia and polyuria. She was admitted elsewhere because of hyperglycaemia, which needed treatment with additional insulin intravenously. Because of a low potassium concentration in the blood (2.1 meq/L), as well as a 'moon'face, the diagnosis of Cushing's syndrome was suspected. Indeed serum cortisol levels were increased to three times normal (levels between 1300 and 1550 nmol/l; normal 300-450 nmol/l), and showed no diurnal variation, while serum cortisol levels failed to decrease adequately after 1 mg of dexamethasone orally (950 nmol/L; normal less than 140 nmol/L). CT scanning of the sellar region revealed no abnormalities, while both adrenal glands seemed slightly enlarged.
Treatment with ketoconazole was started at a dose of 1200 mg per day but no decrease in cortisol levels was found after three days (plasma cortisol 1200 nmol/L). Because of a sudden deterioration in the clinical condition it was decided to transfer the patient to our hospital.

At admission she had signs and symptoms of Cushing's syndrome, as well as a high fever (above 40 °C) and there were signs of meningitis. Platelet levels were lowered (33 x 10^9/l; normal 150-350 x 10^9/l) without evidence of intravascular coagulation. An X-ray examination of the chest (made in bed) seemed normal.
Urinary cortisol level were increased (19800 nmol/day; normal less than 500 nmol/day). Serum cortisol concentration was still elevated: 1048 nmol/l, while ACTH concentration was very high (1930 ng/l; normal < 60 ng/l). In blood cultures a staphylococcus aureus species was isolated and antibiotics were given intravenously (tobramycin, rifampicin and flucloxacillin). In the following two days the situation improved: body temperature normalized, and the signs of meningitis disappeared, serum platelet levels remained low, however. A new problem was a severe psychosis consisting of paranoia and visual and

auditive hallucinations. We were dealing at that moment with a patient with severe Cushing's syndrome without an evident cause. She had just survived a meningitis; she had a persistent low platelet levels (11 x 10^9/L), an acute psychosis and previous therapy for 72 h. with high dose ketoconazole had been shown to be unable to lower circulating cortisol levels.

The decision was made to treat this patient with the cortisol receptor antagonist mifepristone at a dose of 800 mg per day in combination with 2 x 1 mg of dexamethasone in order to prevent the possible development of hypocortisolism. Twelve hours after starting mifepristone administration the psychosis had completely disappeared, while insulin requirement had also decreased considerably. No eosinophilic granulocytes were discovered in the peripheral blood at that time.

To find out what was the cause of Cushing's syndrome, we performed a scanning with ^{111}In-labelled octreotide. This indicated a region with high accumulation of radioactivity in the lung, close to the hart, which was not visible on the X-ray examination of the chest. Also evidence for multiple deposits in the vertebra were present. A bone marrow aspiration was carried out and metastatic tumor cells were found which were compatible with an undifferentiated small cell carcinoma. Also the circulating level of neuron-specific enolase was greatly elevated. The diagnosis of an ACTH secreting small cell carcinoma of the lung with extensive metastases in the bone was made. In the follow-up mifepristone therapy was maintained until the death of the patient. Her mind remained clear until the end, despite of the very high circulating cortisol levels.

Case 4:

This 57 yr old female had been treated elsewhere for ten years with antihypertensive drugs and the diuretic furosemide for a moderate hypertension. Two months before admission in our hospital, hyperglycemia was found after the patient complained about polyuria and thirst. Not long before that, the patient had also been seen by a neurologist because of unexplained proximal weakness of the muscles.
At admission elsewhere a high sodium level in the blood was found (169 mmol/l) in combination of a low potassium level (2.6 mmol/l). Because of here appearance (skin bruises, absence of subcutaneous fat) and these laboratory findings the presence of Cushing's syndrome was suspected. Indeed serum cortisol levels were elevated (1000-1200 nmol/l), while there was no night and day variation. During her stay in the hospital elsewhere, she developed signs of a severe paranoia and threatened to commit suicide.

Therefore she was transferred to our hospital for further analysis and treatment of her Cushing's syndrome.
At admission we found a patient with all clinical signs and symptoms of Cushing's syndrome who was severely psychotic. She was bedridden, but had also extensive decubitus. A dexamethasone suppression test was immediately performed by administration of 7 mg dexamethasone intravenously in 7 hours. This caused a decrease in serum cortisol level from 828 to 343 nmol/l, suggesting that she had pituitary-dependent Cushing's syndrome (Cushing's disease). Then treatment with ketoconazole 1200 mg orally was started, which caused within 24 hours a decrease in cortisol levels to 200 nmol/l. Her mental state improved significantly, although signs of the psychosis reoccurred intermittently. Because of these periods of agitation it was impossible to further analyze her (neuro)radiologically. These psychotic episodes persisted even 7 days after the start of ketoconazole administration. Therefore it was decided to treat her additionally with 600 mg mifepristone orally in combination with 60 mg hydrocortisone. This combined medical therapy resulted in a disappearance of the psychotic episodes. She underwent transsphenoidal hypophysectomy and recovered thereafter.

Discussion

Cushing's syndrome can be the result of overproduction of ACTH by the pituitary (Cushing's disease) or by another (ectopic) source. (e.g. small cell lung cancer or other APUDOMA's). Also excessive production of cortisol by an adrenal adenoma or carcinoma can be the cause of Cushing's syndrome. When the etiology of the syndrome is still unclear, while the severity of the disease necessitates direct treatment, currently choices can be made between drugs which interfere with cortisol synthesis, like aminogluthetimide, o'-p'-DDD, metyrapone and ketoconazole, and the cortisol receptor blocking drug RU 486 (mifepristone).

Metyrapone, aminogluthetimide and o'-p'-DDD are no attractive possibilities, because of their side-effects when used in the dose necessary for a rapid blockade of cortisol production by the adrenal glands. Patients do complain about nausea, vomiting, dizziness and skin rashes (aminogluthetimide), while neurotoxicity is a problem arising in the treatment with o'-p'-DDD. Also the overproduction of adrenal androgens and mineralocorticoids resulting in hypertension, hypokalemic alkalosis, edema and hirsutism, are reasons for avoiding the use of metyrapone if possible.

