Neuroendocrine Tumour Markers

Frank R.E. NOBELS

Printed bij Geers, Oostakker, Belgium

Neuroendocrine Tumour Markers

Neuroendocriene
Tumor Merkers

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus

Prof. Dr. P.W.C. Akkermans

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
22 september 1999 om 13.45 uur

door

Frankie Rachel Eduard Nobels

geboren te Hamme, België

PROMOTIECOMMISSIE

PROMOTORES :	Prof. Dr. S.W.J. Lamberts
	Prof. Dr. R. Bouillon
OVERIGE LEDEN :	Prof. Dr. E.P. Krenning
	Prof. Dr. ir. T.J. Visser
	Prof. Dr. T. H. van der Kwast

Aan mijn ouders

List of Abbreviations

α-SU	alpha-subunit
5-HIAA	5-hydroxyindoleacetic acid
ACTH	adrenocorticotrop(h)ic hormone
ADH	antidiuretic hormone
APUD	amine precursor uptake and decarboxylation
BSIPSS	bilateral simultaneous inferior petrosal sinus sampling
CEA	carcinoembryonic antigen
CgA	chromogranin A
CgB	chromogranin B
CGRP	calcitonin-gene related peptide
CNPA	clinically nonfunctioning pituitary adenomas
CRH	corticotrop(h)in releasing hormone
CT	calcitonin
FSH	follicle stimulating hormone
GEP	gastroenteropancreatic
GH	growth hormone
GHRH	growth hormone releasing hormone
GnRH	gonadotrop(h)in releasing hormone
HCG	human chorionic gonadotrop(h)in
IBZM	(S)-2-hydroxy-3-^{123}iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide
IGFBP-3	insulin-like growth factor binding protein-3
IGF-I	insulin-like growth factor-I
IPS	inferior petrosal sinus
LH	luteinizing hormone
MEN	multiple endocrine neoplasia
MTC	medullary thyroid carcinoma
NFPA	nonfunctioning pituitary adenoma
NSE	neuron-specific enolase
POMC	pro-opiomelanocortin
PP	pancreatic polypeptide
PRL	prolactin
PSA	prostate-specific antigen
PTH	parathyroid hormone
PTHrP	PTH-related protein
SCLC	small cell lung carcinoma
SgII	secretogranin II
SHBG	sex-hormone binding globulin
TRH	thyrotrop(h)in releasing hormone
TSH	thyroid stimulating hormone
VIP	vasoactive intestinal polypeptide
VIPoma	VIP-secreting neuroendocrine tumour
VMA	vanilmandelic acid
VP	vasopressin

Chapter 1

Introduction:
Neuroendocrine tumour markers

F.R.E. Nobels and S.W.J. Lamberts.

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

Published in part in:
Clinical Endocrine Oncology, Blackwell Science Ltd., 1997;60:377-382.

Introduction

The neuroendocrine cells of the gastroenteropancreatic (GEP) axis belong to the APUD-system, because they are capable of amine precursor uptake and decarboxylation, leading to the production of amines and small peptides. Currently, over 50 peptides have been identified, secreted by more than 15 different types of neuroendocrine cells scattered throughout the gut [1]. Tumours of these cells are generally characterized by an excessive production of one or several of these peptides. The presence of peptides in tumour tissue can usually be easily identified with immunohistochemical methods, or by demonstrating their mRNA with in situ hybridization techniques [2,3]. The peptides are also frequently released into the circulation, where they can exert their endocrine effects on various targets, often inducing a typical clinical syndrome of hormonal overproduction. These tumours can be called *clinically functioning neuroendocrine* tumours. The circulating peptides can usually be measured with radioimmunologic methods, allowing them to be used as tumour markers [4]. One tumour generally releases several amines or peptides in the circulation. Therefore the choice of possible tumour markers is much wider than in the case of non-endocrine tumours. The situation is much more difficult in so-called *clinically non-functioning neuroendocrine tumours*, not inducing symptoms or signs relating to hormonal hypersecretion. Sometimes, these tumours remain hormonally active, producing peptides without clinical effect, which still can be used as tumour markers [1,5-8]. When the tumour has lost all abilities to produce hormonally active substances one has to resort to the use of non-endocrine secretion markers, such as certain enzymes or other contents of secretory granules.

In the choice of an adequate tumour marker, the following criteria should be taken into account [9]: the marker must be useful (1) to screen populations for the presence of a tumour, (2) to differentiate between the different types of neuroendocrine tumours, (3) to distinguish between benign, intermediate or malignant tumour types, (4) to provide an estimate of the tumour load, (5) to follow the course of a particular tumour over time, in order to be able to evaluate the response to therapeutic interventions, and to rapidly detect an eventual relapse, and (6) to assess the prognosis. Unfortunately none of the current

tumour markers can fulfill all these goals. Therefore, the search for better markers still goes on, and is at present one of the main activities of neuroendocrine research. In addition to the use of the circulating peptides themselves, the receptors for some peptides have recently been shown to be very valuable markers [4]. Their presence on tumour tissue can be demonstrated in vivo by radioisotopic techniques, using radionuclide labeled peptide, which specifically binds to a specific receptor [10].

Serum markers

Clinically functioning neuroendocrine tumours

Table 1 provides an overview of endocrine syndromes, with the most important peptides being responsible for the clinical expression. Most of these peptides can serve as excellent tumour markers, that can be used both in the diagnosis as well as in the follow-up of these neoplasms.

Table 1. Clinical syndromes and markers of neuroendocrine tumours of the GEP axis

tumours	signs of hormone excess	markers
carcinoid	flushing, diarrhea	serotonin, substance P, urine 5-HIAA
gastrinoma	ulcer disease	gastrin
insulinoma	hypoglycemia	insulin
glucagonoma	dermatosis, dementia, diabetes, DVT	glucagon
somatostatinoma	diabetes, steatorrhea, cholelithiasis	somatostatin
VIPoma	diarrhea, hypokalemia, achlorhydria	VIP
secreting	Cushing	ACTH, CRH
"ectopic" hormones	acromegaly	GHRH
	hypercalcemia	PTH, PTHrp
	SIADH	vasopressin
	flushing, diarrhea	calcitonin
clinically non-functioning	none	PP, HCG-subunits, NSE, CgA

GEP = gastroenteropancreatic, DVT = deep venous thrombosis, SIADH = syndrome of inappropriate antidiuretic hormone secretion, 5-HIAA = 5-hydroxyindoleacetic acid, VIP = vasoactive intestinal polypeptide, ACTH = adrenocorticotropic hormone, CRH = corticotropin releasing hormone, GHRH = growth hormone releasing hormone, PTH = parathyroid hormone, PTHrP = parathyroid hormone related peptide, PP = pancreatic polypeptide, HCG = human chorionic gonadotropin, NSE = neuron-specific enolase, CgA = chromogranin A

In the interpretation of the data one should take into account the feedback system involved. The demonstration of inappropriate secretion is often an important clue to the presence of a tumour: high insulin levels in the presence of hypoglycemia in insulinomas [11], high gastrin levels in the presence of gastric acid hypersecretion in gastrinomas [12], and high glucagon levels in the absence of hypoglycemia in glucagonomas [13]. Generally, the serum concentration of the peptide is positively correlated with tumour mass. Thus, changes in the concentration over time provide information on tumour growth or shrinkage, except during treatment with drugs, such as octreotide, that inhibit peptide secretion [14]. In these cases the relation between changes in the concentration of the marker and changes in the volume of the tumour is lost, so that the marker can unfortunately no longer be used to monitor tumour growth.

Usually the peptide marker can be used to assess the prognosis of its tumour. The prognosis of a particular tumour partially depends on the potential of the secreted peptide(s) to produce clinical symptoms. For instance, most insulin-producing tumours, have a good prognosis, since hypoglycemia usually develops very early in the evolution of the disease, when the tumours are still smaller than 3 cm [5]. On the contrary, glucagon-producing tumours are generally much larger (around 7 cm as a mean) when they develop the glucagonoma syndrome [15]. This may select tumours of higher growing-potential, resulting in a higher malignancy rate (around 60 per cent) as compared to glucagon-producing tumours, lacking the syndrome. Most of the latter tumours ar small, benign adenomas discovered by chance in autopsy or surgical specimens. A similar behaviour is shown by somatostatin-producing tumours [16,17]. The prognosis is also influenced by the localization of the peptide production. Tumours of the endocrine pancreas producing eutopic hormones are less frequently malignant (5-15 per cent in the case of insulinomas), than those producing gut hormones, such as gastrin, vasoactive intestinal polypeptide (VIP) or neurotensin (around 60 per cent), or more apparent ectopic hormones, such as adrenocorticotropic hormone (ACTH), vasopressin (VP) or growth hormone releasing hormone (GHRH) (90-100 per cent malignant) [1-3,18-21]. This obviously suggests that tumours with ectopic hormone production are more

deeply transformed, resulting in a more aggressive behaviour. The concept of ectopic hormone production should be put in perspective, however. Many of the secretion products considered to be ectopic are likely to be eutopic phylogenetically and ontogenetically. For instance, during the last years it has been demonstrated that virtually all normal tissues contain small amounts of a precursor of ACTH, probably pro-opiomelanocortin (POMC) [22]. All cancers produce excessive amounts of this precursor, and some of them are able to convert it into biologically active ACTH. In this context, the word ectopic is a misnomer.

The above discussion demonstrates that the secretion products of most GEP endocrine tumours can relatively easily be detected in the circulation and used as tumour markers. Carcinoids, the most commonly occurring gut endocrine tumours, form an exception to this rule [23,24]. Their clinical expression differs according to their origin from fore-, mid- or hindgut. The typical carcinoid syndrome, consisting of diarrhea, flushing and bronchospasm, is caused by midgut carcinoids [23]. Unfortunately, since their secretions drain into the portal circulation and are degraded by the liver, such tumours usually become symptomatic only after substantial hepatic metastasis. Only in rare cases, when carcinoid tissue is present in the retroperitoneum or the ovaries, which drain directly into the systemic venous circulation, the syndrome can manifest itself in the absence of liver metastases [24]. Serotonin (5-hydroxytryptamine) is one of the major secretion products. The measurement of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in urine is the most frequently used parameter in the diagnosis and follow-up of midgut carcinoids. Using an upper cut-off value of 8 mg / 24 hours the sensitivity in the diagnosis of metastasized carcinoids is nearly 75 per cent with a specificity of 100 per cent [23,25]. Direct measurement of plasma serotonin concentrations, even when determined in platelet-rich plasma, does not provide better results [26]. Although the carcinoid syndrome originally was considered to be due to the effects of serotonin secretion, carcinoid tumours are now known to secrete a mixture of bioactive amines and peptides, including histamine, prostaglandins, tachykinins (most notably substance P and neuropeptide K), neurotensin, kallikreins, bradykinin-like peptides, endorphins, somatostatin, VIP, motilin, etc. [21,25-33]. These secretion products may actually be

responsible for a large proportion of the clinical effects that previously have been attributed to increased levels of serotonin. Unfortunately, the serum concentrations of these peptides are not universally elevated in patients with the carcinoid syndrome, and they can also be produced in other situations where flushing occurs (medullary thyroid carcinoma, VIPoma, idiopathic flushing) [32]. Therefore, it is only recommended to screen for some of the above peptides (e.g. substance P, neurotensin and VIP) if blood serotonin and/or urinary 5-HIAA levels are normal, in the presence of firm clinical suspicion of carcinoid syndrome. The use of provocative tests generally does not add to the information provided by the basal measurements. Due to the rarity of fore- and hindgut carcinoids, no information is presently available on the possible role of these secretion products in the diagnosis and follow-up of these tumours. General neuroendocrine markers, such as neuron-specific enolase (NSE) or chromogranin A (CgA) might provide useful alternatives in these cases, although their concentrations tend to increase rather late in the course of the disease.

Clinically non-functioning neuroendocrine tumours

Many neuroendocrine tumours of the GEP axis are not accompanied by clinical signs of endocrine overactivity, either because the secreted peptides are not able to induce clinically important effects, or because the tumour has lost all endocrine secretory capacity. In the former situation the "silentious" hormonal products can serve as markers, in the latter the use of non-hormonal markers may offer a solution in certain cases.

Silentious hormonal markers

Pancreatic polypeptide (PP)

PP is secreted by the pancreatic islets in response to the presence of nutrients in the gut lumen. Vagal cholinergic pathways fulfill an important role in the regulation of PP release [34]. The function of the peptide has still not been elucidated, since no effects are seen at physiological concentrations. In pharmacological doses PP antagonizes the effects of cholecystokinin (CCK) on gallbladder contraction, choledochal relaxa-

tion and pancreatic exocrine secretion [35,36]. The plasma levels of PP are clearly elevated in around 50 per cent of patients with tumours of the endocrine pancreas, most frequently in combination with other pancreatic hormones, seldomly alone [6,7]. In the former case the clinical picture is dominated by the effects of the concomitantly secreted peptides. When PP is the sole hormonal product of the tumour no endocrine clinical syndrome evolves, even not when plasma concentrations exceed 1000 times the normal levels [7]. Elevated plasma levels of PP must be interpreted with caution, since the concentrations can rise in several conditions of vagal cholinergic stimulation (postprandial, hypoglycemia, exercise, etc.) [37]. These situations can be distinguished from tumoural production by cholinergic blockade with atropine [7,38]. Autonomous neoplastic overproduction of PP will not be influenced by the administration of atropine, while in conditions of an increased endogenous cholinergic tone the concentration significantly decreases.

Subunits of human chorionic gonadotropin (HCG)

HCG is a glycoprotein hormone, synthesized during pregnancy by the throphoblastic cells of the placenta [39]. It consists of an α- and β-subunit. The β-subunit is specific for HCG, while the α-subunit is common to the other hormones of the glycoprotein family (LH, FSH, TSH). Although only the intact hormone is biologically active, the uncombined subunits are also released in the circulation. HCG only fulfills a physiological role during pregnancy, when it is responsible for the preservation of the function of the corpus luteum. It is a well established marker of tumourous throphoblastic or germ-cell tissue in hydatiform mole, choriocarcinoma or testicular cancer [40,41]. These neoplasms secrete large amounts of intact HCG, and to a lesser extent also free α- and/or β-subunits. Ectopic production of HCG subunits, not of intact HCG, is frequently encountered in neuroendocrine tumours of the GEP system [8,42-45]. The serum levels are usually much lower than in trophoblastic neoplasms, requiring the application of highly sensitive detection methods. The prevalences reported in literature vary considerably for similar neuroendocrine tumours, probably due to differences in patient selection and assay characteristics (sensitivity, cross-reaction with other glycoprotein subunits, etc.). High levels occur frequently in patients with endocrine pancreatic tumours and in patients with carcinoids [8].

Elevated levels can also be encountered in nonfunctioning neuroendocrine neoplasms, in the absence of other hormonal markers [8]. Both subunits should be measured, since several tumours only secrete α-subunit and no β-subunit and vice versa. By analogy with other forms of ectopic hormonal secretion, it is postulated that elevated levels of these subunits are markers of malignant behaviour [43,45]. This has not been proven yet, however. Seldomly α- and β-subunits can also be produced by tumours that are not of trophoblastic or neuroendocrine origin [46]. Thus, they are not entirely specific markers.

Non-endocrine markers

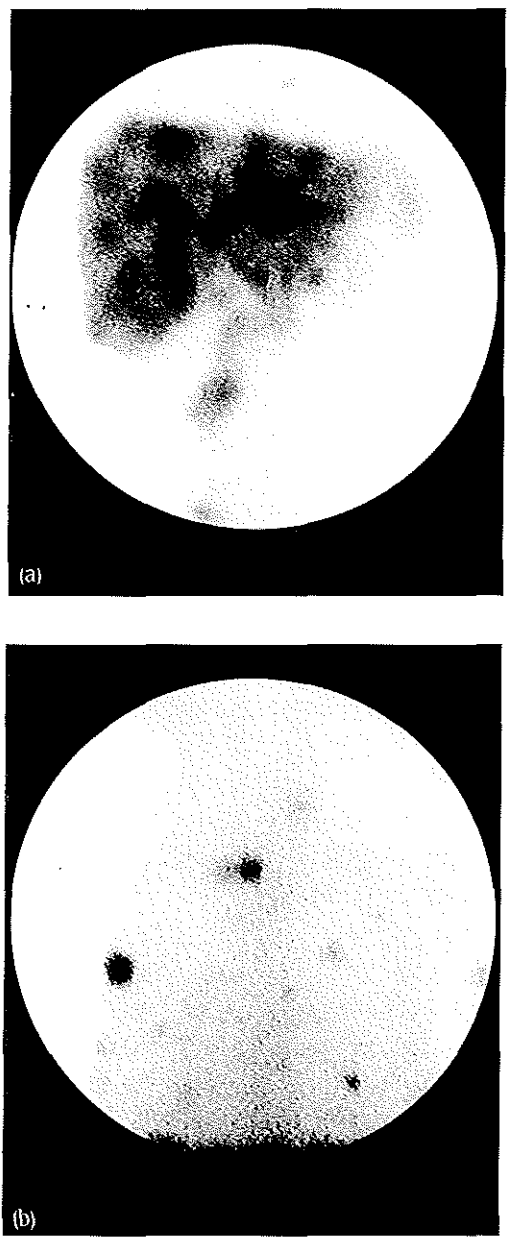
Neuron-specific enolase (NSE)

NSE is a neuron specific isomer of the ubiquitous glycolytic enzyme 2-phospho-D-glycerate hydro-lyase or enolase [47]. This isomer is present in neurons and neuroendocrine cells, and can serve as an immunohistochemical marker for tumours derived from these cells [48]. Elevated serum concentrations of NSE can be detected in all kinds of neuroendocrine neoplasms, regardless of the original cell types or the secreted peptides [49-51]. Most research has concentrated on patients with neuroblastoma and small cell lung carcinoma (SCLC) [52-55]. Levels of NSE correlate with the extent of the disease in patients with SCLC [54,55]. Serum concentrations consistently fall in patients who respond to therapy, and return to normal in patients who are in complete remission [53,55]. Unfortunately, NSE has a poor sensitivity in patients with limited disease [55]. Thus, it cannot be used as a screening marker for early diagnosis or for early detection of recurrence. In functioning neuroendocrine neoplasms it is less sensitive than the classical peptide markers [53-55]. So, its interest is obviously limited to neuroendocrine tumours without hormonal production, or with secretion products that are difficult to quantitate (as is the case in carcinoids or pheochromocytomas). NSE is not entirely specific for neural or neuroendocrine tissues. Positive immunohistochemistry and elevated serum levels are also found in a considerable number of nonneuroendocrine neoplasms [52,56].

Chromogranin A (CgA)

CgA, a protein originally discovered in the chromaffin cells of the adrenal medulla, is widely distributed throughout the neuroendocrine system [57]. Inside the cells it is localized in the electron-dense core secretory granules, where it is co-stored and co-secreted with the local neuropeptides. Although its biological role has not yet been established, several functions have been postulated [57]. It might fulfill regulatory activities in the packaging and processing of peptide hormones and in the modulation of neuroendocrine secretion. CgA is a well-established immunohistochemical marker of normal and neoplastic neuroendocrine tissues [58,59]. During the last years several immunoassays have been developed to measure serum concentrations of CgA, allowing it to be used also as a serum marker of neuroendocrine tumours [60-62]. Even less well differentiated tumours, that lost their ability to secrete neuropeptides, usually retain the capacity to synthesize and secrete CgA [63]. Therefore, serum levels of CgA could be useful in diagnosing and monitoring clinically non-functioning neuroendocrine neoplasms. Unfortunately, it is again not a very sensitive marker. CgA is produced by nearly all normal neuroendocrine tissues throughout the body, resulting in a large circulating plasma pool. Therefore, only extensive tumours are able to induce significant increases in serum concentrations. Extreme elevations can occur in patients with metastatic carcinoids, with values in some patients exceeding 1000 times the upper limit of normal [60,62]. High levels can also be encountered in large pheochromocytomas, clinically functioning and non-functioning endocrine pancreatic tumours and medullary thyroid cancers [60,62]. CgA is a more stable and thus more easily manageable marker for carcinoids and pheochromocytomas than the existing determinations of respectively serotonin and catecholamines or their urinary metabolites [61]. In the interpretation of the data one should take into account that hepatic and renal failure result in increased serum CgA levels, with severe renal failure (creatinin clearance lower than 25 ml/min) leading to concentrations otherwise only seen in patients with neuroendocrine neoplasia [61].

Figure 1. (a) Anterior view of the abdomen, showing multiple somatostatin receptor-positive metastases in an enlarged liver, as well as the primary carcinoid tumour in the wall of the jejunum of a patient with severe attacks of flushing and diarrhea. Pictures made 24 hours after [^{111}In]-DTPA-octreotide administration. (b) Posterior view of the chest and neck of this same patient showing a metastasis in a lymph node on the left side of the neck (top), as well as multiple metastases in ribs and pleura. (Reproduced from [4] with permission.)



Peptide receptors as markers for neuroendocrine tumours

The synthesis and secretion of peptide hormones by most human neuroendocrine tumours allows the measurement of circulating hormone concentrations as a marker of tumour presence, size and/or activity (see above). In recent years it has become evident that virtually all tumours with neuroendocrine characteristics also express membrane receptors for small peptides like somatostatin, VIP, bombesin and substance P. The demonstration of peptide receptors on tumours by ligand binding studies or autoradiography has extended the number of "neuroendocrine markers" which can be used in the pathological examination of tumours [4]. However, apart from these *in vitro* investigations, peptide receptor expression by neuroendocrine tumours can also be studied *in vivo* after the administration of tracer amounts of peptides coupled to radionuclides. This technique of peptide receptor scintigraphy has been developed successfully for the visualization of somatostatin receptors on (neuro)endocrine tumours [10,64]. After the intravenous administration of ^{111}In-DTPA-octreotide (Octreoscan) the primary tumours, but also the previously unrecognized metastases of most carcinoids, islet cell tumours, paragangliomas, pheochromocytomas, medullary thyroid cancers and SCLC can be visualized [10,64] (Fig. 1). Also other tumour types containing neuroendocrine cells are often positive on the scan. The technique of peptide receptor scintigraphy is a new addition to the armament of different tumour markers, which gives information about the spread of the disease, but often also predicts a beneficial effect of therapy with somatostatin analogs.

References

1. Green DW, Gomez G, Greeley Jr GH. Gastrointestinal peptides. Gastroenterol Clin North Am 1989;18(4):695-733.
2. Heitz PhU, Kasper M, Polak JM, *et al.* Pancreatic endocrine tumours: immunocytochemical analysis of 125 tumours. Hum Pathol 1982;13:263-271.
3. Mukai K, Greider MH, Grotting JC, *et al.* Retrospective study of 77 pancreatic endocrine tumours using the immunoperoxidase method. Am J Surg Pathol 1982;6:387-399.
4. Reubi J-C. The role of peptides and their receptors as tumour markers. Endocrinol Metab Clin North Am 1993;22(4):917-939.
5. Grimelius L, Hultquist GT, Stenkvist B. Cytological differentiation of asymptomatic pancreatic islet cell tumours in autopsy material. Virchows Arch (A) 1975:365: 275-288.
6. Polak JM, Bloom SR, Adrian TE, *et al.* Pancreatic polypeptide in insulinomas, gastrinomas, VIPomas and glucagonomas. Lancet 1976;1:328-330.
7. Adrian TE, Uttenthal LO, Williams SJ, *et al.* Secretion of pancreatic polypeptide in patients with pancreatic endocrine tumours. N Engl J Med 1986;315:287-91.
8. Eriksson B, Oberg K, Skogseid B. Neuroendocrine pancreatic tumours. Clinical findings in a prospective study of 84 patrients. Acta Oncol. 1989;28:373-377.
9. Modlin IM, Basson MD. Clinical applications of gastrointestinal hormones. Endocrinol Metab Clin North Am 1993;22(4):823-844.
10. Lamberts SWJ, Krenning EP, Reubi J-C. The role of somatostatin and its analogs in the diagnosis and treatment of tumours. Endocr Rev 1991;12:450-482.
11. Service FJ. Hypoglycemic disorders. N Engl J Med 1995;332:1144-1152.
12. Frucht H, Howard JM, Slaff JI, Wank SA, McCarthy DM, Maton PN, Vinayek R, Gardner JD, Jensen RT. Secretin and calcium provocative tests in the Zollinger-Ellison Syndrome. A prospective study. Ann Intern Med 1989;111:713-722.
13. Wermers RA, Fatourechi V, Wynne AG, Kvols K, Lloyd RV. The glucagonoma syndrome. Clinical and pathologic features in 21 patients. Medicine 1996;75:53-63.
14. Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ. Octreotide. N Engl J Med 1996;334:246-254.
15. Ruttman E, Klöppel G, Bommer G, *et al.* Pancreatic glucagonoma with and without syndrome. Virchows Arch (A) 1980;388:51-67.
16. Krejs GJ, Orci L, Conlon JM, *et al.* Somatostatinoma syndrome. Biochemical, morphologic and clinical features. N Engl J Med 1979;301:285-292.
17. O'Brien TD, Chejfec G, Prinz RA. Clinical features of duodenal somatostatinomas. Surgery 1993;114:1144-1147.
18. Ellison EH, Wilson SD. The Zollinger-Ellison syndrome : re-appraisal and evolution of 260 registered cases. Ann Surg 1964;160:512-528.
19. Capella C, Polak JM, Buffa R, *et al.* Morphological patterns and diagnostic criteria of VIP-producing endocrine tumours. A histological, histochemical, ultratstructural and biochemical study of 32 cases. Cancer 1983;52:1860-1874.
20. Maton PN, Gardner JD, Jensen RT. Cushing's syndrome in patients with the Zollinger-Ellison syndrome. N Engl J Med 1986;315:1-6.
21. Kapuscinski M, Shulkes A, Read D, Hardy KJ. Expression of neurotensin in endocrine tumours. J Clin Endocrinol Metab 1990;70:100-106.

22. Odell WD. Ectopic ACTH secretion. A misnomer. Endocrinol Metab Clin North Am 1991;20:371-379.
23. Feldman JM. Carcinoid tumours and syndrome. Semin Oncol 1987;14:237-246.
24. Moertel CG. An Odyssey in the land of small tumours. Karnofsky Memorial Lecture. J Clin Oncol 1987;5:1503-1522.
25. Pernow B, Waldenstrom J. Determination of 5-hydroxytryptamine, 5-hydroxyindoloacetic acid and histamine in 33 cases of carcinoid tumour (argentaffinoma); Am J Med 1957;23:16-25.
26. Feldman JM, O'Dorisio TM. Role of neuropeptides and serotonin in the diagnosis of carcinoid tumours. Am J Med 1986;81(Suppl.6B):41-48.
27. Sandler M, Karim SMM, William ED. Prostaglandin in amine-peptide-secreting tumours. Lancet 1968;2:1053-1054.
28. Theodrosson-Norheim E, Norheim KO, Brodin E, *et al.* Neuropeptide K: a major tachykinin in plasma and tumour tissues from carcinoid patients. Biochem Biophys Res Commun 1985;131:77-83.
29. Conlon JF, Deacon CF, Richter G, *et al.* Measurement and partial characterization of the multiple forms of neurokinin A-like immunoreactivity in carcinoid tumours. Regul Pept 1986;13:183-196.
30. Lucas KG, Feldman JM. Flushing in the carcinoid syndrome and plasma kallikrein. Cancer 1986;58:2290-2293.
31. Grouzmann E, Comoy E, Bohuon C. Plasma neuropeptide Y concentrations in patients with neuroendocrine tumours. J Clin Endocrinol Metab 1989;68:808-813.
32. Vinik AI, Gonin J, England BG, Jackson T, McLeod MK, Cho K. Plasma substance P in neuroendocrine tumours and idiopathic flushing: the value of pentagastrin stimulation tests and the effects of somatostatin analog. J Clin Endocrinol Metab 1990;70:1702-1709.
33. Koch TR, Michener SR, Go VLW. Plasma vasoactive intestinal polypeptide concentration determination in patients with diarrhea. Gastroenterology 1991;100: 99-106.
34. Hosotani R, Chowdhury P, Huang YS, Rayford PL. Neural mechanisms of pancreatic polypeptide release in conscious dogs. Am J Physiol 1989;257:G134-137.
35. Langlois A, Corring T, Cuber JC, Gueugneau AM, Levenez F, Chayvialle JA. Effects of pancreatic polypeptide on the pancreatic exocrine secretion stimulated by secretin and cholecystokinin in the conscious pig. Regul Pept 1989;24:55-65.
36. Langlois A, Corring T, Levenez F, Cuber JC, Chayvialle JA. Effects of pancreatic polypeptide on biliary flow and bile acid secretion stimulated by secretin and cholecystokinin in the conscious pig. Regul Pept 1990;27:139-147.
37. Inui A, Okita M, Miura M, Hirosue Y, Mizuno N, Baba S, Kasuga M. Plasma and cerebroventricular fluid levels of pancreatic polypeptide in the dog: effects of feeding, insulin-induced hypoglycemia, and physical exercise. Endocrinology 1993;132:1235-1239.
38. Schwartz TW. Atropine suppression test for pancreatic polypeptide. Lancet 1978;2:43-44.
39. Mann K, Saller R, Hoermann R. Clinical use of hCG and hCGβ determinations. Scand J Clin Lab Invest 1993;53 (Suppl 216):97-104.
40. Olt G, Berchuck A, Bast RC Jr. The role of tumour markers in gynecologic oncology. Obstet Gynecol Surv 1990;45:570-577.

41. Saller B, Clara R, Spotti G, Siddle K, Mann K. Testicular cancer secretes intact human choriogonadotropin (hCG) and its free beta-subunit: evidence that hCG (+ hCG-beta) assays are the most reliable in diagnosis and follow-up. Clin Chem 1990;36:234-9.
42. Öberg K, Wide L. HCG and hCG subunits as tumour markers in patients with endocrine pancreatic tumours and carcinoids. Acta Endocrinol 1981;98:256-260.
43. Heitz PhU, Kasper M, Klöppel G, *et al.* Glycoprotein-hormone alpha-chain production by pancreatic endocrine tumours : a specific marker for malignancy. Cancer 1983;51:277-282.
44. Heitz PU, von Herbay G, Klöppel G, Komminoth P, Kasper M, Höfler H, Müller K-M, Oberholzer M. The expression of subunits of human chorionic gonadotropin (hCG) by nonthrophoblastic, non-endocrine and endocrine tumours. Am J Clin Pathol 1987;88:467-472.
45. Grossmann M, Trautmann ME, Poertl S, Hoermann R, Berger P, Arnold R, Mann K. Alpha-subunit and human chorionic gonadotropin-beta immunoreactivity in patients with malignant endocrine gastroenteropancreatic tumours. Eur J Clin Invest 1994;24:131-6.
46. Iles RK, Purkis PE, Whitehead PC, Oliver RT, Leigh I, Chard T. Expression of beta human chorionic gonadotrophin by non-trophoblastic non-endocrine 'normal' and malignant epithelial cells. Br J Cancer 1990;61:663-666.
47. Schmechel D, Marangos PJ, Brightman M. Neuron-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature 1978;276:834-6.
48. Vyberg M, Hron T, Francis D, Askaa J. Immunohistochemical identification of neuron-specific enolase, synaptophysin, chromogranin and endocrine granule constituent in neuroendocrine tumours. Acta Histochem Suppl 1990;38:179-181.
49. Oishi S, Sato T. Elevated serum neuron-specific enolase in patients with malignant pheochromocytoma. Cancer 1988;61:1167-1170.
50. Cunningham RT, Johnston CF, Irvine GB, Buchanan KD. Serum neuron-specific enolase levels in patients with neuroendocrine and carcinoid tumours. Clin Chim Acta 1992;212:123-131.
51. D'Alessandro M, Mariani P, Lomanto D, Carlei F, Lezoche E, Speranza V. Serum neuron-specific enolase in diagnosis and follow-up of gastrointestinal neuroendocrine tumours. Tumour Biol 1992;13:352-357.
52. Viallard JL, Tiget F, Hartmann O, Lemerle J, Demeocq F, Malpuech G, Dastugue B. Serum neuron-specific / non-neuronal enolase ratio in the diagnosis of neuroblastomas. Cancer 1988;62:2546-2553.
53. Akoun GM, Scarna HM, Milleron BJ, Bénichou MP, Herman DP. Serum neuron-specific enolase. A marker for disease extent and response to therapy for small-cell lung cancer. Chest 1985;87:39-43.
54. Harding M, McAllister J, Hulks G, Vernon D, Monie R, Paul J. Neuron specific enolase (NSE) in small cell lung cancer: a tumour marker of prognostic significance? Br J Cancer 1990;61:605-607.
55. Van Zandwijk N, Jassem E, Bonfrer JM, van Tinteren H. Value of neuron specific enolase in early detection of relapse in small cell lung carcinoma. Eur J Cancer 1990;26:373-376.
56. Thomas P, Battifora H, Manderino GL, Patrick J. A monoclonal antibody against neuron-specific enolase. Immunohistochemical comparison with a polyclonal antiserum. Am J Clin Pathol 1987;82:146-152.