Ketoconazole and mifepristone can both be used in the treatment of (severe)

Cushing's syndrome, and the choice and (dis)advantages of both drugs are discussed below. Ketoconazole is an orally active antimycotic drug, which turned out to have also potent inhibitory effects on gonadal and adrenal steroid synthesis by interfering with several cytochrome P-450 dependent enzyme systems, e.g. desmolase and 17-α hydroxylase.[1 2]

Several side-effects can occur when treatment with ketoconazole is initiated. Although the drug is generally well tolerated, these side-effects do occur especially at the higher dose necessary to control cortisol hypersecretion. Skin rashes, dyspepsia and episodic diarrhea are minor side-effects, but (reversible) severe liver toxicity is a major side effect which occurs rather frequently.

It has been demonstrated that ketoconazole effectively controls hypercortisolism in all types of Cushing's syndrome, but mostly case reports and only a few small series have been reported.[3 4 5 6 7]

A larger prospective study published recently by Sonino and coworkers observed 34 patients.[8] These authors show that in patients with pituitary dependent Cushing's disease, ketoconazole (400-800 mg daily) was capable of reducing cortisol levels to normal in almost all patients (90%). In two patients ,however, cortisol levels did increase again to above normal level after 6 months of continuous ketoconazole therapy. In one patient with an adrenal carcinoma and one with ectopic ACTH syndrome ketoconazole doses of 1000-1200 mg daily were unable to control hypercortisolism. Tabarin and coworkers also reported that in case of Cushing's syndrome, due to ectopic ACTH production, ketoconazole was unable to reduce hypercortisolism effectively in a number of patients.[9]

In our patient with Cushing's syndrome caused by ectopic ACTH secretion by a small cell carcinoma of the lung (patient 3), ketoconazole also failed to reduce cortisol levels, while in patient 4 (with Cushing's disease) cortisol levels did decrease to normal levels during ketoconazole therapy although psychiatric disturbances did only disappear after the additional administration of mifepristone.

Synthesized in 1980 as a pure antagonist of progesterone, mifepristone (RU 486) was found to have a high affinity for progesterone receptors (without agonist activity) as well as for glucocorticoid receptors [10 11]. By 1982 it was tested in humans for its ability to interrupt either the luteal phase of the menstrual cycle or early pregnancy [12 13 14]. A single 400 mg dose of RU 486 induces a glucocorticoid-receptor blocking response that lasts at least 34 h. There is a dose-dependent competition between RU 486 and dexamethasone [15].

There seems at present no way how to distinguish between adequate or excessive blockade of glucocorticoid action, which makes the recognition of the development of

adrenal insufficiency in these patients difficult or impossible[16]. Recently it was shown that excessive cortisol receptor blockade during mifepristone therapy can be antagonized successfully by low dose prednisone and dexamethasone, however.[17]

In conclusion the acute blockade of the biological effects of hypercortisolism is sometimes acutely mandatory in patients with Cushing's syndrome of as yet unknown etiology, especially in the case of acute psychosis or when another life-threatening disorders develop. At present we have in these cases available two therapeutic pharmacological options: the first is via a blockade of adrenal cortisol production, and the second is via blockade of the biological effects of cortisol at its receptors. Treatment with ketoconazole is the first choice of treatment, because of the relatively high efficacy and low incidence of side-effects. However, the effects of ketoconazole appears in most instances only after a few days, while even high doses of the drug are in minority of cases unable to reduce cortisol production. Both in the last situation (i.e. when ketoconazole is unable to lower cortisol production) and if the clinical condition requires a rapid, more or less acute total blockade of cortisol receptors (i.e. acute psychosis), the use of mifepristone should be considered. Mifepristone seems more potent in reducing the clinical symptomatology of hypercortisolism than ketoconazole, but the drug cannot be titrated very well because there are no objective biological parameters to measure its effects. Only the development of hypoglycemia and the appearance of eosinophilic granulocytes in the peripheral circulation are indirect signs of the development of clinical adrenal insufficiency in the presence of high circulating cortisol levels.

References:

1. Sonino N. The use of ketoconazole as an inhibitor of steroid production. N Eng J Med 1987;317: 812-818.

2. Lamberts SWJ, Bons EG, Bruining HA, de Jong FH. Differential effects of the imidazole derivates etomidate, ketoconazole and miconazole and of metyrapone on the secretion of cortisol and its precursurs by human adrenocortical cells. J Pharmacol Exp Ther 1987;240: 259-264.

3. Loli P, Berselli ME, Tagliaferri M. Use of ketoconazole in the treatment of Cushing's syndrome. J Clin Endocrinol Metab 1986;63: 1365-1371.

4. McCance DR, Hadden DR, Kennedy L, Sheridan B, Atkinson AB. Clinical experience with ketoconazole as a therapy for patients with Cushing's syndrome. Clin Endocrinol (Oxf) 1987;27: 593-599.

5. Tabarin A, Navarranne A, Guerin J, Corcuff JB, Parneix M, Roger P. Use of ketoconazole in the treatment of Cushing's disease and ectopic ACTH syndrome. Clin Endocrinol (Oxf) 1991;34: 63-69.

6. Khanderia U. Use of ketoconazole in the treatment of Cushing's syndrome. Clin Pharm 1991;10: 12-13.

7. Hoffman DM, Brigham B. The use of ketoconazole in ectopic adrenocorticotropic hormone syndrome. Cancer 1991;1: 1447-1449.

8. Sonino N, Boscaro M, Paoletta A, Mantero F, Ziliotto D. Ketoconazole treatment in Cushing's syndrome: experience in 34 patients. Clin Endocrinol 1991;35: 347-352.

9. Tabarin A, Navarranne A, Guerin J, Corcuff JB, Parneix M, Roger P. Use of ketoconazole in the treatment of Cushing's disease and ectopic ACTH syndrome. Clin Endocrinol (Oxf) 1991;34: 63-69.

10. Philibert D, Deraedt R, Teutsch G. RU 38486. A potent antiglucocorticoid in vivo. Int Congr of Pharmacol Tokyo 1981;668: abstract 1463.

11. Proulx-Terland L, Cote J, Philibert D, Deraedt R. Potent antiglucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat. J Steroid Biochem 1982;17: xvii.

12. Herrmann W, Wyss R, Riondel A. Effet d'un steroide antiprogesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut. C R Seances Acad Sci [III] 1982;294: 933-938.

13. Ulmann A. The antiprogestins: a recent advance in fertility regulation. J Steroid Biochem 1987;27: 1009-1012.

14. Couzinet B, Le Strat N, Ulmann A, Baulieu EE, Schaison G. Termination of early pregnancy by the progesterone antagonist RU 486 (Mifepristone). N Eng J Med 1986;315: 1565-1569.