57. Deftos LJ. Chromogranin A: its role in endocrine function, and as an endocrine and neuroendocrine tumour marker. Endocr Rev 1991;12:181-187.
58. O'Connor DT, Burton D, Deftos LJ. Immunoreactive chromogranin A in diverse polypeptide hormone producing tumours and normal endocrine tissues. J Clin Endocrinol Metab 1983;57:1084-1086.
59. Weiler R, Fischer-Colbrie R, Schmid KW, *et al.* Immunological studies on the occurrence and properties of chromogranin A and B and secretogranin II in endocrine tumours. Am J Surg Pathol 1988;12:877-884.
60. O'Connor DT, Deftos LJ. Secretion of chromogranin A by peptide-producing endocrine neoplasms. N Engl J Med 1986;314:1145-1151.
61. O'Connor DT, Pandian MR, Carlton E, Cervenka JH, Hsiao RJ. Rapid radioimmunoassay of circulating chromogranin A: *in vitro* stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. Clin Chem. 1989;35:1631-1637.
62. Eriksson B, Arnberg H, Öberg K, *et al.* A polyclonal antiserum against chromogranin A and B - a new sensitive marker for neuroendocrine tumours. Acta Endocrinol (Copenh) 1990;122:145-155.
63. Sobol RE, Memoli V, Deftos LJ. Hormone-negative, chromogranin A-positive endocrine tumours. N Engl J Med 1989;320:444-447.
64. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumours. N Engl J Med 1990;323:1246-1249.

Chapter 2

Aims of the thesis

From the literature discussed in the previous introductory chapter it is apparent that, although many amines or peptides are available that can be used as serum markers for tumours of neuroendocrine origin, several clinical situations remain where these markers are either unavailable or provide unsatisfactory results.

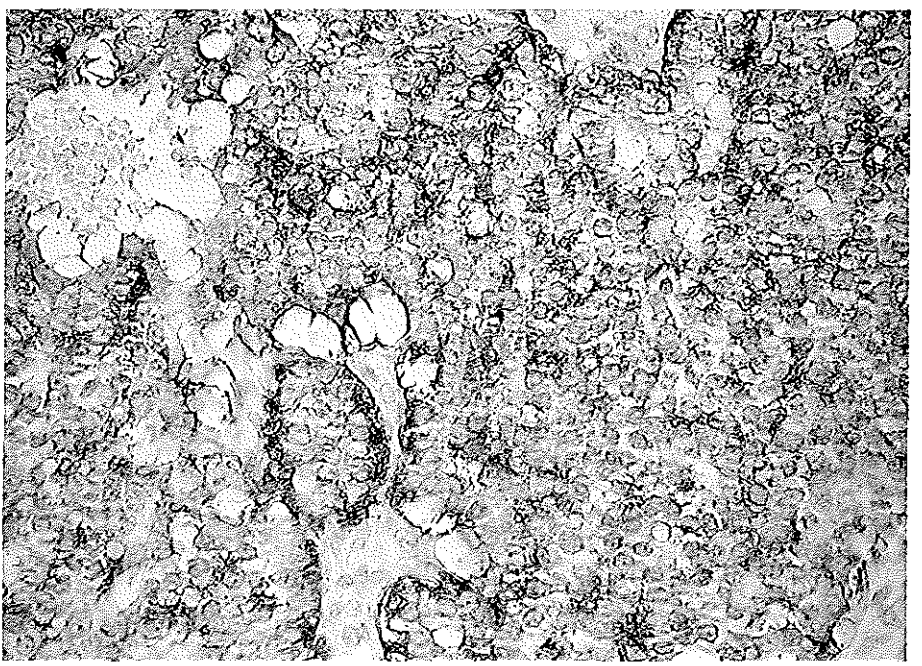
Examples of such situations encountered in endocrine clinical practice are:

1. when the existing markers are either unstable or rapidly fluctuating or are inconvenient for clinical use. Examples of these are serotonin levels and 24-h urine collections for 5-hydroxy-indole-acetic acid (5-HIAA) in patients with carcinoid tumours and catecholamine levels and 24-h urine collections for catecholamines and their degradation products in patients with pheochromocytomas.
2. when no peptide marker is available, as is the case in so-called "non-functioning" neuroendocrine tumours. Examples of these are clinically non-functioning pituitary adenomas (CNPA), silent gastro-entero-pancreatic neuroendocrine tumours and small-cell lung carcinomas (SCLC).
3. when available markers fail to differentiate between different causes of an endocrine syndrome. Cushing's syndrome is an example of such a condition, where the distinction between a pituitary, adrenal or ectopic source of hormonal overproduction can be very difficult, but is nevertheless essential to be able to provide adequate treatment.
4. when several neuroendocrine tissues are simultaneously involved in neoplastic disease, as is the case in the multiple endocrine neoplasia syndromes. In this situation the availability of a universal marker of neuro-endocrine tissue would be preferable over the use of several tissue-specific peptide markers.

As was briefly mentioned in the previous chapter, two developments of recent years provide possibilities to resolve these clinical problems :

1. the introduction of chromogranin A (CgA) as a general marker of neuroendocrine tissues.
2. the development of amine or peptide receptor scintigraphy as a tool to demonstrate receptor expression on neuroendocrine tumours *in vivo*.

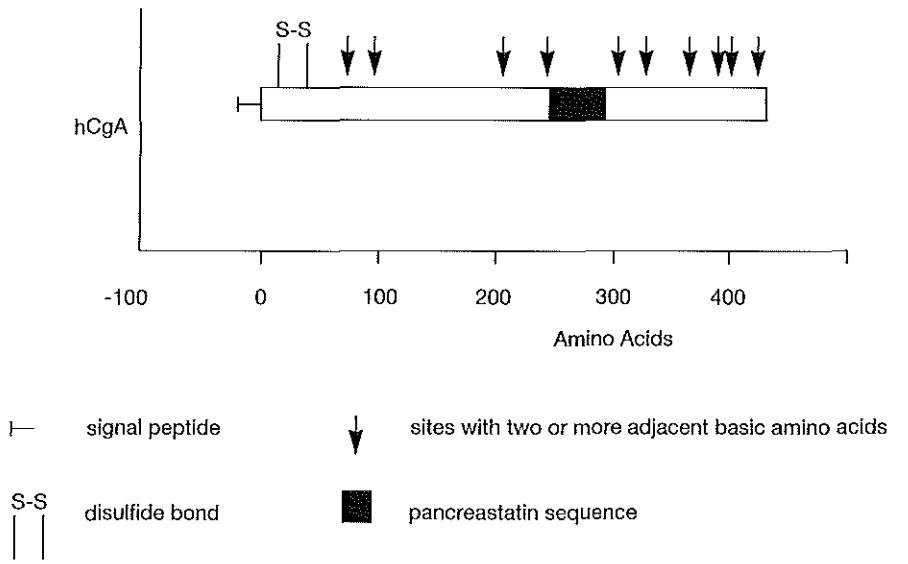
Figure 1. Presence of neuroendocrine cells in tumour tissue detected by immunohistochemistry for chromogranin A.



The aim of this thesis is to evaluate whether these techniques can provide useful solutions in the above-mentioned clinical problem-situations.

CgA is a protein that is uniquely present in neuronal and neuroendocrine cells. It can be detected in neuroendocrine tissues by immunohistochemical techniques. Monoclonal antibodies for CgA are commercially available, and are used by clinical pathologists throughout the world to verify the presence of neuroendocrine cells in tumour tissues (Fig.1). Inside the cell CgA is located in the neuroendocrine secretory granules. The primary structure of the protein has been deduced from its corresponding cDNA sequence. It contains multiple sites for proteolytic processing to smaller peptides (Fig.2). During the secretion process CgA and its cleavage products are co-released with the neuroendocrine hormones. This provides the opportunity to use CgA and/or its proteolytic derivatives as serum markers of neuroendocrine neoplasms. Several

Figure 2. Diagram of the primary structure of human chromogranin A (hCgA), with indication of the dibasic amino acid sites where proteolytic processing may take place.



polyclonal- and monoclonal-based immuno-assays have been developed for measuring serum levels of CgA and/or its cleavage products. Polyclonal assays are more suitable for screening the secretion activity of neuroendocrine cells, since they detect CgA and cross-react to its proteolytic products. We will use a polyclonal assay developed by the group of Prof. Bouillon (Leuven, Belgium) to measure CgA in serum of patients with a broad range of neuroendocrine tumours.

The use of CgA as serum marker of peripheral (non-pituitary) neuroendocrine tumours

Several groups have reported results of determination of serum levels of CgA in small series of neuroendocrine tumours. Information on its sensitivity to detect small tumours, its specificity in differentiating between neuroendocrine and non-endocrine tumours, and its clinical value compared with other neuroendocrine markers is sparse or lacking, however.

The aim of the first part of the thesis is to evaluate the clinical usefulness of CgA as serum marker in a large group of patients with non-pituitary neuroendocrine tumours, including the following subgroups:

- silent gastro-entero-pancreatic neuroendocrine tumours and SCLC, for which no peptide marker is available yet.
- carcinoids and pheochromocytomas, whose existing markers are inconvenient for clinical use.
- patients with biochemical proof of the presence of a neuroendocrine tumour, but where conventional radiography remains negative. One can often detect small neuroendocrine tumours in these patients by using [^{111}In-DTPA-D-Phe^1]-octreotide scanning. The inclusion of these patients provides the opportunity to evaluate the usefulness of CgA in the early detection of neuroendocrine tumours, as would be necessary in screening for multiple endocrine neoplasia.

CgA will be compared with the specific neuroendocrine markers of these tumours as well as with the general neuroendocrine markers neuron-specific enolase (NSE) and the α-subunit of glycoprotein hormones (α-SU).

The effect of tumour extent on CgA levels will be explored by measuring tumour volume by conventional radiographic techniques and by [^{111}In-DTPA-D-Phe^1]-octreotide scanning.
A large control group of patients with a wide variety of non-endocrine tumours will be used to evaluate the ability of serum CgA to differentiate between neuroendocrine and non-endocrine tumours.

Serum CgA in the differential diagnosis of Cushing's syndrome

Imaging techniques are generally unreliable in localizing the causative tumour in patients with Cushing's syndrome. Corticotroph adenomas are often too small to be detected on pituitary CT- or MR-imaging. On the other hand, the presence of a small lesion in the pituitary gland, is no proof of pituitary ACTH overproduction, since it can as well be an insignificant "incidentaloma". The demonstration of a lesion in an adrenal gland should also be cautiously interpreted, since it can be a

sign of ACTH-induced macronodular hyperplasia. Several endocrine tests have been developed to differentiate between the various forms of Cushing's syndrome. They frequently fail, however, in cases of ectopic production of ACTH or CRH. Only the invasive technique of bilateral simultaneous inferior petrosal sinus sampling for ACTH determination can reliably distinguish ectopic from pituitary ACTH production, but still fails in cases of ectopic CRH secretion.

We hypothesize that CgA levels will usually remain normal in patients with pituitary corticotroph adenomas. These tumours are generally very small and will therefore be unable to elevate the CgA levels above the physiological background secretion. Adrenal cortisol-producing tumours obviously do not produce CgA, because the adrenal cortex does not belong to the neuroendocrine system. On the contrary, tumours such as carcinoid tumours, medullary thyroid carcinomas and SCLC, that can be involved in ectopic production of ACTH or CRH, will probably secrete substantial amounts of CgA. CgA might thus prove to be a serum marker of ectopic Cushing's syndrome.

In order to evaluate this hypothesis CgA levels will be measured in patients with various well-proved causes of cortisol overproduction.

CNPA, as a paradigm of neuro-endocrine tumours for which no classical markers are available

The use of CgA as serum marker of CNPA

The majority of so-called CNPA are in fact poorly secreting rather than completely non-secreting. In cell culture they usually release gonadotropins or their subunits in the incubation medium. In most cases, however, this secretion is to weak to substantially increase the serum concentrations of LH, FSH or subunits. The diagnostic yield can be enhanced by demonstrating a response of gonadotropins and/or their subunits to the intravenous administration of TRH. However, in a fair number of patients the diagnosis cannot be made by these means. Moreover, data on the specificity of the TRH-test to differentiate between CNPA and other (endocrine and non-endocrine) tumours of the pituitary region are lacking.

We hypothesize that CgA might be a suitable serum marker for CNPA, since they are generally large tumours with a well-developed neurosecretory apparatus. To evaluate this hypothesis the following experiments will be done in a well-defined group of patients with CNPA:

- immunohistochemistry for CgA on tissue specimens.
- determination of *in vitro* secretion of gonadotropins, α-subunit and CgA in cell culture.
- measurement of serum gonadotropin, α-subunit and CgA levels before and during dynamic testing with TRH.

Patients with "clinically functioning" pituitary adenomas and with nonendocrine tumours of the (peri)pituitary region will serve as controls to evaluate the specificity of the measurements.

Pharmacological treatment of CNPA

The dopamine agonist bromocriptine can suppress the synthesis and secretion of gonadotropins and α-subunits by CNPA cells *in vitro*. The results of bromocriptine treatment in patients with CNPA are rather disappointing, however, showing improvement of visual field defects and/or reduction of tumour mass in only a small number of patients. Since bromocriptine is generally not well tolerated in high doses, rather small doses were used. The new generation of more powerful and more selective dopamine agonists are better tolerated than bromocriptine, allowing the use of higher doses. They might thus be useful alternatives for bromocriptine in the treatment of patients with CNPA.

We aim to prospectively evaluate the effect of the dopamine agonist quinagolide (CV 205-502) on the growth of CNPA *in vivo*. Since these tumours are generally slowly growing a long-term study with a follow-up of at least 3 years is needed. Tumour volume will be carefully measured using CT- and MR-imaging of the pituitary region. The effect on visual function will be evaluated by Goldmann perimetry.

Dopamine receptor scintigrapy in patients with CNPA

The technique of peptide receptor scintigraphy has been developed succesfully for the visualization of somatostatin receptors on neuroendocrine tumours. Other receptors can be visualised in the same way using similar techniques. Recently new *in vivo* dopamine D2 receptor scinti-

graphies with iodinated benzamides (^{123}I-IBZM and ^{123}I-epidepride) have been developed. Since most CNPA express dopamine D2 receptors on their cell membranes, this offers an opportunity to visualize CNPA.

We aim to evaluate the value of pituitary dopamine D2 receptor scintigraphy in the diagnostic evaluation of patients with pituitary tumours. In addition, the possibility to predict the clinical response to dopamine agonists in patients with CNPA will be evaluated.

Chapter 3

Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the α-subunit of glycoprotein hormones.

F.R.E. Nobels[1], D.J. Kwekkeboom[2], W. Coopmans[4], C.H.H. Schoenmakers[3], J.Lindemans[3], W.W. de Herder[1], E.P. Krenning[2], R. Bouillon[4] and S.W.J. Lamberts[1].

Departments of Medicine[1], Nuclear Medicine[2] and Clinical Laboratory[3], University Hospital Dijkzigt, Rotterdam, The Netherlands and Laboratory of Experimental Medicine and Endocrinology[4], University Hospital Gasthuisberg, Leuven, Belgium.

J Clin Endocrinol Metab 1997;82:2622-2628.

Summary

Chromogranin A (CgA) is gaining acceptance as a serum marker of neuroendocrine tumours. Its specificity in differentiating between neuroendocrine and nonneuroendocrine tumours, its sensitivity to detect small tumours, and its clinical value, compared with other neuroendocrine markers, have not clearly been defined, however. The objectives of this study were to evaluate the clinical usefulness of CgA as neuroendocrine serum marker. Serum levels of CgA, neuron-specific enolase (NSE), and the α-subunit of glycoprotein hormones (α-SU) were determined in 211 patients with neuroendocrine tumours and 180 control subjects with nonendocrine tumours. The concentrations of CgA, NSE and α-SU were elevated in 50%, 43%, and 24% of patients with neuroendocrine tumours, respectively. Serum CgA was most frequently increased in subjects with gastrinomas (100%), pheochromocytomas (89%), carcinoid tumours (80%), nonfunctioning tumours of the endocrine pancreas (69%), and medullary thyroid carcinomas (50%). The highest levels were observed in subjects with carcinoid tumours. NSE was most frequently elevated in patients with small cell lung carcinoma (74%), and α-SU was most frequently elevated in patients with carcinoid tumours (39%). Most subjects with elevated α-SU levels also had elevated CgA concentrations. A significant positive relationship was demonstrated between the tumour load and serum CgA levels ($p < 0.01$ by χ^2-testing). Elevated concentrations of CgA, NSE, and α-SU were present in, respectively, 7%, 35% and 15% of control subjects. Markedly elevated serum levels of CgA, exceeding 300 µg/l, were observed in only 2% of control patients (n=3) compared to 40% of patients with neuroendocrine tumours (n=76). We conclude that CgA is the best general neuroendocrine serum marker available. It has the highest specificity for the detection of neuroendocrine tumours compared to the other neuroendocrine markers NSE and α-SU. Elevated levels are strongly correlated with tumour volume; therefore, small tumours may go undetected. Although its specificity cannot compete with that of the specific hormonal secretion products of most neuroendocrine tumours, it can have useful clinical applications in subjects with neuroendocrine tumours for whom either no marker is available, or the marker is inconvenient for routine clinical use.

Introduction

Chromogranin A (CgA) is a protein that is present in the secretory dense core granules of neuroendocrine tissues [1]. It is widely used as an immunohistochemical marker of neuroendocrine tumours. It can also serve as a serum marker, as it is cosecreted with the amines and peptides that are present in the neurosecretory granules [2]. It is at present considered to be a very sensitive and specific serum marker of neuroendocrine tumours. The concentrations often remain increased in cases of less well differentiated tumours of neuroendocrine origin that do not secrete known hormones [3]. The publications on CgA as a serum marker of neuroendocrine neoplasia deal with rather small numbers of patients and small numbers of control subjects with nonneuroendocrine neoplasms [2,4,5]. Most neuroendocrine tumours included are far advanced, with a large tumour volume. Data on the specificity of CgA for the differentiation between neuroendocrine and nonneuroendocrine tumours, and on its sensitivity for the detection of small neuroendocrine tumours are sparse or lacking. There are also no large studies available in which CgA is compared with other neuroendocrine markers, such as neuron-specific enolase (NSE) and the α-subunit of glycoprotein hormones (α-SU).

We investigated the roles of the serum concentrations of CgA, NSE and α-SU in a large study group of patients with neuroendocrine tumours, including tumours with a small volume, and in a control group consisting of patients with several nonendocrine tumours. The results suggest that the determination of CgA is useful in selected clinical conditions when either no known specific peptide markers are available or when the available markers are inconvenient for routine clinical practice.

Subjects and methods

Patients

Serum samples were obtained from 211 subjects with the following neuroendocrine neoplasms : carcinoid tumour (n=62), medullary thyroid carcinoma (n=26), paraganglioma (n=25), pheochromocytoma (n=9),

neuroblastoma (n=3), small cell lung carcinoma (n=23), insulinoma (n=21), gastrinoma (n=9), nonfunctioning pancreatic islet cell tumour (n=13), Merkel cell tumour (n=4), clinically nonfunctioning pituitary adenoma (n=10) and GH-secreting pituitary adenoma (n=6). All diagnoses were made histologically, except in a few patients with small tumours of the neuroendocrine pancreas. In these cases the following diagnostic criteria were used : paradoxical rise in gastrin levels after stimulation by intravenous injection of secretin in gastrinoma, and hypoglycemia with inappropriate hypersecretion of insulin and C-peptide during a diagnostic fast in insulinoma. All plasma samples were obtained before operation.

Serum samples were also obtained from 180 subjects with a variety of "control" neoplasms of nonendocrine origin, both benign and malignant, including hematological and neurological tumours. This control group consisted of patients with breast carcinoma (n=64), nonsmall cell lung cancer (n=24), pancreatic adenocarcinoma (n=21), adenocarcinoma of unknown origin (n=12), non-Hodgkin lymphoma (n=25), Hodgkin lymphoma (n=13), multiple myeloma (n=7), meningioma (n=10) and astrocytoma (n=4). All these diagnoses were confirmed by histological examination.

Immunoassays

CgA was measured in serum samples, stored at - 20°C, by a polyclonal RIA, using human CgA isolated from pheochromocytomas as tracer and standard, as previously described [6]. The within-assay coefficients of variation were 6.5% and 8.6% for mean concentrations of 95 and 1160 ng/ml (n=18), respectively. The between-assay coefficients of variation were 6.9% and 6.3% for mean concentrations of 90 and 698 ng/ml (n = 38), respectively. The detection sensitivity was 1.6 µg/l. The CgA immunoreactivity remained stable whether the serum samples were immediately frozen or kept at 4°C or at room temperature for 24 h, or whether blood was centrifuged immediately to obtain serum or only after 24-h storage at room temperature. The reference value in 568 normal subjects of both sexes, aged 6-50 yr, is 90 ± 24 µg/l (range 35-176); in 33 normal men older than 50 yr, it is 106 ± 22 µg/l (range 70-159); in 249 normal postmenopausal women older than 50 yr, it is 110.1 ± 35.5 µg/l

(range 54-220). In men and premenopausal women, 175 µg/l was chosen as the upper cut-off value, and in postmenopausal women, 220 µg/L was used, to avoid overlapping values with normal subjects. This corresponds to slightly more than 3 SD above the mean.

NSE was measured by RIA. The upper cut-off value is 12.5 µg/l.

α-Subunit was measured by RIA using antibodies purchased from UCB (Brussels, Belgium). The upper cut-off values are 1.1 µg/l in men, 2.3 µg/l in premenopausal women, and 4.0 µg/l in postmenopausal women.

Determination of tumour mass

The number of neuroendocrine tumour localizations was counted using computed tomography scan images and [^{111}In-DTPA-D-Phe^1]octreotide scanning [7]. The tumour load was considered limited when one or two localizations were found; it was considered extensive when more than three localizations were demonstrated.

Statistical analysis

Results are reported as the means ± SD. To compare the different markers, χ^2-tests and Spearman rank correlations were used. To study the effect of tumour load on circulating concentrations of the markers, χ^2-tests were used.

Results

Serum concentrations of the markers CgA, α-SU and NSE were determined in 211 patients with neuroendocrine tumours and compared to levels in a control group, consisting of 180 patients with nonendocrine neoplasms. The study and control groups showed comparable age distributions (53 ± 14 and 54 ± 13 yr, respectively). The sex distribution showed a higher male/female ratio for the study group (1.67 vs. 0.68), which can be ascribed to the high number of patients with breast carcinoma in the control group.

Because renal failure can increase circulating CgA concentrations [4], we evaluated whether this could cause falsely elevated levels. In the control group a significant relationship was demonstrated between a serum creatinine level higher than 133 µmol/l and increased levels of CgA ($p < 0.001$ by χ^2-test). A creatinine concentration above 133 µmol/l was found in seven patients in the control group. Elevated serum concentrations of CgA (maximum, 371 µg/l) were present in six of these patients. A creatinine level above 133 µmol/l was also present in three subjects in the study group (all with carcinoid tumour). One of these patients had normal and one had slightly increased CgA concentrations (268 µg/l). The third patient, with an extensively metastasized carcinoid tumour, had a creatinine level of 220 µmol/l and very high levels of CgA (188,160 µg/l). Although these extreme elevations probably cannot be attributed to the diminished renal function [4], these three study patients and seven control subjects with creatinine levels above 133 µmol/l were eliminated for further analysis of the data. Slightly elevated CgA concentrations can also occur in cases of severe liver dysfunction [4]. This was not encountered in any of our study or control patients.

The results are summarized in Tables 1 and 2 and Figure 1. The serum concentrations of CgA were elevated in 103 of 208 patients with neuroendocrine tumours. They were more frequently increased (in 50% of the subjects) than the concentrations of NSE and α-SU (in 43% and 24% of the subjects, respectively). The highest elevations of CgA were observed in subjects with carcinoid tumours (up to a maximum of 52,340 µg/l). Very high levels (> 1,000 µg/l) were also seen in subjects with nonfunctioning pancreatic islet cell tumour, medullary thyroid carcinoma, pheochromocytoma, paraganglioma, small cell lung carcinoma, gastrinoma, and Merkel cell tumour. The levels were most frequently elevated in subjects with gastrinoma (100%), pheochromocytoma (89%) and carcinoid tumour (80%). In subjects with pituitary adenoma (13%), insulinoma (10%) and paraganglioma (8%), elevated CgA levels were only rarely present (Tab. 1 and 2, Fig. 1).

The highest levels of NSE were recorded in patients with small cell lung carcinoma and Merkel cell tumour (up to a maximum of 558 µg/l). NSE was more frequently elevated than CgA in subjects with small cell lung

Table 1. Serum levels of CgA, NSE and α-SU in patients with neuroendocrine tumours and in controls with nonendocrine tumours.

Type of tumour	No. of subjects	CgA (µg/l)		NSE (µg/l)		α-SU (µg/l)	
		Median	Range	Median	Range	Median	Range
Neuroendocrine tumours (n=208)							
Carcinoid tumour	59	688	33 - 52,340	12	6 - 156	1.5	0.6 - 353.0
Medullary thyroid carcinoma	26	184	80 - 13,900	11	4 - 146	1.1	0.6 - 3.7
Paraganglioma	25	106	50 - 11,590	10	6 - 35	0.9	0.6 - 2.0
Pheochromocytoma	9	275	110 - 4,674	11	1 - 19	0.9	0.6 - 1.8
Neuroblastoma	3	133	117 - 238	36	12 - 100	0.6	0.3 - 3.0
Small cell lung carcinoma	23	149	45 - 2,948	27	6 - 511	1.2	0.5 - 2.5
Insulinoma	21	105	63 - 236	12	5 - 19	1.1	0.5 - 3.4
Gastrinoma	9	772	289 - 1,933	13	8 - 23	1.6	0.5 - 4.7
NF pancreatic islet cell tumour	13	306	85 - 14,750	10	5 - 91	1.1	0.7 - 2.1
Merkel cell tumour	4	109	84 - 1,056	23	7 - 558	1.0	0.6 - 2.5
Clinically NF pituitary adenoma	10	131	85 - 240	10	8 - 12	0.9	0.6 - 3.6
GH-secreting pituitary adenoma	6	71	53 - 115	8	6 - 10	0.9	0.5 - 1.7
Nonendocrine tumours (n=173)							
Breast carcinoma	62	96	55 - 8,307	11	4 - 26	1.5	0.4 - 13.0
Nonsmall cell lung cancer	23	95	47 - 219	11	5 - 41	1.1	0.6 - 6.7
Pancreatic adenocarcinoma	20	116	51 - 395	11	6 - 24	1.1	0.5 - 2.9
Adenocarcinoma uo	12	119	68 - 154	12	7 - 44	1.3	0.7 - 2.7
Non-Hodgkin lymphoma	24	99	67 - 185	10	5 - 22	1.1	0.5 - 2.3
Hodgkin lymphoma	13	75	22 - 161	10	6 - 52	1.0	0.7 - 1.4
Multiple myeloma	5	162	98 - 215	13	9 - 15	1.1	0.8 - 1.7
Meningioma	10	89	57 - 134	11	6 - 32	1.0	0.4 - 2.6
Astrocytoma	4	86	66 - 121	6	5 - 52	0.8	0.6 - 0.8

NF, Nonfunctioning; uo, unknown origin

carcinoma (in 74% and 39%, respectively), Merkel cell tumour (in 50% and 25%, respectively), insulinoma (in 38% and 10%, respectively), paraganglioma (in 36% and 8%, respectively) and neuroblastoma (in 67% and 33%, respectively).

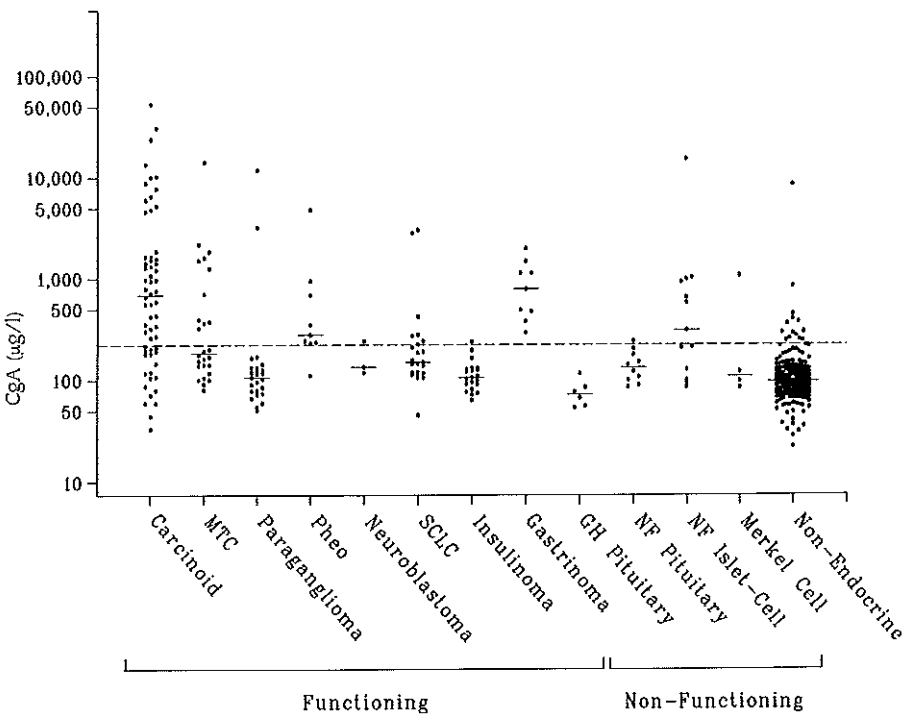
The levels of α-SU were most frequently elevated in patients with carcinoid tumours (39%). Very high levels (up to a maximum of 353 µg/l) were found in these patients. α-SU concentrations higher than 10 µg/l were found in 7 of 59 subjects with carcinoid tumours (12%), whereas they were never encountered in subjects with other neuroendocrine neoplasms.