15. Raux-Demay MC, Pierret T, Bouvier d'Yvoire M, Bertagna X, Girard F. Transient inhibition of RU 486 antiglucocorticoid action by dexamethasone. J Clin Endocrinol Metab 1990;70: 230-233.

16. Laue L, Callucci W, Loriaux DL, Udelman R, Chrousos GP. The antiglucocorticoid and antiprogestin steroid RU 486: its glucocorticoid agonist effect is inadequate to prevent adrenal insufficiency in primates. J Clin Endocrinol Metab 1988;67: 602-606.

17. Lamberts SWJ, Koper JW, de Jong FH. The endocrine effects of long-term treatment with metyrapone (RU 486). J Clin Endocrinol Metab 1991;73: 187-191.

CHAPTER 10.

CURRENT THOUGHTS ABOUT OPTIMAL MEDICAL THERAPY OF FUNCTIONING PITUITARY ADENOMAS.

This thesis provides some new information on the treatment of patients with acromegaly, prolactinoma patients and patients with Cushing's syndrome.

The treatment with acromegaly has considerably improved after the introduction of octreotide. It has been shown in the past to be very difficult to "cure" a patient with acromegaly by transsphenoidal operation. Control of hormonal hypersecretion can also be achieved in part of the patients by octreotide therapy. Good selection of patients by age, sex and tumor size is necessary. Not all patients need a transsphenoidal operation, which has its own morbidity and appears to be seldom radical in large invasive adenomas. Especially in older (male) acromegalic patients one can probably primarily choose for octreotide therapy and omit transsphenoidal surgery. Also one can predict the ultimate efficacy of octreotide therapy in controlling hormonal hypersecretion by monitoring the degree of decrease of GH secretion after a single gift of octreotide. The efficacy of bromocriptine can be predicted roughly by measuring the PRL concentration together with the paradoxal rise of GH after TRH administration.
Pretreatment with octreotide of those patients, in whom a transsphenoidal operation is indicated has been suggested to improve the outcome of surgery. However, in the 10 patients we investigated, we could not find such a positive effect.

If tumor size is a problem on its own, octreotide is not the treatment of choice, because the extent of tumor shrinkage is in most instances disappointing, possibly due to an elevation of intracellular GH concentration during octreotide therapy. In this case surgery (often in combination with radiotherapy) is mandatory. After surgery octreotide therapy should be considered in order to control clinical symptomatology related to residual acromegaly. It is uncertain as yet, whether this treatment is safe enough to allow omission of post-operative radiotherapy.

The treatment of prolactinomas has improved dramatically in the last 20 years. Transsphenoidal hypophysectomy as primary treatment is almost never indicated any more. Dopaminergic drugs are effective enough to control increased PRL production and tumor growth in the vast majority of patients. Also considerable tumor size shrinkage occurs in most patients.This by itself can be a problem in patients with very large adenomas which infiltrate the base of the skull. Tumor size reduction in these patients can lead to severe problems as liquorrhea and meningitis.

An important problem remains that bromocriptine, as the first clinically introduced dopaminergic drug, has considerable side effects, which result in a decrease

in patient compliance in part of the patients. The development of newer, more specific dopaminergic drugs e.g. CV 205-502, were shown to be an improvement in the therapy of (macro)prolactinomas, with regard to the lower incidence of side-effects, as well as with regard to their compliance, because this drug can be taken once daily.

The treatment of Cushing's syndrome depends entirely on its cause. The diagnosis of Cushing's syndrome due to ectopic ACTH or CRH production seems to be improved by the introduction of new procedures, like petrosal sinus catheterisation and the use of NMR. In the case of ectopic hormone production, radioactive labelled somatostatin analogues are promising new tools, both as a rapid and sensitive diagnostic procedure, as well as a therapeutic approach.

Also several other new drugs have become available. Both drugs blocking adrenal cortisol production (e.g. ketoconazole) as well as cortisol receptor blocking agents like mifepristone (RU 486) seem to have a place in the medical treatment of Cushing's syndrome. These drugs are better capable of controlling the clinical effects of hypercortisolism in Cushing's syndrome than the older ones. They still have considerable side-effects and, in the case of mifepristone treatment, monitoring the degree of cortisol receptor blockade is difficult, if not impossible.

CHAPTER 11.

SUMMARY

Treatment of pituitary diseases has changed considerably over the last 35 years. Apart from the introduction of transsphenoidal surgery the development of dopaminergic drugs, as well as of the somatostatin analog octreotide have been major steps forward towards the control of the growth and hormonal hyperproduction by pituitary adenomas. The improved understanding of the interaction between hormones and their receptors, the knowledge about hypothalamic releasing factors and hormones, as well as the development of RIA- and ELISA techniques for the measurement of circulating hormone and growth factor levels have also contributed to this progress in treatment.

This thesis is dealing with some aspects of the medical treatment of neuroendocrine disorders.

In **Chapter 1**, a survey of the literature is presented, which deals with the diagnosis and current treatment of acromegaly, prolactinomas, Cushing's syndrome and TSH- and gonadotrophin-secreting adenomas.

In **Chapter 2**, the aims and scope of the thesis were listed.

In **Chapter 3** we tried to answer the first question, which was: Is it possible to predict the sensitivity of tumorous GH release in acromegalic patients to treatment with either bromocriptine or octreotide?

In this chapter we describe the results of a study, involving a group of 100 untreated acromegalic patients, in which we investigated the relationship between age, sex, pituitary tumor volume, serum GH, PRL and IGF-I levels with the responsiveness of GH to TRH, bromocriptine and octreotide.

The results confirmed that bigger pituitary adenomas secrete higher quantities of GH ($r=0.46$; $p<0.01$). GH and IGF-I levels were related up till a certain cut-off level of serum GH concentrations (in our case 40 μg/L), indicating that at this GH level IGF-I production is maximally stimulated.

The severity of the disease tends to be less in elderly, especially male acromegalic patients (using IGF-I levels and circulating GH levels as criteria). There was a homogeneity in the responsiveness of tumorous hormone secretion to TRH, bromocriptine and octreotide. There was a close correlation between the responsiveness of GH release to octreotide and bromocriptine ($r=0.60$; $p<0.01$), which might be of clinical importance. (see also in chapter 4).

The most important conclusion from our study was that elderly, especially male patients show the highest sensitivity to the GH release inhibitory effects of octreotide. Together with the conclusion that the activity of the disease in these patients tends to be the lowest, we suggest that octreotide might be used successfully as a primary medical therapy in these elderly acromegalics.