Table 2. Presence of elevated serum levels of CgA, NSE and α-SU in patients with neuroendocrine tumours and controls with nonendocrine tumours.

Type of tumour	No. of subjects	↑ CgA		↑ NSE		↑ α-SU	
		n	%	n	%	n	%
Neuroendocrine tumours							
Carcinoid tumour	59	47	**80**	28	**47**	23	**39**
Medullary thyroid carcinoma	26	13	**50**	11	**42**	5	**19**
Paraganglioma	25	2	**8**	9	**36**	3	**12**
Pheochromocytoma	9	8	**89**	4	**44**	1	**11**
Neuroblastoma	3	1	**33**	2	**67**	1	**33**
Small cell lung carcinoma	23	9	**39**	17	**74**	8	**35**
Insulinoma	21	2	**10**	8	**38**	0	**0**
Gastrinoma	9	9	**100**	4	**44**	3	**33**
NF pancreatic islet cell tumour	13	9	**69**	4	**31**	3	**23**
Merkel cell tumour	4	1	**25**	2	**50**	0	**0**
Clinically NF pituitary adenoma	10	2	**20**	0	**0**	2	**20**
GH-secreting pituitary adenoma	6	0	**0**	0	**0**	0	**0**
total:	*208*	*103*	***50***	*89*	***43***	*49*	***24***
Nonendocrine tumours							
Breast carcinoma	62	5	**8**	23	**37**	6	**10**
Nonsmall cell lung carcinoma	23	1	**4**	9	**39**	4	**17**
Pancreatic adenocarcinoma	20	3	**15**	7	**35**	2	**10**
Adenocarcinoma uo	12	0	**0**	6	**50**	2	**17**
Non-Hodgkin lymphoma	24	1	**4**	5	**21**	5	**21**
Hodgkin lymphoma	13	0	**0**	3	**23**	4	**31**
Multiple myeloma	5	2	**40**	3	**60**	2	**40**
Meningioma	10	0	**0**	4	**40**	1	**10**
Astrocytoma	4	0	**0**	1	**25**	0	**0**
total:	*173*	*12*	***7***	*61*	***35***	*26*	***15***

n, Number of subjects with elevated levels

Figure 1. Serum concentrations of CgA in patients with neuroendocrine tumours and in controls with nonendocrine tumours. Individual levels are presented as dots; median levels as lines. The dashed line represents the upper cut-off level of 220 µg/l. The results are plotted logarithmically to accomodate extreme values. MTC, Medullary thyroid carcinoma; Pheo, Pheochromocytoma; SCLC, Small cell lung carcinoma; GH, GH- producing; NF, Nonfunctioning.



Elevated levels of CgA, α-SU, and NSE were present in respectively 9 (69%), 4 (31%) and 3 (23%) of 13 patients with nonfunctioning pancreatic islet cell tumours (Table 3). In 7 (54%) of these 13 patients, CgA levels were markedly elevated (>300 µg/l).

Elevated levels of CgA, α-SU, and NSE were present in, respectively, 7%, 15% and 35% of control subjects with nonendocrine neoplasms.

When these control subjects were used as reference population, the sensitivities of CgA, NSE, and α-SU for the diagnosis of peripheral neuroendocrine tumours (pituitary adenomas excluded) were, respectively, 53%, 46%, and 26%, with specificities of 93%, 65%, and 85%. We applied, however, a rather high upper cut-off value for CgA, corresponding to

Table 3. Serum levels of CgA, NSE, α-SU in patients with nonfunctioning tumours of the endocrine pancreas.

Patient no.	Sex	Age (yr)	CgA (µg/l)	NSE (µg/l)	α-SU (µg/l)
1	M	56	969*	11.4	1.9*
2	V	67	306*	4.6	1.7
3	M	23	999*	5.6	1.1
4	V	55	14,750*	10.3	2.1
5	V	51	94	12.6*	2.0
6	M	72	641*	6.8	1.3*
7	M	70	207*	15.5*	1.0
8	V	46	99	11.0	1.0
9	M	51	910*	90.8*	1.1
10	M	64	211*	8.1	1.0
11	M	59	85	5.3	0.7
12	M	51	126	15.6*	1.2*
13	M	63	567*	8.0	0.7

* = elevated levels

Upper cut-off values: CgA (µg/l), 175 in men and premenopausal women, 220 in postmenopausal women; NSE (µg/l), 12.5; α-SU (µg/l), 1.1 in men, 2.3 in premenopausal women, and 4.0 in postmenopausal women.

slightly more than 3 SD above the mean, to avoid overlapping values with normal subjects. Usually 2 SD above the mean is used as the upper cut-off level, increasing the risk of overlap. When we reanalysed our data using 2 SD as the upper cut-off level, the sensitivity hardly improved to 58% with a specificity of 90%. When using 300 µg/L as the upper cut-off concentration for CgA, elevated levels were found in only 3 of 173 patients (2%) with nonneuroendocrine control tumours compared to 76 of 192 patients (40%) with peripheral neuroendocrine tumours (sensitivity, 40%; specificity, 98%). Thus, finding an excessively elevated level of CgA firmly suggests the presence of a neuroendocrine tumour.

Relationships among the general neuroendocrine markers CgA, NSE and α-SU

In subjects with neuroendocrine tumours a statistically significant relationship was demonstrated between the presence and absence of elevated serum levels of CgA and α-SU ($p < 0.001$ by χ^2-test), but not

between CgA and NSE nor between α-SU and NSE (Tab. 4). A weak, but significant, relationship was present between the presence and absence of elevated serum concentrations of CgA and α-SU ($p = 0.05$, by χ^2-test) in subjects with carcinoid tumours, who frequently had elevated α-SU levels. In patients with small cell lung carcinoma, who frequently had elevated NSE levels, no significant relationship could be shown between CgA and NSE concentrations.

Relationship with other neuroendocrine markers

Measurements of 24-h urinary 5-hydroxyindole acetic acid (5-HIAA) excretions were available in 46 of 59 patients with carcinoid tumours. Increased levels (> 40 μmol/24 h) were present in 31 patients (67%). Elevated serum concentrations of CgA were demonstrated in 30 of these 31 subjects (97%; $p < 0.01$ by χ^2-test). A significant correlation was also present between the absolute values of serum CgA and 24-h urinary 5-HIAA excretion (Spearman rank correlation test; $r = 0.65$; $p < 0.01$). No significant relationships were demonstrated between α-SU and NSE concentrations, on the one hand, and urinary 5-HIAA excretions, on the other hand ($p > 0.05$, by χ^2-tests).

Table 4. Relations between serum levels of CgA, NSE, and α-SU in subjects with neuroendocrine tumours.

$\chi^2 = 18.10$ ($p < 0.001$)	**CgA** Normal	Elevated	No. of subjects
Normal	93	66	159
α-SU Elevated	12	37	49
No. of subjects	105	103	208

$\chi^2 = 1.08$ (p = NS)	**CgA** Normal	Elevated	No. of subjects
Normal	64	55	119
NSE Elevated	41	48	89
No. of subjects	105	103	208

$\chi^2 = 0.05$ (p = NS	**NSE** Normal	Elevated	No. of subjects
Normal	92	67	159
α-SU Elevated	27	22	49
No. of subjects	119	89	208

NS, $p > 0,05$

Determinations of serum calcitonin and carcinoembryonic antigen (CEA) concentrations were available in, respectively, 20 and 21 of 26 subjects with medullary thyroid carcinoma. Calcitonin was elevated (> 0.14 μg/l) in 18 of 20 patients (90%), and CEA (> 10 μg/l) was elevated in 18 of 21 patients (86%). Elevated CEA levels were present in the 2 patients with normal calcitonin levels. In 1 of these 2 subjects, slightly elevated concentrations of CgA (192 μg/l) and α-SU (1.5 μg/l) were found. CgA, α-SU, and NSE levels were not increased in the 3 patients with normal CEA levels. Significant correlations were demonstrated between serum CgA, on the one hand, and calcitonin (by Spearman rank correlation test: $r = 0.79$; $p < 0.01$) and CEA ($r = 0.84$; $p < 0.01$), on the other hand, as well as between NSE, on the one hand, and calcitonin ($r = 0.71$; $p < 0.01$) and CEA ($r = 0.82$; $p < 0.01$), on the other hand. α-SU showed a correlation with calcitonin ($r = 0.63$; $p < 0.01$), but no significant correlation with CEA ($r = 0.33$; $p > 0.05$).

Determinations of 24-h urinary excretions of vanilmandelic acid (VMA) were only available in five of nine patients with pheochromocytoma. The highest levels of CgA were found in the patients with the highest urinary VMA excretion, although the small number of cases did not permit statistical evaluation.

Relationship with tumour load

Using computed tomography scan images and octreotide scintigrams, information on tumour volume could be obtained in subjects with the following neuroendocrine neoplasms: 60 carcinoid tumours, 26 medullary thyroid carcinomas, 25 paragangliomas, 11 small-cell lung carcinomas, 9 gastrinomas and 12 nonfunctioning pancreatic islet cell tumours. Tumour load was considered to be limited when 1 or 2 localizations were found and was considered extensive when more than 3 localizations were demonstrated. A highly significant positive relationship was demonstrated between the tumour load and serum CgA levels ($p < 0.01$, by χ^2-test). Such a relationship could not be shown for α-SU or NSE. In the individual neuroendocrine neoplasms, the relationship between tumour load and CgA levels was only significant in subjects with carcinoid tumours. Because they represent the largest subgroup, statistical significance is more easily reached. Gastrinomas form an exception to

the rule that small neuroendocrine tumours have low CgA levels; elevated CgA levels were detected in all patients with gastrinomas, although they all presented with limited neoplastic disease.

Discussion

We evaluated the clinical usefulness of CgA, NSE, and α-SU as serum markers of neuroendocrine neoplasia in general. Serum concentrations were measured in a large group of patients with several neuroendocrine tumours and compared with those in a large control group with a variety of nonendocrine tumours.

The highest concentrations of CgA, with values up to 250 times the upper limit of normal, were observed in subjects with carcinoid tumours, medullary thyroid carcinomas, pheochromocytomas, and some tumours of the endocrine pancreas. This confirms the results of previous smaller studies [2,4,5]. Elevated levels were also frequently encountered in subjects with peripheral (nonpituitary) neuroendocrine tumours without detectable hormonal secretion. These so-called chromograninomas were first described by Sobol and co-workers [3]. Serum concentrations of CgA are only rarely increased, however, in cases of clinically nonfunctioning pituitary adenomas. This is probably due to the small volume of these adenomas [6].

We demonstrated a significant positive relation between the serum levels of CgA and the tumour mass of the neuroendocrine neoplasms. This confirms our earlier findings in Cushing's syndrome caused by ectopic ACTH production by extrapituitary neuroendocrine tumours [8] and the findings by O'Connor and Deftos [2] and Hsiao and co-workers [9] in pheochromocytomas. The serum concentrations of CgA are only rarely slightly elevated in subjects with small neuroendocrine tumours, such as insulinomas, paragangliomas, or pituitary adenomas [6,8,10]. These tumours are usually detected at an early stage of oncological evolution, because they rapidly induce symptoms due to active hormonal secretion or compression of important surrounding tissues. The presence of CgA or its messenger ribonucleic acid can nearly allways be

demonstrated in the cells of these tumours by immunohistochemistry or *in situ* hybridization [6,11,12]. Nevertheless, it must be assumed that the small amount of CgA released by these neoplasms usually fails to elevate the serum concentration above the physiological background level. Increased CgA concentrations were detected, however, in all of our patients with gastrinoma, although they all had a very limited tumour burden. It is well known that chronic elevation of gastrin levels provokes hyperplasia of the neuroendocrine cells of the stomach [13]. As these cells are able to secrete CgA, they might be responsible for the elevated CgA concentrations. Stabile and co-workers demonstrated that the CgA concentrations can be normalized by gastrectomy alone, without resection of the gastrin-producing tumour (and thus without correction of the elevated gastrin levels) [14] .

In our hands, CgA had a smaller sensitivity for the detection of neuroendocrine neoplasms than reported in previous studies [2,4,5]. However, the technical characteristics of our RIA for CgA are very similar to those of the other assays used in frequently cited publications [2,4,5]. A small neuroendocrine tumour mass was present in a rather large percentage of our patients, in contrast to the hitherto published series, in which almost all tumours were extensively metastasized [2,4,5]. This can probably be explained by the fact that patients tend to be transferred earlier in their oncological evolution, after the development of [^{111}In-DTPA-D-Phe^1]octreotide scanning for the visualization of neuroendocrine tumours in our hospital. Many patients with biochemical proof of a neuroendocrine tumour were transferred for somatostatin receptor scintigraphy after conventional radiography failed to elucidate the location of the tumour. The inclusion of a number of patients with these smaller tumours decreased the overall sensitivity of serum CgA in our series.

The specificity of elevated levels of CgA in the diagnosis of neuroendocrine tumours, was also lower in our study than in previous ones [2,4,5]. O'Connor and co-workers [2,4] and Erikkson and co-workers [5] reported specificities of 100 %. By contrast, we used a much larger control group, consisting of patients with a greater variety of nonendocrine tumours. After excluding patients with decreased renal function, eleva-

ted serum concentrations of CgA were demonstrated in 12 of 173 nonendocrine neoplasms (7%). The serum levels in these control patients were usually only slightly elevated. They exceeded 300 µg/l in only 3 of 173 patients (2%) compared to 76 of 208 patients (37%) with neuroendocrine tumours. Thus, finding an excessively elevated level of CgA firmly suggests the presence of a neuroendocrine tumour.

It is well established that many nonendocrine tissues contain neuroendocrine cells, belonging to the amino-precursor-uptake-decarboxylation system. A substantial body of data has accumulated in the literature during recent years, revealing that these cells are also present in most tumours of nonendocrine origin [16-20]. They are either diffusely scattered throughout the tumour or multifocally located in small nests. In malignant tumours these neuroendocrine cells even participate in the neoplastic growth, as they show nuclear abberations and are present in locally invasive or metastatic tumour tissue. The number of tumours harboring these neuroendocrine cells or the percentage of neuroendocrine cells in a tumour depend on the tumour type, the number of neuroendocrine markers used, and the detection technique (histochemistry for argyrophilia, immunohistochemistry, or detection of messenger ribonucleic acid of neuroendocrine markers). These cells probably secrete CgA, as it is present in their dense core secretory granules. There are only scarce data available in the literature concerning serum levels of CgA in subjects with nonendocrine tumours. Elevated levels were reported in patients with carcinomas of the prostate gland [21,22] and in cases of nonsmall cell lung cancer [23]. Whether proliferation of neuroendocrine cells also occurs in hematological neoplasms is not known. One study reported the presence of scarcely distributed CgA-positive cells in the normal spleen, lymph nodes, and thymus [24].

As a general neuroendocrine marker, CgA cannot differentiate between different subtypes of neuroendocrine neoplasms. Most tumours of neuroendocrine origin release typical secretion products that can be used as specific serum markers. These markers usually provide a higher sensitivity and specificity than CgA, as illustrated by our data comparing calcitonin and CEA with CgA in subjects with medullary thyroid carcinoma. In these situations the usefulness of CgA is limited, because it does

not provide additional information. By contrast, CgA can have interesting clinical applications in so-called nonfunctioning neuroendocrine tumours that are either not able to secrete hormonal products or release products that cannot be detected by current techniques. It can also be useful in neuroendocrine tumours in which other diagnostic procedures have their limitations (*e.g.* fluctuating levels of serum catecholamines in pheochromocytoma) or are inconvenient (*e.g.* 24-h urine collections for 5-HIAA determination in carcinoid tumours). Our data illustrate the value of CgA in these conditions : increased levels were found in 69% of nonpituitary, hormone-negative neuroendocrine tumours, 89% of pheochromocytomas, and 80% of carcinoid tumours. Very high concentrations were frequently encountered in these patients.