In **Chapter 4** the results of a study in 51 acromegalic patients are presented, in whom the dynamics of the acute effects of 50 μg octreotide subcutaneously, 2.5 mg bromocriptine orally and the combination of these drugs were tested. In this way, we tried to give an answer to question 2, which was: While both bromocriptine and octreotide are proven to be useful in the treatment of acromegaly, one can wonder whether long-term treatment with a combination of both drugs might be of benefit in clinical practice.

It appeared that octreotide produces in most patients a stronger and faster suppression of GH levels than bromocriptine, while the combination of both drugs had an additive effect in lowering GH levels, especially between 7 and 10 hours after drug administration. Previous studies had shown that both the acute reaction of GH to a single dose of 2.5 mg bromocriptine, as well as that to a single subcutaneous injection of 50 μg octreotide closely predict the ultimate suppressive effect of chronic therapy with these two drugs on circulating GH and IGF-I levels. GH secretion tends to escape from suppression by octreotide towards every next injection when given in the usual three times daily treatment scheme, therefore causing an insufficient suppression of IGF-I production in part of the patients. Combination therapy of octreotide together with bromocriptine may be effective especially in those acromegalic patients, in whom GH levels show this "escape" towards each next injection of octreotide.

In **Chapter 5**, we tried to answer the next question: Octreotide therapy results in pituitary tumor shrinkage in about half of acromegalic patients. However, the degree of shrinkage is often marginal. Which factors play a role in the mechanism of octreotide mediated tumor shrinkage?

In trying to answer this question we investigated the effects of incubation of human GH secreting pituitary adenoma cells for 4 days with 10nM octreotide on GH release, intracellular GH concentrations, and GH mRNA levels. In all adenomas GH release was significantly reduced. However octreotide induced a dose-dependent increase (range 24-517%) in intracellular GH levels in 6 out of 7 adenomas. Also bromocriptine

induced an increase in intracellular GH levels in adenomas where it inhibited GH secretion. These changes in secretion of GH (decrease) and intracellular GH content (increase) were still present after three weeks of exposure to 10nM octreotide. The changes appeared to be reversible, because withdrawal of octreotide after 6 days of incubation resulted in a decrease in the accumulated intracellular GH concentration to control levels. A time-dependent increase in mRNA concentration was found during incubation with 10nM octreotide in 2 of the 3 adenomas tested. The third one showed a slight decrease in the intracellular mRNA levels.

In conclusion it was demonstrated that long-term exposure of GH secreting pituitary adenoma cells to octreotide in vitro causes an increase in the intracellular GH concentration in a majority of the adenomas tested. Probably this is caused by an increase in GH mRNA within the tumor cell.

Although octreotide turns out to be quite effective in reducing the serum GH concentrations, the corresponding reduction in pituitary tumor size was frequently disappointing. This study gives a possible explanation of this phenomenon because we found that the intracellular GH content increases during octreotide therapy. We hypothesize that the relatively small tumor shrinkage in acromegalic patients as observed during chronic octreotide therapy is the end result of a balance in which somatostatin (analog) primarily inhibits GH secretion, without affecting its synthesis. This is initially accompanied by a piling up of hormone within the tumor cells. This, however, is secondarily followed by increased lysosomal degradation of the stored hormone. The efficacy of this last intracellular mechanism probably defines the degree of tumor shrinkage which is eventually reached during octreotide therapy.

In Chapter 6, we tried to answer the third question, which was: Is it possible to improve the outcome of surgical treatment by pretreatment of acromegalic patients for several months with octreotide?

After transsphenoidal surgery, GH levels of less than 5 μg/L are reached in less than 30% of the patients with extrasellar extension of the adenoma. In the literature it has been claimed recently that the result of transsphenoidal operation for GH secreting pituitary tumors was better after treating several small groups of patients preoperatively with octreotide.

To investigate these findings properly, a prospective multicentre open label randomized comparative study is currently performed by the Sandoz Company (Basel; Switzerland) of 100 previously untreated acromegalic patients. These 100 patients were randomly allocated to receive either transsphenoidal surgery or octreotide (administered

subcutaneously 100 μg t.i.d. for a period of 4 months) prior to surgery. This international study has not been finished yet.
We participated in this study by entering 20 patients and have analyzed the results. Mean GH levels before and after 16 weeks of octreotide therapy were reduced significantly by 51% (24.4 ± 16.5 vs 14.6 ± 16.2 μg/l; $p<0.05$) in the group of 10 patients who were treated preoperatively, while IGF-I levels had reduced from 7.5 ± 1.4 mU/L before to 3.3 ± 1.5 mU/l after 16 weeks of octreotide therapy ($p<0.05$). Postoperatively, GH levels were similarly reduced in the octreotide pretreated (24.4 ± 16.5 to 14.3 ± 12.5 μg/l; $p<0.05$) and in the control group (22.3 ± 19.8 to 9.7 ± 6.0 μg/l; $p<0.05$). Immediately after surgery, IGF-I levels were reduced significantly in the octreotide pretreated group (7.5 ± 1.4 to 4.1 ± 1.6 mU/L; $p<0.05$), while no reduction was found in IGF-I concentrations in the control group (7.2 ± 1.6 to 7.9 ± 2.4 mU/l). Eight to ten weeks after surgery, however, the reduction in IGF-I levels in the pretreated group were not significant any more, in comparison with pretreatment values.

These findings suggest that pretreatment of acromegalic patients with octreotide before surgery does not improve the outcome of surgery if circulating IGF-I levels are used as criterium. We want to emphasize, however, that final conclusions can only be drawn when the results of all 100 patients who entered this study are analyzed.

In **Chapter 7**, the following question was answered: The occurrence of side-effects, as well as the need for two or three daily doses of bromocriptine remain important problems in the long-term treatment of prolactinomas. Can new dopaminergic drugs, like CV 205-502, be used in macroprolactinoma patients, intolerant to bromocriptine, in order to reduce side-effects, and is a once daily administration of this new drug as effective as two or three times daily administration of bromocriptine?

In this chapter we describe the effect of therapy with CV 205-502 of 12 macroprolactinoma patients and 8 patients with PRL secreting tumors, who were selected because of previous repeatedly shown intolerance to bromocriptine. In the patients with a macroprolactinoma, therapy with 0.075-0.450 mg CV 205-502 for 12 months reduced PRL levels by 91.2 ± 5.4 %. In the bromocriptine-intolerant patients, 0.075-0.300 mg CV 205-502 reduced PRL levels by 80.2 ± 6.3 %.

There was a close correlation between pretreatment serum PRL levels and pituitary tumor size while the decrease in tumor size after 12 months of CV 205-502 therapy (-74 ± 6%) correlated closely with the decrease in serum PRL levels ($p<0.01$). CV 205-502 caused only mild and transient side-effects in a minority of the patients. Therefore we conclude that this drug can be used to treat patients who have shown

previous intolerance to bromocriptine. It is a potent dopaminergic drug, which at a single once daily administration effectively controls PRL secretion and induces tumor shrinkage in (macro)prolactinoma patients.

In macroprolactinoma patients with normal anterior pituitary function CV 205-502 caused a significant rise in serum IGF-I concentrations. Although the cause of this is still controversial, this increase in IGF-I levels and the possible ensuing anabolic effects might contribute to the sense of well-being reported by most of the patients in this study.

In **Chapter 8**, we tried to answer the next question, which was: What is the explanation for the fact that some patients with huge hypophyseal tumors present themselves primarily with pharyngeal problems, and why do most of them have normal or almost normal anterior pituitary functions?

The findings of Erdheim (1904) of the existence of a pharyngeal pituitary in man gave rise to the suspicion that remnants of the craniopharyngeal duct or the pharyngeal pituitary might be the primary focus of tumor growth. There is uncertainty about the physiological role of the pharyngeal pituitary which was shown to contain in the normal situation at least seven hormone-producing cell types.

In previous studies by Klijn in our clinic it was found in 62 patients that there was a close relationship between tumor size of prolactinomas and the occurrence of secondary anterior pituitary insufficiency. If tumor size was greater than 3 cm^2 (lateral area) the incidence of disturbances in anterior pituitary hormone secretion in the reaction to TRH, LHRH and metyrapone was very high. In the study presented in chapter 8, we report on six patients with very large chromophobe pituitary tumors (all exceeding 3 cm^2) in whom no or only discrete abnormalities of anterior pituitary functional reserve were found. In the four patients with macroprolactinomas the very large tumors had caused radiological destruction of the sellar region. However, in the two patients with chromophobe non-functioning pituitary tumors the pituitary fossa was normal at radiological examination. The nasal and/or paranasal (sinal) localization of these very big pituitary tumors in patients with (virtually) normal anterior pituitary function suggest that tumor growth in these patients originated from another than the sellar region, i.e. from the pharyngeal pituitary.

The potential primary nasal origin of these tumors raises specific problems, because shrinkage of the part of the tumor infiltrating the base of the skull and/or pituitary fossa can result in liquorrhea. In two macroprolactinoma patients, central necrosis of the tumor (with air-trapping) during dopaminergic drug therapy might have been related to previous radiotherapy, but the localization poses extra risks for the

development of local infections and/or meningitis.

In **Chapter 9**, we discussed the next question: In patients with severe Cushing's syndrome, acute psychiatric disturbances can be threatening, while they interfere with normal diagnostic and therapeutic steps. Is it possible to improve the mental status in these patients by the use of the cortisol receptor antagonist RU 486 (mifepristone)?

Firstly we describe 2 patients with severe Cushing's syndrome with acute psychosis related to hypercortisolism originating from (inoperable) adrenal cancers. Thereafter two other patients are described with Cushing's syndrome complicated by severe psychiatric disturbances due to ectopic ACTH secretion from small cell lung cancer and to Cushing's disease. In all four patients mifepristone (400-800 mg/day) rapidly reversed these severe bouts of psychosis.

When the etiology of the syndrome is still unclear, but the severity of the disease necessitates direct medical treatment, one chooses in general primarily for treatment with ketoconazole. Several side-effects can occur after treatment with ketoconazole is initiated. Especially liver function disturbances limit its use. Also the effect of lowering adrenal cortisol production in patients with Cushing's syndrome occurs rather slowly over days, while in part of patients with adrenal cancer and with ectopic ACTH syndrome ketoconazole seems unable to control hypercortisolism, even if doses of 1000-1200 mg daily are used.

A single 400-800 mg dose of RU 486 induces a rapidly occurring glucocorticoid receptor blocking response which lasts at least 34 h. There is a dose-dependent competition at the glucocorticoid receptor between RU 486 and glucocorticoids, like prednisone and dexamethasone.

There is currently, however, no way to distinguish between adequate or excessive blockade of glucocorticoid action, making the recognition of adrenal insufficiency in these patients difficult. Mifepristone seems a drug which can be effectively used in the control of acute psychosis of patients with Cushing's syndrome or in the case of other life-threatening complications. Close clinical supervision is needed, however, in order to monitor the clinical course in these patients.

CHAPTER 12.

SAMENVATTING

In de laatste 25 jaar is er veel veranderd in de behandeling van hypofysaire aandoeningen. Behalve de introductie van de transnasale hypofysectomie zijn vooral de ontwikkeling van dopaminerge farmaca en het somatostatine-analogon octreotide belangrijk geweest voor een verbetering in behandeling van hypofysaire hormonale overproduktie door hypofyse-adenomen. Ook de grotere kennis van interacties tussen hormonen en hun receptoren, en de ontwikkeling van RIA en ELISA laboratoriumtechnieken ter bepaling van hormoon en groeifactor concentraties hebben bijgedragen tot deze verbetering in de behandeling.

In dit proefschrift worden enkele nieuwe aspecten van de behandeling van neuroendocriene stoornissen besproken.

In **Hoofdstuk 1**, wordt een overzicht van de literatuur gegeven, die de diagnostiek en behandeling van acromegalie, prolactinomen, het syndroom van Cushing en TSH- en gonadotrofine-producerende adenomen beschrijft.

In **Hoofdstuk 2**, worden de vraagstellingen van dit proefschrift vermeld.

In **Hoofdstuk 3** is geprobeerd antwoord te geven op de vraag of het mogelijk is de gevoeligheid van GH producerende tumoren voor bromocriptine of octreotide te voorspellen.

In dit hoofdstuk beschrijven we de resultaten van een studie van 100 onbehandelde acromegalie-patiënten, waarbij de relatie werd onderzocht tussen leeftijd, geslacht, tumorgrootte, serum GH- en IGF-I concentraties en de gevoeligheid van de GH-secretie na toediening van TRH, bromocriptine en octreotide.