NSE is the neuron-specific isomer of the glycolytic enzyme 2-phospho-D-glycerate hydrolase or enolase [25]. It is a widely used immunohistochemical and serum marker for neuroendocrine tissues and is especially known as a marker for small cell lung carcinoma [26]. Our data confirm the frequent elevation of its serum concentrations in patients with several neuroendocrine tumours [27-29]. The highest levels were encountered in small-cell lung carcinoma and in the rare cases of Merkel cell tumours. Serum concentrations of NSE are more often elevated than those of CgA in subjects with these tumours and in those with insulinomas, paragangliomas and neuroblastomas. The specificity of serum NSE for the diagnosis of neuroendocrine tumours is, however, much lower than that of serum CgA. Increased NSE levels were demonstrated in 61 of 173 subjects with nonneuroendocrine neoplasms (35%), as compared to 89 of 192 with peripheral (nonpituitary) neuroendocrine tumours (46%). Unlike CgA, the specificity of NSE can hardly be improved by increasing the upper cut-off value. Therefore, NSE cannot be considered a good diagnostic marker for neuroendocrine tumours, but can be very useful as follow-up marker, especially for small cell lung carcinoma and Merkel cell tumours.

The α-SU of the glycoprotein hormones is a well known marker of pituitary adenomas of gonadotroph origin [6]. Recent studies suggest that determination of the serum concentrations of α-SU might also be of value in patients with peripheral neuroendocrine neoplasms [30-32].

Our data confirm the presence of elevated serum levels in several subjects with these neoplasms. Again, as with CgA and NSE, the marker lacks specificity. Serum levels were elevated in 26 of 173 subjects with nonneuroendocrine neoplasms (15%) compared to 47 of 192 with peripheral neuroendocrine tumours (24%). Increasing the cut-off level again failed to improve the specificity. Very high levels were frequently detected in patients with carcinoid tumours; 7 of 59 subjects with carcinoid tumours (12%) had levels higher than 10 μg/l. Such high levels were only encountered once in the control group, in a patient with breast carcinoma. Thus, the finding of very high serum concentrations of α-SU suggests the presence of a carcinoid tumour, when tumours of germ cell or trophoblastic origin are excluded. The clinical usefulness of α-SU as a marker for neuroendocrine tumours is limited, however, because most subjects with elevated levels also have elevated CgA concentrations.

In conclusion, CgA is the best general neuroendocrine serum marker available. It had the highest specificity for the detection of neuroendocrine tumours of the three tested markers. Unfortunately, it is not a very sensitive marker; its serum concentrations seem to rise relatively late in the evolution of the tumour. Although its specificity cannot compete with that of the specific hormonal secretion products of most neuroendocrine tumours, it can have useful clinical applications in subjects with neuroendocrine tumours for which either no marker is available (so-called nonfunctioning neuroendocrine tumours), or the marker is inconvenient for daily clinical use (e.g. 24-h urinary 5-HIAA excretions and plasma catecholamines).

References

1. Deftos LJ. Chromogranin A : its role in endocrine function, and as an endocrine and neuroendocrine tumour marker. Endocr Rev 1991;12:181-187.
2. O'Connor DT, Deftos LJ. Secretion of chromogranin A by peptide-producing endocrine neoplasms. N Engl J Med 1986;314:1145-1151.
3. Sobol RE, Memoli V, Deftos LJ. Hormone-negative, chromogranin A-positive endocrine tumours. N Engl J Med 1989;320:444-447.
4. O'Connor DT, Pandian MR, Carlton E, Cervenka JH, Hsiao RJ. Rapid radioimmunoassay of circulating chromogranin A: *in vitro* stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. Clin Chem 1989;35:1631-1637.
5. Eriksson B, Arnberg H, Öberg K, *et al.* A polyclonal antiserum against chromogranin A and B - a new sensitive marker for neuroendocrine tumours. Acta Endocrinol (Copenh) 1990;122:145-155.
6. Nobels FRE, Kwekkeboom DJ, Coopmans W, Hoekstra R, Bouillon R, Lamberts SWJ. A comparison between the diagnostic value of the measurement of gonadotropins, α-subunits and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. J Clin Endocrinol Metab 1993;77:784-789.
7. Lamberts SWJ, Bakker WH, Reubi J-C, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumours. N Engl J Med 1990;323:1246-1249.
8. Nobels FRE, de Herder WW, Kwekkeboom DJ, *et al.* Serum chromogranin A in the differential diagnosis of Cushing's syndrome. Eur J Endocrinol 1994;131:589-93.
9. Hsiao RJ, Neumann HPH, Parmer RJ, Barbosa JA, O'Connor DT. Chromogranin A in familial pheochromocytoma: diagnostic screening value, prediction of tumour mass, and post-resection kinetics indicating two-compartment distribution. Am J Med 1990;88:607-613.
10. Deftos LJ, O'Connor DT, Wilson CB, Fitzgerald PA. Human pituitary tumours secrete chromogranin-A. J Clin Endocrinol Metab 1989;68:869-872.
11. Weiler R, Fischer-Colbrie R, Schmid KW, *et al.* Immunological studies on the occurrence and properties of chromogranin A and B and secretogranin II in endocrine tumours. Am J Surg Pathol 1988;12:877-884.
12. Lloyd RV, Iacangelo A, Eiden LE, Cano M, Jin L, Grimes M. Chromogranin A and B messenger ribonucleic acids in pituitary and other normal and neoplastic human endocrine tissues. Lab Invest 1989;60:548-556.
13. D'Adda T, Corleto V, Pilato FP, *et al.* Quantitative ultrastructure of endocrine cells of oxyntic mucosa in Zollinger-Ellison syndrome. Gastroenterology 1990;99:17-26.
14. Stabile BE, Howard TJ, Passaro E, O'Connor DT. Source of plasma chromogranin A elevation in gastrinoma patients. Arch Surg 1990;125:451-453.
15. O'Connor DT, Bernstein KN. Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. N Engl J Med 1984;311:764-770.

16. Sundaresan V, Reeve JG, Stenning S, Stewart S, Bleehen NM. Neuroendocrine differentiation and clinical behaviour in non-small cell lung tumours. Br J Cancer 1991;64:333-338.
17. Scopsi L, Andreola S, Pilotti S, *et al.* Argyrophylia and granin (chromogranin/secretogranin) expression in female breast carcinomas. Their relationship to survival and other disease parameters. Am J Surg Pathol 1992;16:561-576.
18. di Sant'Agnese PA. Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic and therapeutic implications. Cancer 1992;70:254-268.
19. De Bruïne AP, Wiggers T, Beek C, *et al.* Endocrine cells in colorectal adenocarcinomas : incidence, hormone profile and prognostic relevance. Int J Cancer 1993;54:765-771.
20. Pour PM, Permert J, Mogaki M, Fujii H, Kazakoff K. Endocrine aspects of exocrine cancer of the pancreas. Their patterns and suggested biologic significance. Am J Clin Pathol 1993;100:223-230.
21. Kadmon D, Thompson TC, Lynch GR, Scardino PT. Elevated plasma chromogranin-A concentrations in prostatic carcinoma. J Urol 1991;146:358-61.
22. Deftos LJ, Nakada S, Burton DW, di Sant'Agnese PA, Cockett AT, Abrahamsson PA. Immunoassay and immunohistology studies of chromogranin A as a neuroendocrine marker in patients with carcinoma of the prostate. Urology 1996;48:58-62.
23. Sobol RE, O'Connor DT, Addison J, Suchocki K, Royston I, Deftos LJ. Elevated serum chromogranin A concentrations in small-cell lung carcinoma. Ann Intern Med 1986;105:698-700.
24. Hogue-Angeletti R, Hickey WF. A neuroendocrine marker in tissues of the immune system. Science 1985;230:89-90.
25. Schmechel D, Marangos PJ, Brightman M. Neuron-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature 1978;276:834-6.
26. Akoun GM, Scarna HM, Milleron BJ, Bénichou MP, Herman DP. Serum neuron-specific enolase. A marker for disease extent and response to therapy for small-cell lung cancer. Chest 1985;87:39-43.
27. Oishi S, Sato T. Elevated serum neuron-specific enolase in patients with malignant pheochromocytoma. Cancer 1988;61:1167-1170.
28. Cunningham RT, Johnston CF, Irvine GB, Buchanan KD. Serum neuron-specific enolase levels in patients with neuroendocrine and carcinoid tumours. Clin Chim Acta 1992;212:123-131.
29. D'Alessandro M, Mariani P, Lomanto D, Carlei F, Lezoche E, Speranza V. Serum neuron-specific enolase in diagnosis and follow-up of gastrointestinal neuroendocrine tumours. Tumour Biol 1992;13:352-357.
30. Heitz PU, von Herbay G, Klöppel G, et al. The expression of subunits of human chorionic gonadotropin (hCG) by nonthrophoblastic, non-endocrine and endocrine tumours. Am J Clin Pathol 1987;88:467-472.
31. Eriksson B, Oberg K, Skogseid B. Neuroendocrine pancreatic tumours. Clinical findings in a prospective study of 84 patrients. Acta Oncol 1989;28:373-377.
32. Grossmann M, Trautmann ME, Poertl S, *et al.* Alpha-subunit and human chorionic gonadotropin-beta immunoreactivity in patients with malignant endocrine gastroenteropancreatic tumours. Eur J Clin Invest 1994;24:131-6.

Chapter 4

Serum chromogranin A in the differential diagnosis of Cushing's syndrome

F.R.E. Nobels[1], W.W. de Herder[1], D.J. Kwekkeboom[1], W. Coopmans[3], A. Mulder[2], R. Bouillon[3] and S.W.J. Lamberts[1].

Departments of Medicine[1] and Pathology[2], University Hospital Dijkzigt, Rotterdam, The Netherlands and Laboratory of Experimental Medicine and Endocrinology[3], University Hospital Gasthuisberg, Leuven, Belgium.

Eur J Endocrinol 1994;131:589-593.

Summary

We evaluated whether measuring serum levels of chromogranin A, a marker of neuroendocrine tumours, could be useful in the differential diagnosis between pituitary, adrenal and ectopic causes of Cushing's syndrome. Thirty patients with Cushing's syndrome were studied. The localization of the tumours responsible was pituitary in 15, adrenal in 5 and ectopic in 10 patients. Serum concentrations of chromogranin A were measured in all patients. Petrosal sinus sampling for chromogranin A was performed in the cases with pituitary-dependent Cushing's syndrome. Immunohistochemical staining for chromogranin A was carried out on part of the tumour specimens. Slightly elevated serum levels of chromogranin A (range 223 - 262 µg/l) were detected in inferior petrosal sinus and peripheral venous samples from 3 patients with pituitary-dependent Cushing's syndrome. Serum chromogranin A showed no significant pituitary to peripheral gradient in these patients. Chromogranin A levels were not elevated in cases of adrenal Cushing's syndrome. Markedly elevated concentrations (range 270 - 13,900 µg/l) were shown in 7 of 10 patients with neuroendocrine tumours with ectopic adrenocorticotrophin (ACTH) and/or corticotrophin-releasing hormone (CRH) production. Widespread metastasis was present in all these cases. Subjects with "occult" carcinoid tumours, with limited spread, had normal chromogranin A levels. Immunohistochemical staining for chromogranin A was positive in 3 out of 5 pituitary adenomas and in all neuroendocrine tumours with ectopic ACTH and/or CRH production, while it was negative in all adrenocortical tumour specimens. It is concluded that elevated serum levels of chromogranin A can serve as markers of neuroendocrine tumours with ectopic ACTH and/or CRH production. The circulating levels are dependent mainly on the size of the tumours. Serum chromogranin A is not useful in the diagnosis of so-called occult Cushing's syndrome, caused by ectopic ACTH and/or CRH secretion by small neuroendocrine tumours.

Introduction

The diagnostic evaluation of patients presenting with cortisol excess remains one of the most difficult challenges of internal medicine. Over the years, several endocrine tests have been developed in order to be able to localize the causative tumour [1]. While these tests usually perform well in differentiating between the adrenal and pituitary-dependent forms, they fail frequently in cases of ectopic adrenocorticotrophin (ACTH) and/or corticotrophin-releasing hormone (CRH) production [2,3]. An ACTH or cortisol response to an intravenous injection of CRH, or suppression of cortisol levels by a high dose of dexamethasone, although typical for pituitary-dependent Cushing's syndrome, is encountered frequently in patients with ectopic ACTH- and/or CRH-secreting tumours [4,5]. Pituitary computed tomography (CT) or magnetic resonance imaging (MRI) often is not able to reveal the corticotroph adenoma or hyperplasia in cases of Cushing's disease [6,7]. On the other hand, abnormalities on pituitary CT or MRI examination have been reported in patients with ectopic ACTH-secreting neoplasms [8]. These probably represent clinically insignificant "incidentalomas" [9]. Only the complex invasive technique of bilateral simultaneous inferior petrosal sinus sampling (BSIPSS) for ACTH determination can differentiate reliably between ectopic and pituitary ACTH production, but it still fails in cases of ectopic CRH secretion [10]. Because of these difficulties, many patients with so-called "occult" Cushing's syndrome, caused by ectopic ACTH and/or CRH secretion, have undergone unnecessary pituitary surgery [8].

Chromogranin A (CgA), a 439-amino acid protein originally discovered in the chromaffin cells of the adrenal medulla, is present in the electron-dense core secretory granules of a wide variety of neuroendocrine tissues [11]. Although its biological role is not entirely clear, it is used increasingly as a marker for neuroendocrine neoplasia. CgA is overproduced and released into the circulation by some of these tumours. High serum levels are encountered frequently in patients with carcinoid tumours, medullary thyroid carcinomas (MTC) and small-cell lung cancers (SCLC) [12-15]. These endocrine tumours can be responsible for ectopic ACTH and/or CRH secretion. Therefore, CgA might prove to be a serum marker of ectopic Cushing's syndrome. In the present study we evaluate the use of CgA in the differential diagnosis of the various causes of cortisol overproduction.

Subjects and methods

Patients

The study was carried out in 30 patients with Cushing's syndrome, characterized by the typical clinical picture of cortisol excess, high urinary cortisol excretion, absent cortisol diurnal rhythm and lack of suppression of serum cortisol by low dose dexamethasone. All patients had normal renal function. The localization of the tumours responsible was pituitary in 15, adrenal in 5 and ectopic in 10 patients. Cushing's disease was confirmed by BSIPSS, showing a significant inferior petrosal sinus (IPS) to peripheral (P) serum ACTH gradient, according to the criteria of Oldfield and coworkers (basal IPS/P ratio ≥ 2.0 and/or CRH-stimulated peak IPS/P ratio ≥ 3.0) [16]. The adrenocortical tumours were adenomas in 2, ACTH-independent macronodular hyperplasia in 1 and carcinomas in 2 patients. Neuroendocrine tumours causing ectopic ACTH and/or CRH secretion were SCLC in 1, bronchial carcinoids in 4, thymic carcinoid in 1 and MTC in 4 patients. Immunostaining of the tumour cells was positive for ACTH in all but one tumour (MTC), that stained positive for CRH.

Bilateral simultaneous inferior petrosal sinus sampling

Petrosal sinus sampling was performed according to the technique descibed by Oldfield and coworkers [16]. Synchronous blood samples were taken from the catheters in each IPS and from a femoral vein for measurement of ACTH and CgA before and 5, 10, 15 and 20 minutes after the intravenous administration of CRH at a dose of 1 μg/kg. A positive response to CRH was defined arbitrarely as an increase of over 40 % above the basal level.

Immunoassays

ACTH was measured by radioimmunoassay (RIA) and PRL, FSH, LH and GH by immunoradiometric assay (IRMA) (IRE-Medgenix, Fleurus, Belgium). α-Subunit was measured by RIA, using antibodies purchased from UCB (Brussels, Belgium). CgA was measured by a polyclonal RIA, using human CgA isolated from pheochromocytomas, as tracer and standard [17]. The upper limit of normal was 175 μg/l in men and premenopausal women and 220 μg/l in postmenopausal women.

Immunohistochemistry

Tumour tissue for immunohistochemistry was available from 5/15 corticotroph adenomas, 4/5 adrenocortical tumours and 8/10 neuroendocrine tumours with ectopic ACTH and/or CRH production. Paraffin-embedded specimens were immunostained with a monoclonal CgA antibody, purchased from Eurodiagnostics BV (Apeldoorn, the Netherlands) [18]. The extent of immunoreactivity was recorded on a scale from "-" (no staining) to "+++" (heavy staining). Human pancreatic tissue served as a positive control.

Results

Table I summarizes the hormonal and immunohistochemical data of the patients with pituitary dependent Cushing's syndrome. A basal IPS/P ACTH ratio ≥ 2.0 and/or a CRH-stimulated peak IPS/P ACTH ratio ≥ 3.0 was present in all patients, confirming the pituitary source of ACTH production. When applying these criteria to the other hormonal products, significant IPS/P gradients were demonstrated in 14/15 (93%) of the patients for PRL, 13/15 (87%) for GH, 9/15 (60%) for LH, 1/13 (8%) for FSH, and 7/15 (7%) for α-subunit (data not shown). Using the same criteria, CgA never showed a significant IPS/P gradient. Moreabove, CgA concentrations of the IPS samples were not statistically different from peripheral values ($p > 0.05$ by paired Student's t-test). Intravenous injection of CRH induced marked increases (> 40% above baseline levels) of IPS levels of ACTH in 14/15 (93%) of the patients, of PRL in 14/15 (93%), of GH in 12/15 (80%), of LH in 8/15 (53%), of FSH in 6/13 (46%), and of α-subunit in 6/15 (40%) of the patients. The IPS concentrations of CgA did not respond to CRH injection ($p > 0.05$ by paired Student's t-test). Three patients showed slightly elevated CgA levels in peripheral and IPS samples, without an IPS/P gradient. In none of these patients we could find evidence for the presence of other neuroendocrine tumours as the cause of these increased circulating CgA levels. Immunohistochemical staining for CgA was performed in 5 of the 15 patients with pituitary dependent Cushing's syndrome: it was slightly positive in 1 and markedly positive in 2 of 5 basophil adenoma's.

Table 1. Serum ACTH and chromogranin A (CgA) concentrations during bilateral simultaneous inferior petrosal sinus sampling, and immunohistochemical data in patients with pituitary-dependent Cushing's disease.[a]

patient		**ACTH** (pmol/l)						**CgA** (µg/l)						CgA immuno-histo-chemistry
		P		left IPS		right IPS		P		left IPS		right IPS		
		before CRH	after CRH	before CRH	after CRH	before CRH	after CRH	before CRH	after CRH	before CRH	after CRH	before CRH	after CRH	
post-menopausal women	1	6.8	20.9	6.8	29.5	4.8	190.0	86	96	96	96	92	98	n.d.
	2	15.9	24.2	60.6	106.4	70.2	111.6	91	91	101	101	118	118	n.d.
	3	2.2	14.3	4.4	12.6	13.7	58.4	235[b]	246[b]	220[b]	266[b]	210	240[b]	n.d.
	4	2.0	2.0	32.6	39.9	74.0	94.0	262[b]	287[b]	258[b]	283[b]	254[b]	281[b]	n.d.
pre-menopausal women	5	2.0	17.8	83.7	532.7	143.0	288.9	61	63	60	68	72	72	n.d.
	6	9.0	18.9	62.3	203.9	49.3	210.5	67	87	69	79	88	88	n.d.
	7	8.8	18.9	33.3	311.8	11.0	45.8	69	85	68	76	84	84	n.d.
	8	3.1	7.3	78.4	316.0	12.1	28.4	77	87	114	114	83	90	n.d.
	9	2.0	31.9	5.7	112.7	65.2	621.2	84	96	84	100	87	104	-
	10	3.7	16.3	9.2	658.4	49.1	443.0	90	91	84	84	69	85	-
	11	2.0	11.9	35.5	351.9	34.8	351.4	223[b]	235[b]	224[b]	233[b]	236[b]	236[b]	++
men	12	37.0	72.2	57.5	261.6	56.4	158.5	68	73	65	83	74	76	+
	13	2.0	2.0	21.1	87.6	29.7	80.2	66	73	74	77	71	79	+++
	14	6.2	20.5	29.1	367.5	4.4	27.3	103	108	104	108	103	112	n.d.
	15	2.0	4.6	5.7	51.5	32.8	114.7	103	116	106	117	110	121	n.d.

[a] P: peripheral venous levels; IPS: inferior petrosal sinus levels; n.d.: not done. Immunohistochemical staining for CgA was performed on 5 adenomas. The extent of immunoreactivity was recorded on the following scale: - = all cells negative (0%), + = occasional cells positive (< 20%), ++ = numerous cells positive (< 20-50%), +++ = widespread positivity (> 50%)

[b] Elevated CgA concentrations. The upper limit of normal is 175 µg/l in men and premenopausal women and 220 µg/l in postmenopausal women.

None of the patients with adrenal Cushing's syndrome had elevated CgA levels, and all tumours examined were negative immunohistochemically for CgA (Tab. 2). The CgA levels were elevated markedly in several patients with neuroendocrine tumours with ectopic ACTH and/or CRH production (Tab. 2). Immunohistochemical staining for CgA on tumour specimens from 8 patients was diffusely positive in all. The highest serum concentrations were seen in patients with extensive metastatic disease. Normal levels were found in patients with small carcinoid tumours. In patients with MTC, the highest levels were found in those with the highest plasma concentrations of the tumour markers calcitonin and carcinoembryonic antigen (CEA), although the number of cases investigated (4) did not permit statistical analysis. The lowest CgA concentrations were present in patients with small bronchial carcinoids, with no or only limited lymphatic spread. No association could be demonstrated between serum CgA concentrations and the severity of the hypercortisolism, expressed by mean 24-h urinary free cortisol excretion, serum cortisol after overnight dexamethasone suppression or mean serum ACTH levels, in any of the patients.

Discussion

Immunohistochemical staining for CgA has been demonstrated in many tumours of the anterior pituitary, with the exception of prolactinomas [17,19-24]. The cell types that produce glycoprotein hormones and/or their subunits are frequently positive [17,19,23]. Even so-called null cell adenomas, which lack any hormonal production, can still express CgA [17,19-24]. Data on the presence of CgA in corticotroph adenomas are scarse. This is probably due to the difficulty in obtaining sufficient histological material from this type of tumour [25]. Schmid and coworkers found CgA, in a focal distribution, in 1/3 ACTH-secreting adenomas [24]. Deftos and coworkers reported the presence of CgA in more than 75 % of the cells of 2 corticotroph adenomas [20]. We were able to perform immunohistochemistry for CgA on tissue specimens from 5 corticotroph adenomas. CgA immunostaining could be detected in 3 tumours, with a focal pattern in 1 and diffuse patterns in 2 cases.

Table 2. Hormonal and immunohistochemical data in patients with adrenal and ectopic Cushing's syndrome.[a]

diagnosis		sex	age (years)	disease extent	serum CgA (μg/l)	CgA immuno-staining	low dex. (nmol/l)	mean cortisoluria (nmol/24h)	ACTH (pmol/l)	other secretion products
adrenal	adenoma	F	37		63	-	505	900	0	
		F	39		98	-	750	3650	0	
	hyperplasia	F	36		74	-	696	1483	0	
	carcinoma	F	76	N	143	-	860	n.d.	3.5	
		F	62	E	167	n.d.	812	3500	10.8	androgens
ectopic	bronchial carcinoid	M	42	N	122	+++	641	4365	20.0	5-HIAA 40
		M	65	N	165	+++	1320	26867	78.2	5-HIAA 30
		F	26	L	33	n.d.	n.d.	8334	39.2	5-HIAA 45
		F	36	E	378[b]	+++	549	292	24.4	5-HIAA 246
	thymic carcinoid	F	35	E	719[b]	++	579	1540	44.3	5-HIAA 36
	medullary thyroid carcinoma	M	32	E	699[b]	++	n.d.	n.d.	n.d.	calcit. 28 ; CEA 185
		F	42	E	1248[b]	+++	498	1690	n.d.	calcit. 150 ; CEA 1740
		M[c]	38	E	1822[b]	+++	n.d.	25000	19.8	calcit. 107 ; CEA 511
		M	43	E	13900[b]	+++	1018	1400	4.2	calcit. 1092 ; CEA 3500
	small cell lung cancer	F	61	E	270[b]	n.d.	1820	28000	425.0	

[a] CgA: chromogranin A; low dex: serum cortisol after 1 mg of dexamethasone suppression overnight (normal < 145 nmol/l); F: female; M: male; N: no metastasis; L: lymphatic spread; E: extensive metastasis (liver and/or lung); n.d.: not done; 5-HIAA: urinary 5-hydroxyindoleacetic acid excretion (normal ≤ 40 μmol/day); calcit.: serum calcitonin concentration (normal < 0.1 μg/l); CEA: serum carcinoembryonic antigen concentration (normal < 5.0 μg/l in non-smokers, < 10.0 μg/l in smokers). See legend to Table 1 for immunohistochemical staining for CgA.

[b] Elevated CgA concentrations (see legend to Table 1).

[c] Medullary thyroid carcinoma with ectopic CRH production.

Probably, the use of more sensitive molecular biological techniques would have allowed CgA to be detected in more, if not in all, corticotroph adenomas.

Deftos and coworkers reported elevated serum levels of CgA in 3/5 patients with pituitary-dependent Cushing's syndrome [20]. We observed only slightly elevated concentrations in 3/15 patients. BSIPSS failed to reveal an IPS/P gradient for CgA in these cases, suggesting overproduction by an extrapituitary source. In none of these patients, however, could we find evidence for the presence of other neuroendocrine tumours that might be responsible for the overproduction of CgA. The adrenal cortex cannot be the source of the CgA overproduction, because this tissue does not originate from the neural crest. Only tissues from neuroendocrine origin are able to produce CgA. This is illustrated by the fact that we demonstrated normal serum concentrations and negative immunohistochemistry for CgA in our patients with adrenal cortisol-producing tumours. Probably, corticotroph adenomas do produce CgA, but as these microadenomas frequently have a diameter of only a few millimeters, their small CgA production may not be enough to raise the concentrations in the petrosal venous circulation. CgA is produced by a wide variety of normal neuroendocrine tissues throughout the body [11]. Its circulating plasma pool is substantially greater than that of many other peptide hormones and it has a relatively long plasma half-life of 16-18 minutes [26]. Therefore, it is no surprise that small tumours cannot perturb the serum levels of CgA. Thus, it is clear from these data that CgA cannot be used as a reliable serum marker of pituitary Cushing's disease.

In contrast, serum levels of CgA frequently were elevated markedly in patients with tumours with ectopic ACTH and/or CRH production. Several groups described high serum values of CgA in patients with metastatic carcinoid tumours, MTC and SCLC [12-15]. We extend these data to neuroendocrine tumours responsible for ectopic Cushing's syndrome. The highest concentrations were seen in patients with metastatic MTC. The serum levels of CgA in these cases seem to be correlated with the tumour markers calcitonin and CEA. A weak correlation between serum calcitonin and CgA in patients with tumours of thyroid C-cell origin, has been described previously [12].

The finding of markedly elevated CgA concentrations in 7/10 patients with Cushing's syndrome due to ectopic ACTH and/or CRH secretion may be explained by the big tumour bulk, because all showed widespread metastasis. By contrast, all subjects with "occult" carcinoid tumours, presenting with fairly limited disease (small tumours without distant metastases), had normal CgA levels. In the patient with SCLC, only a slight elevation of serum CgA was found, despite diffuse dissemination of tumour cells in the bone marrow. This might suggest that less well-differentiated tumours secrete smaller amounts of CgA.

The circulating CgA levels produced by "peripheral" neuroendocrine tumours seem mainly dependent on the size of these tumours. Increased CgA levels were found only in cases with extensively metastasized tumours, which usually are detected easily by physical examination or routine imaging techniques. Thus, the clinical challenge of making an early diagnosis of Cushing's syndrome due to ectopic ACTH and/or CRH production by a small "occult" neuroendocrine tumour, cannot be resolved by measurement of serum CgA.

References

1. Kaye TB, Crapo L. The Cushing syndrome: an update on diagnostic tests. Ann Intern Med 1990;112:434-444.
2. Jex RK, van Heerden JA, Carpenter PC, Grant CS. Ectopic ACTH syndrome. Diagnostic and therapeutic aspects. Am J Surg 1985;149:276-282.
3. 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.
4. Suda T, Kondo M, Totani R, Hashimoto N, Suzuki M, Imaki T *et al.* Ectopic adrenocorticotropin syndrome caused by lung cancer that responded to corticotropin-releasing hormone. J Clin Endocrinol Metab 1986;63:1047-1051.
5. Malchoff CD, Orth DN, Abboud C, Carney JA, Pairolero PC, Carey RM. Ectopic ACTH syndrome caused by a bronchial carcinoid tumour responsive to dexamethasone, metyrapone, and corticotropin-releasing factor. Am J Med 1988;84:760-764.
6. Doppman JL, Frank JA, Dwyer AJ, Oldfield EH, Miller DL, Nieman LK *et al.* Gadolinium-DTPA enhanced MR imaging of ACTH-secreting microadenomas of the pituitary gland. J Comp Ass Tomog 1988;12:728-735.
7. De Herder WW, Uitterlinden P, Pieterman H, Tanghe HLJ, Kwekkeboom DJ, Pols HAP *et al.* Pituitary tumour localisation in patients with Cushing's disease with magnetic resonance imaging. Is there a place for petrosal sinus sampling? Clin Endocrinol 1993;40:87-92.
8. Findling JW, Tyrrell JB. Occult ectopic secretion of corticotropin. Arch Intern Med 1986;146:929-933.
9. Molitch ME, Russel EJ. The pituitary "incidentaloma". Ann Intern Med 1990;112: 925-931.
10. 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.
11. Deftos, LJ. Chromogranin A: its role in endocrine function and as an endocrine and neuroendocrine tumour marker. Endocr Rev 1991;12:181-187.
12. O'Connor DT, Deftos LJ. Secretion of Chromogranin A by peptide-producing endocrine neoplasms. N Engl J Med 1986;314:1145-1151.
13. Eriksson B, Arnberg H, Öberg K, Hellman U, Lundqvist G, Wernstedt C, *et al.* A polyclonal antiserum against chromogranin A and B - a new sensitive marker for neuroendocrine tumours. Acta Endocrinol (Copenh) 1990;122:145-155.
14. Blind E, Schmidt-Gayk H, Sinn HP, O'Connor DT, Raue F. Chromogranin A as tumour marker in medullary thyroid carcinoma. Thyroid 1992;2:5-10.
15. Sobol RE, O'Connor DT, Addison J, Suchocki K, Royston I, Deftos LJ. Elevated serum chromogranin A concentrations in small-cell lung carcinoma. Ann Intern Med 1986;105:698-700.