De resultaten bevestigen de eerdere bevinding dat grotere hypofyse tumoren grotere hoeveelheden GH secerneren ($r=0.46$; $p<0.01$). Serum GH- en IGF-I concentraties vertoonden een significante correlatie, echter alleen bij patiënten met een GH concentratie van 40 μg/L of minder. Bij deze GH concentratie is de IGF-I productie waarschijnlijk maximaal gestimuleerd.

De ernst van de ziekte lijkt minder bij oudere, met name mannelijke acromegalie-patiënten (hierbij IGF-I en GH als criterium gebruikend). Er was een significant positieve correlatie aanwezig tussen de gevoeligheid van de hormoonsecretie door de tumor voor TRH, bromocriptine en octreotide. Ook werd een correlatie gevonden tussen de gevoeligheid van de tumor voor octreotide en bromocriptine ($r=0.60$; $p<0.01$) voor

wat betreft de daling in GH concentratie. (zie ook Hoofdstuk 4).

De belangrijkste bevinding van ons onderzoek is dat de GH secretie bij oudere, met name mannelijke acromegalen de hoogste gevoeligheid voor octreotide vertoont. Dit gegeven, tezamen met het feit dat de activiteit van de ziekte in deze groep patiënten het laagst lijkt te zijn heeft ons doen besluiten octreotide-therapie als een potentieel succesvolle primaire therapie voor oudere patiënten te zien.

In **Hoofdstuk 4** worden de resultaten van een studie van 51 acromegalie-patiënten gepresenteerd, bij wie de acute effecten van 50 μg octreotide, subcutaan toegediend, werden vergeleken met het acute effect van 2.5 mg oraal toegediende bromocriptine, alsook met de combinatie van deze medicamenten. Op deze manier hebben we getracht een antwoord op vraag 2 te geven, welke luidde: Omdat zowel bromocriptine en octreotide een bewezen gunstig effect hebben bij de behandeling van acromegalie, kan men zich afvragen of wellicht langdurige therapie met een combinatie van deze farmaca een klinisch bruikbare methode kan zijn.

Het bleek dat bij de meeste patiënten octreotide een sterkere en meer langdurige onderdrukking van de GH secretie bewerkstelligde, terwijl de combinatietherapie een additief effect bleek te hebben, speciaal tussen 7 en 10 uur na toediening. Eerdere studies hadden aangetoond dat de acute reactie van GH op zowel een enkele dosis van 2.5 mg bromocriptine als een enkele injectie van 50 μg octreotide vrij nauwkeurig het uiteindelijke effect van chronische therapie met deze medicamenten voorspellen. GH secretie neigt onder de suppressie van octreotide te ontsnappen, met name vlak voor elke nieuwe injectie, wanneer het standaard schema van drie maal daags een injectie wordt aangehouden. Dit kan een onvolledige suppressie van de IGF-I productie veroorzaken bij een deel van de patiënten. Combinatie-therapie met zowel octreotide als bromocriptine zou derhalve van nut kunnen zijn vooral voor patiënten bij wie de GH secretie onder de octreotide suppressie lijkt te ontsnappen vlak voor elke nieuwe injectie.

In **Hoofdstuk 5** is geprobeerd antwoord te geven op de volgende vraag: Therapie met octreotide resulteert in afname van de tumorgrootte in ongeveer de helft van de acromegalie-patiënten. De mate van afname in grootte is echter vaak gering. Welke factoren spelen een rol in het mechanisme van tumorschrompeling tijdens therapie met octreotide.

In een poging deze vraag te beantwoorden hebben we de effecten onderzocht van incubatie van menselijke, GH-secernerende tumorcellen met 10 nM octreotide gedurende

4 dagen op de intracellulaire GH- en GH mRNA concentraties.

In alle adenomen werd de GH secretie significant geremd. In 6 van de 7 adenomen veroorzaakte octreotide echter een dosisafhankelijke toename van de intracellulaire GH concentratie (range 21-517%). Ook bromocriptine veroorzaakte een toename van de intracellulaire GH concentratie in die adenoomcellen waarin het een remming van de GH secretie bewerkstelligde. Deze bovengenoemde veranderingen in GH secretie en intracellulaire GH concentratie waren na 3 weken blootstelling aan 10 nM octreotide nog steeds aanwezig. De veranderingen bleken reversibel, omdat het verwijderen van octreotide uit het medium na een incubatie van 6 dagen leidde tot een afname van het in de tumorcel opgehoopte GH tot het controleniveau.

In 2 van de 3 onderzochte tumoren werd een tijdsafhankelijke toename van de intracellulaire mRNA concentraties tijdens incubatie met octreotide gevonden. De derde tumor toonde echter een geringe afname van de mRNA concentratie.

Concluderend kan gesteld worden dat chronische blootstelling van menselijk GH producerende hypofyse-tumorcellen aan octreotide leidt tot een toename van de intracellulaire GH concentratie in een meerderheid van de onderzochte adenomen. Mogelijkerwijze is dit een gevolg van de toename in GH-mRNA in de tumorcel.

Hoewel octreotidetherapie goed in staat is een daling in serum GH concentraties te bewerkstelligen, blijkt de corresponderende reductie in tumorgrootte vaak teleurstellend. Deze studie verschaft een mogelijke verklaring voor dit fenomeen, omdat wij een toename in de intracellulaire GH concentratie vonden tijdens behandeling met octreotide.

Wij vermoeden dat de relatief geringe afname van de tumorgrootte bij acromegalie-patiënten tijdens behandeling met octreotide het eindresultaat is van een balans, waarbij somatostatine (analogon) aanvankelijk de GH secretie remt zonder de synthese te beïnvloeden. Dit gaat aanvankelijk gepaard met een stapeling van het GH in de tumorcel. Dit wordt echter gevolgd door een toename in de lysosomale afbraak van het opgestapelde hormoon. De effectiviteit van dit laatstgenoemde mechanisme bepaalt waarschijnlijk de uiteindelijke mate van afname in tumorgrootte welke wordt bereikt tijdens langdurige behandeling met octreotide.

In **Hoofdstuk 6** is geprobeerd om antwoord op de derde vraag te verkrijgen, welke als volgt luidde: Is het mogelijk om de postoperatieve resultaten van een transsfenoïdale operatie ter behandeling van acromegalie te verbeteren door patiënten verscheidene maanden voor te behandelen met octreotide.

Bij slechts 30% van de patiënten met een hypofyseproces met extrasellaire

uitbreiding van het adenoom wordt na transsfenoïdale chirurgie een GH concentratie van kleiner dan 5 μg/L bereikt. Uit recent onderzoek bij kleine groepen patiënten kwam naar voren dat het resultaat van een transsfenoidale operatie voor GH producerende hypofysetumoren zou kunnen worden verbeterd door preoperatieve voorbehandeling met octreotide.

Om dit gegeven nader te onderzoeken werd een prospectief, open, vergelijkend en gerandomiseerd onderzoek gestart door de firma Sandoz (Bazel; Zwitserland), waarin 100 onbehandelde acromegalie patiënten werden opgenomen. Deze 100 patiënten werden gerandomiseerd om òf direct te worden geopereerd middels transsfenoïdale chirurgie, òf eerst gedurende 4 maanden te worden voorbehandeld met octreotide (subcutaan toegediend in een dosering van 100 μg drie maal per dag). Deze internationale zgn "multicentre" trial is op dit moment nog niet beëindigd. In deze studie werd door ons met 20 patiënten deelgenomen en de resultaten werden gedeeltelijk geanalyseerd. In de groep patiënten welke werden voorbehandeld daalde de gemiddelde GH concentratie na 16 weken behandeling met octreotide daalde met 51% in vergelijking met de uitgangswaarden voor therapie. (24,4 ± 16,5 vs 14,6 ± 16,2 μg/L; $p<0,05$). In deze groep patiënten daalde het IGF-I gehalte in dezelfde periode van 7,5 ± 1,4 mU/L naar 3,3 ± 1,5 mU/L ($p<0,05$). Het postoperatieve GH gehalte was zowel in de met octreotide voorbehandelde (24,4 ± 16,5 naar 14,3 ± 12,5 μg/L; $p<0,05$), als in de niet voorbehandelde groep patiënten (22,3 ± 19,8 naar 9,7 ± 6,0 μg/L; $p<0,05$) gedaald. Direct (7 dagen) na chirurgie waren de IGF-I concentraties significant gedaald in de met octreotide voorbehandelde groep (7,5 ± 1,4 naar 4,1 ± 1,6 mU/L; $p<0,05$); maar de niet voorbehandelde groep liet direct na operatie geen significante daling zien in de IGF-I concentratie (7,2 ± 1,6 naar 7,9 ± 2,4 mU/L). Acht tot tien weken na operatie echter was er in de voorbehandelde groep geen significante reductie in IGF-I concentratie ten opzichte van de uitgangsconcentratie meer meetbaar.

Dit suggereert dat voorbehandeling van acromegalie-patiënten met octreotide voor transsfenoidale chirurgie de postoperatieve resultaten niet beïnvloedt, wanneer het IGF-I gehalte als parameter gebruikt wordt. We wijzen er echter op dat de slotconclusie van dit onderzoek pas getrokken kan worden wanneer de resultaten van alle 100 patiënten die in het onderzoek werden opgenomen bekend en geanalyseerd zijn.

In **Hoofdstuk 7** werd de volgende vraag beantwoord: Het optreden van bijwerkingen, zowel als de noodzaak om bromocriptine drie maal daags toe te dienen, blijven belangrijke problemen bij de lange termijn behandeling van prolactinoompatiënten. Kunnen bij patiënten intolerant voor bromocriptine wellicht beter

meer moderne dopaminerge medicamenten, zoals bijvoorbeeld het CV 205-502, gebruikt worden, waardoor er minder bijwerkingen optreden en kan in zo'n geval de toediening slechts één maal per dag geschieden in plaats van drie maal per dag zoals dat bij de behandeling met bromocriptine het geval is?

In dit hoofdstuk zijn de resultaten beschreven van een onderzoek naar de effectiviteit van de behandeling met CV 205-502 van 12 macroprolactinoompatiënten en 8 patiënten met PRL-secernerende hypofysetumoren, die waren geselecteerd op grond van een herhaald opgetreden intolerantie tegen bromocriptine. In de macroprolactinoompatiënten leidde behandeling gedurende één jaar met CV 205-502 tot een daling van het PRL gehalte met 91,2 ± 5,4% (doses 0,075-0,450 mg). Na één jaar behandeling met 0,075-0,300 mg CV 205-502 werd het serum PRL gehalte in de patiënten groep, intolerant voor bromocriptine, gereduceerd met 80,2 ± 6,3%.

Er bestond een duidelijke correlatie tussen de serum PRL concentratie voor behandeling en de tumorgrootte, terwijl de afname in de tumorgrootte na 12 maanden behandeling met CV 205-502 (-74 ± 6%) een correlatie vertoonde met de mate van afname van het serum PRL gehalte ($p<0,01$). Slechts een klein aantal patiënten vertoonde geringe voorbijgaande bijwerkingen tijdens de behandeling met CV 205-502.

Wij concluderen derhalve dat CV 205-502 gebruikt kan worden bij patiënten met intolerantie voor bromocriptine. Daarnaast verlaagt het in een één maal daagse dosering de PRL secretie van macroprolactinoompatiënten effectief, terwijl daarbij ook de tumorgrootte aanzienlijk wordt gereduceerd.

In macroprolactinoompatiënten met een intacte hypofysevoorkwabsfunctie veroorzaakt CV 205-502 een significante stijging van de serum IGF-I concentratie. Alhoewel het werkingsmechanisme van dit fenomeen nog onduidelijk is, kan deze stijging in de serum IGF-I concentratie, en de daarbij passende positieve metabole uitwerking bijdragen aan de toename van het gevoel van algemeen welbehagen, dat werd gerapporteerd bij de meeste patiënten in deze studie.

In **Hoofdstuk 8** werd getracht de volgende vraag te beantwoorden, welke luidde: Wat is de verklaring voor het feit dat sommige patiënten met zeer grote hypofysetumoren zich aanvankelijk presenteren met primaire neus-keel problemen, en waarom hebben deze patiënten een normale- of bijna normale hypofysevoorkwabsfunctie?

Erdheim beschreef in 1904 voor het eerst het bestaan van een pharyngeale hypofyse in de mens. Wellicht is het mogelijk dat overblijfselen van de ductus craniopharyngeus of deze pharyngeale hypofyse het primaire focus van tumorgroei zijn.

Of er een fysiologische rol voor de pharyngeale hypofyse bestaat is niet duidelijk, maar wel zijn er in de normale situatie in dit orgaan tenminste 7 typen hormoon-producerende cellen aangetoond.

In een eerdere studie in ons instituut door Klijn en medewerkers in 62 patiënten is gebleken dat er een sterk verband bestaat tussen tumorgrootte van prolactinomen en het optreden van een hypofysevoorkwabsinsufficiëntie. Als de oppervlakte van een tumor (laterale CT meting) de 3 cm^2 te boven gaat was de incidentie van een gestoorde reactie op TRH,LHRH en metopiron zeer hoog. In de studie, vermeld in hoofdstuk 8 beschrijven wij 6 patiënten met zeer grote chromofobe hypofysetumoren, alle met een oppervlakte van meer dan 3 cm^2, waarbij geen- of slechts geringe afwijkingen in de hypofysevoorkwabsfunctie werden aangetoond. Bij de 4 onderzochte patiënten met zeer grote macroprolactinomen had de tumor radiologisch een destructie van de regio rond de sella turcica hadden veroorzaakt. Bij de 2 patiënten met een niet producerend hypofyseproces echter was de hypofysaire fossa radiologisch geheel intact. De nasale en/of paranasale (sinus) lokalisatie van deze zeer grote tumoren, waarbij de hypofysevoorkwabsfunctie geheel of praktisch geheel intact blijft, suggereert dat de oorsprong van deze tumoren bij deze patiënten in een andere regio dan de sella turcica is gelegen, bijvoorbeeld de pharyngeale hypofyse.

De mogelijk primair nasale oorsprong van deze tumoren levert specifieke problemen op, omdat tumorschrompeling van het gedeelte van de tumor dat de schedelbasis infiltreert liquorrhoea kan veroorzaken. Bij 2 patiënten ontstond tijdens therapie met dopaminerge medicamenten centrale tumornecrose, waarbij bij radiologisch onderzoek lucht aanwezig bleek binnen de tumor. De lokalisatie geeft een verhoogd risico op het ontwikkelen van locale infecties en/of een meningitis.

In **Hoofdstuk 9** wordt de volgende vraag bediscussieerd: Bij patiënten met een ernstig syndroom van Cushing kunnen levensbedreigende psychiatrische stoornissen optreden, welke kunnen interfereren met de diagnostische procedures, nodig voor een uiteindelijke behandeling. Is het mogelijk de geestelijke situatie bij deze patiënten te verbeteren door gebruik te maken van het glucocorticoidreceptor-blokkeerende geneesmiddel RU 486 (mifepriston)?

Allereerst beschreven we in dit hoofdstuk twee patiënten met een ernstig syndroom van Cushing, gecompliceerd door een acute psychose, tengevolge van hypercortisolisme door (inoperabele) bijnierschorscarcinomen. Daarna beschreven we twee andere patiënten waarvan één met een syndroom van Cushing tengevolge van ectopische ACTH productie door een kleincellig ongedifferentieërd longcarcinoom en

de ander met de ziekte van Cushing. Bij alle 4 patiënten was mifepriston (400-800 mg/dag) in staat om de ernstige psychiatrische stoornissen snel te laten verdwijnen.

Wanneer de oorzaak van het syndroom van Cushing nog niet is opgeklaard, terwijl de ernst van de ziekte direct medisch ingrijpen noodzakelijk maakt, zal men in het algemeen kiezen voor een primaire behandeling met ketoconazol. Tijdens behandeling met ketoconazol kunnen echter ernstige bijwerkingen optreden. Met name leverfunctiestoornissen kunnen het gebruik ervan onmogelijk maken. Ook het gewenste resultaat, te weten het verminderen van de cortisolproductie door de bijnierschors kan dagen op zich laten wachten, terwijl daarnaast bij een aantal patiënten met een bijnierschorscarcinoom of een ectopisch ACTH syndroom ketoconazol niet in staat bleek te zijn om een effectieve controle van de productie van cortisol te bewerkstelligen, zelfs niet in doses van 1000 tot 1200 mg per dag.

Een enkelvoudige dosis van 400-800 mg mifepriston veroorzaakt een snelle blokkade van de glucocorticoidreceptoren, welke tenminste 34 uur aanhoudt. Er blijkt een dosis-afhankelijke competitie te bestaan van de glucocorticoidreceptoren tussen mifepriston en glucocorticoiden als dexamethason en prednison. Op dit moment echter is er geen methode om een onderscheid te maken tussen een gedeeltelijke, of een te ver doorgevoerde blokkade van de glucocorticoidreceptoren door mifepriston. Hierdoor is het uitermate moeilijk om een optredende bijnierschorsinsufficiëntie bij deze patiënten goed te herkennen.

Mifepriston lijkt een medicament dat effectief gebruikt kan worden bij patiënten met een syndroom van Cushing ter controle van een acute psychose, of in het geval van een andere levensbedreigende complicatie. Intensieve klinische observatie is echter noodzakelijk om het klinisch verloop van deze patiënten goed te kunnen bewaken.

CURRICULUM VITAE:

De schrijver van dit proefschrift werd op 6 juni 1957 geboren te 's Gravenhage. Na het behalen van het Gymnasium-ß diploma aan de Groen van Prinsterer Scholengemeenschap te Vlaardingen werd in 1975 begonnen met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam, alwaar in 1982 het artsexamen werd afgelegd. Van 1983 tot februari 1986 was hij werkzaam als arts-assistent Inwendige Geneeskunde in het voormalige Eudokia ziekenhuis te Rotterdam (hoofd G. van der Waa), waarna van februari 1986 tot februari 1991 de opleiding tot internist werd gevolgd op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam (hoofd Prof. J.H.P Wilson). Op 1 februari 1991 werd hij als internist ingeschreven in het specialisten register. Sindsdien als internist werkzaam op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam.

DANKWOORD

Er was niets van dit proefschrift terechtgekomen als de onderstaande personen zich niet ook zeer hadden ingespannen, en voor die inspanning ben ik hen ook zeer erkentelijk.

Met name wil ik noemen mijn promotor Steven, die ondanks recidiverend gestuntel mijnerzijds, vol humor er bij mij altijd de moed in wist te houden om door te zetten en mij wist te leren om uit een brij van meetresultaten uiteindelijk een acceptabel manuscript te distilleren.

Anke de Graaff die menigmaal, op voor haar zeer ongelegen tijdstippen, toch weer tijd wist te vinden om 'even' een artikel te corrigeren of te wijzigen.

Paula Schuijff en al het verplegend personeel van de afdeling 4 Noord, die een groot deel van de gegevens voor mij hebben verzameld door op zeer kundige wijze bij patiënten allerlei metingen te verrichten, vaak volgens omslachtige protocollen.

En tenslotte de mensen van het laboratorium Interne III die al die honderden bepalingen moesten doen die nodig waren voor dit proefschrift.