16. Oldfield EH, Chrousos GP, Schulte HM, Schaaf M, McKeever PE, Krudy AG *et al.* Preoperative lateralization of ACTH-secreting pituitary microadenomas by bilateral and simultaneous inferior petrosal venous sinus sampling. N Engl J Med 1985;312:100-103.
17. Nobels FRE, Kwekkeboom DJ, Coopmans W, De Herder WW, Hoekstra R, Bouillon R *et al.* A comparison between the diagnostic value of the measurement of gonadotropins, α-subunits and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. J Clin Endocrinol Metab 1993;77:784-89.
18. Lloyd RV, Wilson BS. Specific endocrine tissue marker defined by a monoclonal antibody. Science 1984;22:628-630.
19. DeStephano DB, Lloyd RV, Pike AM, Wilson BS. Pituitary adenomas: an immunohistochemical study of hormone production and chromogranin localization. Am J Pathol 1984;116:464-472.
20. Deftos LJ, O'Connor DT, Wilson CB, Fitzgerald PA. Human pituitary tumours secrete chromogranin-A. J Clin Endocrinol Metab 1989;68:869-872.
21. Lloyd RV, Iacangelo A, Eiden LE, Cano M, Jin L, Grimes M. Chromogranin A and B messenger ribonucleic acids in pituitary and other normal and neoplastic human endocrine tissues. Lab Invest 1989;60:548-556.
22. Lloyd RV, Jin L, Fields K, Chandler WF, Horvath E, Stefaneanu L *et al.* Analysis of pituitary hormones and chromogranin A mRNAs in null cell adenomas, oncocytomas and gonadotroph adenomas by in situ hybridization. Am J Pathol 1991;139: 553-564.
23. Oda K, Inada K, Osamura RY. Immunohistochemical studies of chromogranins (A and C) in pituitary adenomas. Pathol Res Pract 1991;187:626-628.
24. Schmid KW, Kroll M, Hittmair A, Maier H, Totsch M, Gasser R *et al.* Chromogranin A and B in adenomas of the pituitary. An immunohistochemical study of 42 cases. Am J Surg Pathol 1991;15:1072-1077.
25. Kruse A, Klinken L, Holch S, Lindholm J. Pituitary histology in Cushing's disease. Clin Endocrinol 1992;37:254-259.
26. Hsiao RJ, Neumann HPH, Parmer RJ, Barbosa JA, O'Connor DT. Chromogranin A in familial pheochromocytoma: diagnostic screening value, prediction of tumor mass, and post-resection kinetics indicating two-compartment distribution. Am J Med 1990;88:607-613.

Chapter 5

A comparison between the diagnostic value of gonadotropins, α-subunit and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas.

F.R.E. Nobels[1], D.J. Kwekkeboom[1], W. Coopmans[3], R. Hoekstra[2], W.W. de Herder[1], R. Bouillon[3], S.W.J. Lamberts[1].

Departments of Medicine[1] and Pathology[2], Erasmus University Rotterdam, The Netherlands and Laboratory of Experimental Medicine and Endocrinology[3], University Hospital Gasthuisberg, B-3000 Leuven, Belgium.

J Clin Endocrinol Metab 1993;77:2622-8.

Summary

We tested the hypothesis of whether chromogranin A (CgA), an immunohistochemical marker of neuroendocrine tumours, could serve as a serum marker for clinically nonfunctioning pituitary adenomas.
Basal and TRH-stimulated concentrations of LH, FSH, α-subunit, and CgA were measured in 22 patients with clinically nonfunctioning pituitary adenomas and in 20 control patients with other pituitary tumours. The control group consisted of 9 patients with prolactin (PRL) and/or growth hormone (GH) secreting adenomas and 11 patients with non-endocrine tumours (5 craniopharyngiomas, 2 (dys)germinomas, 1 astrocytoma, 1 meningioma, 1 neurinoma of the acoustic nerve and 1 dermoid cyst). Immunohistochemical staining for CgA was performed on tumour tissue obtained at transsphenoidal surgery in 18 study and 12 control patients. Tissue from 19 of the 22 clinically nonfunctioning adenomas was cultured, and concentrations of LH, FSH, α-subunit and CgA were measured.
Immunohistochemical staining for CgA was positive in 15 of 18 clinically nonfunctioning adenomas and negative in all examined control tumours (n=12). CgA was present in culture medium of 16 of 18 adenomas *in vitro*. In 3 adenomas it was present in the absence of detectable amounts of gonadotropins or α-subunit. Basal serum levels of gonadotropins and/or α-subunit were elevated in 7 of 22 patients with clinically nonfunctioning adenomas and in 4 of 9 control patients with PRL- and/or GH-secreting adenomas. Basal CgA was elevated in 2 study patients and in 1 prolactinoma patient. Significant increases in serum gonadotropin and/or α-subunit levels in response to TRH occurred in 14 of 21 patients with clinically nonfunctioning adenomas and in 13 of 20 control patients. A significant CgA peak after TRH administration was demonstrated in 6 patients with clinically nonfunctioning pituitary tumours and in none of the controls.
We conclude that 1) immunohistochemical staining for CgA seems an excellent tool to prove the endocrine origin of clinically nonfunctioning pituitary tumours; 2) *in vivo*, the gonadotroph origin can be recognized in only a minority of patients who have elevated basal levels of LH, FSH, or α-subunit; 3) examination of the effect of TRH on CgA release is a rather insensitive, but specific diagnostic test, allowing differentiation

from nonendocrine pituitary tumours; 4) the responses of gonadotropins and α-subunit to TRH, although more sensitive, are not specific for clinically nonfunctioning pituitary adenomas and probably only reliable in cases of total hypopituitarism.

Introduction

Clinically nonfunctioning pituitary adenomas are characterized by the absence of symptoms of hormone excess. In cell culture, however, virtually all these adenomas produce and secrete gonadotropins and/or their subunits [1-3]. During the last years, effective medical treatment became available for most hormone-secreting pituitary adenomas. Obviously, medical treatment might also be an attractive therapeutic option for adenomas of gonadotroph origin [4]. To be able to evaluate the effects of various drugs on the growth of these adenomas, a precise diagnosis is very important. Reliable distinction from nonendocrine tumours in the pituitary region, such as craniopharyngiomas and (dys)germinomas, is necessary to avoid inadvertent medical treatment in such patients. Increased serum concentrations of LH, FSH, or α-subunit are present in only a minority of cases of nonfunctioning pituitary adenomas [2,3,5-7]. The presence of a so-called paradoxical response of gonadotropins and/or their subunits to the intravenous administration of TRH enhances the diagnostic yield [3,6,7]. Even so, there remains a fair number of patients who cannot be characterized in these ways. Chromogranin A (CgA), a protein present in neuroendocrine secretory granules, is currently used as a serum and immunohistochemical marker of a wide variety of neuroendocrine tumours [8,9]. Even nonhormone-producing tumours of neuroendocrine origin retain the ability to synthesize and secrete CgA [10,11]. Therefore, serum levels of CgA could be useful in diagnosing and monitoring clinically nonfunctioning pituitary adenomas [12]. The aim of this study was to evaluate the value of CgA as a tumour marker in a welldefined group of patients with clinically nonfunctioning pituitary adenomas and compare it with the measurement of gonadotropins and α-subunit. Patients with clinically functioning pituitary adenomas, and with nonendocrine pituitary tumours served as controls.

Subjects and methods

Patients

The study was carried out in 22 patients with a clinically nonfunctioning, α-subunit-secreting, or gonadotroph pituitary adenoma, diagnosed after 1988. In none of these patients was there evidence that the adenoma originated from lactotroph cells (serum PRL < 100 µg/l), somatotroph cells (no clinical manifestations of acromegaly and serum insulin-like growth factor-I (IGF-I) < 45 nmol/l) or corticotroph cells (no clinical manifestations of Cushing's syndrome). In every patient, the diagnosis of pituitary adenoma was confirmed by histological examination of surgically excised tissue, and immunochemistry for PRL, GH and ACTH was negative. Patients with pituitary macroadenomas (n=9), secreting PRL and/or GH, and patients with intra- or suprasellar tumours of nonendocrine origin (n=11) served as controls. Five adenomas originated from somatotroph cells (clinical manifestations of acromegaly, serum IGF-I > 50 nmol/l), and 4 from lactotroph cells (serum PRL > 1000 µg/l). In all acromegalics and in 1 patient with prolactinoma who were treated by transsphenoidal surgery, histological examination and immunohistochemistry confirmed the diagnosis of pituitary adenoma. All patients with nonendocrine pituitary tumours underwent surgical excision. The histological diagnoses were 5 craniopharyngiomas, 2 (dys)germinomas, 1 astrocytoma, 1 meningioma, 1 neurinoma of the acoustic nerve and 1 dermoid cyst.

TRH tests

The response to 200 µg TRH was studied in each patient. Blood samples were collected 30, 15, and 0 minutes before and 10, 20, 30, 60, and 120 minutes after iv bolus injection of TRH (Hoechst, Amsterdam, The Netherlands). All TRH tests were performed before treatment.

Immunoassays

PRL and IGF-I were measured using immunoradiometric assay (IRMA) kits obtained from IRE-Medgenix (Brussels Belgium), and TSH was measured using an IRMA from Behring (Marburg, Germany). The cross-reactivities of α-subunit and gonadotropins in the TSH assay were less than 1% and less than 0.01%, respectively.

FSH and LH were measured using IRMA kits supplied by IRE-Medgenix. The sensitivity of these assays was 0.5 IU/l. α-Subunit was measured by RIA using antibodies purchased from UCB (Brussels, Belgium]. The sensitivity of the α-subunit assay was 0.3 µg/l. The cross-reactivities were: LH assay: FSH, 0.4%; α-subunit, 2.7%; FSH assay: LH, 0.1%; α-subunit, 0.5%; α-subunit assay: LH, 0.6%; FSH, 2.3%. The intra- and interassay coefficients of variation (CVs) were, respectively, less than 5% and less than 15% for LH, less than 3% and less than 8% for FSH, and less than 6% and less than 11% for α-subunit. Reference values in men are: LH, 1.9-9.2 U/l; FSH, 1.6-11.1 U/l; α-subunit, 0.4-1.1 µg/l; those in premenopausal women are: LH, 1.1-49.5 U/l; FSH, 1.8-46.0 U/l; α-subunit, 0.3-2.3 µg/l; those in postmenopausal women are: LH, 17.5-86.6 U/l; FSH, 26.2-107.7 U/l; α-subunit, 1.3-4.0 µg/l (13,14].

Chromogranin A

CgA was purified from human pheochromocytomas using two anion exchange chromatographic steps (diethylaminoethyl cellulose and Zorbax SAX high pressure liquid chromatography). The final purification step was a reverse phase high pressure liquid chromatography on a wide pore Bakerbond Butyl column. During all purification steps, fractions were monitored with dot blots, using monoclonal antibody LK2H10 against human CgA (Clonatec, Paris, France). Purity was confirmed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The peak area at 280 nm was used to quantitate human chromogranin, with human serum albumin as a reference standard.

Human CgA was iodinated with $Na^{125}I$ (ICN Biochemicals, Costa Mesa, CA) by the chloramine-T method and separated from unincorporated iodine by gel filtration on a PD10-column (Pharmacia, Uppsala, Sweden). The specific activity ranged from 270 to 330 Ci/g.
The RIA used 25 µL of serum, diluted 10-fold in assay buffer (23 mM / l barbital sodium, 0.02% sodium azide, and 1% BSA), 0.1 ml of rabbit antiserum (V-6; diluted 12,500-fold), and 0.1 ml labeled chromogranin (15,000 cpm). After overnight incubation at 4°C, phase separaration was obtained by second antibody solid phase (0.1 ml; A-SAC1, IDS Ltd., Boldon, United Kingdom]. The within-assay CV ranged from 6.5% (mean, 95 ng/ml; n = 18) to 8.6% (mean, 1160 ng/ml). The between-assay CVs were 6.9% and 6.3% for mean concentrations of 90 and 698 ng/ml (n = 38), respectively. The sensitivity of the assay was 1.6 µg/l.

The reference value in 568 normal subjects of both sexes, aged 6 - 50 yr, is 90 ± 24 µg/l (range, 35-176); in 33 normal men older than 50 yr, it is 106 ± 22 µg/l (range, 70-159); in 249 normal postmenopausal women older than 50 yr, it is 110.1 ± 35.5 µg/l (range 54-220). In men and premenopausal women, 175 µg/l was chosen as upper cutoff value, and in postmenopausal women, 220 µg/l was used, to avoid overlapping values with normal subjects. This corresponds to 3 SD above the mean.

Cell cultures

Tumour cells from the 22 study patients were cultured as previously described [15]. In short, surgically removed pituitary tumour tissue was washed several times and incubated with dispase (Boehringer, Mannheim, Germany), and the cells were dispersed using a Dounce type homogenizer (Kantes co., Vineland, NJ). The tumour cells were separated from blood cells by discontinuous Ficoll-Isopaque gradient centrifugation and then suspended in Eagle's Minimum Essential Medium containing 100 g/l fetal calf serum. Cell viability, determined by the trypan blue dye exclusion method, was over 90% in each experiment. Cells were cultured at 37°C in Costar multiwell plates (Costar, Cambridge, MA) at a concentration of 200,000 cells /well. In addition, in most instances cells were also lysed after Ficoll separation in distilled water containing 1 g/l BSA by repeated freezing and thawing. Medium harvested after the first 3 - 4 days in culture and cell lysates were stored at -20°C for hormone assay. The fetal calf serum used in the culture medium contained small amounts of CgA (<10 µg/l). Therefore the detection limit of CgA in culture samples was set at 10 µg/l.

Immunohistochemistry

Surgically excised tumour tissues were investigated by immunohistochemical methods using the avidin-biotin-peroxidase complex method. All tissues were fixed in 4% buffered neutral formaldehyde, embedded in paraffin, and cut in 4- to 6-µm slices. Mouse monoclonal antibodies against CgA, isolated from human pheochromocytomas, were purchased from Eurodiagnostics BV (Apeldoorn, the Netherlands) [16]. The antiserum was used in a dilution of 1:20. Human pancreatic tissue served as a positive control. Interpretation was performed blindly, without knowledge of the endocrine characteristics of the patients. The extent of immunoreactivity was recorded on a scale from - (no staining) to +++ (heavy staining].

Results

In vivo hormonal results in study patients

Basal serum gonadotropin, α-subunit and CgA levels of the 22 patients with clinically nonfunctioning pituitary macroadenomas are listed in Table 1. LH levels were in the low normal range or decreased in all patients. FSH levels were elevated in 2 male patients (9%). Both patients also presented elevated circulating α-subunit concentrations. In 2 of the 6 postmenopausal women, FSH levels were in the normal postmenopausal range in the presence of lowered LH levels and hypopituitarism. Serum concentrations of α-subunit were increased in 6 patients (27%), 5 males and 1 female. In total, 7 of the 22 patients (32%) presented with a gonadotroph- and/or α-subunit-secreting adenoma, defined by increased circulating FSH and/or α-subunit levels. An elevated basal CgA concentration was found in 2 male patients. Both also had elevated α-subunit levels, and in 1 of them, an elevated FSH level was present.

TRH tests were performed in 21 patients (Table 1). Because of the large variability in basal values of the gonadotropins, α-subunit, and CgA, all post-TRH values were expressed as a percentage of the 3 pre-TRH values. Basal serum levels of LH, FSH, α-subunit, and CgA varied by up to 28%, 22%, 30%, and 20% of the respective baseline values. Therefore, increases after TRH over 30% of the baseline level were arbitrarely considered to be significant.

LH levels increased significantly after the administration of TRH in 5 of 21 patients (24%). All had normal basal LH levels. FSH increased significantly in 4 patients (19%). In 3 of them, the basal FSH value was elevated. An increase in α-subunit after TRH treatment occurred in 11 patients (52%). No significant correlation was present between the absolute increases of α-subunit and those in TSH in response to TRH (Spearman rank test). A significant increase in CgA levels after TRH was demonstrated in 6 patients (29%), all of whom presented normal basal CgA levels. The presence or absence of a significant increase in hormone levels after TRH treatment was not related to the respective basal serum concentrations (by χ^2-tests). The presence or absence of a significant increase in CgA levels was not related to the presence or absence of a reaction of gonadotropin or α-subunit levels to TRH (by χ^2-test).

Table 1. Serum LH, FSH, α-subunit, and CgA concentrations at baseline and in response to TRH in 22 patients with clinically nonfunctioning, α-subunit-secreting or gonadotroph pituitary macroadenomas.

			LH		FSH		α-subunit		CgA	
patient	sex	age	basal level (U/l)	% increase after TRH	basal level (U/l)	% increase after TRH	basal level (µg/l)	% increase after TRH	basal level (µg/l)	% increase after TRH
1	M	49	2.8	61[a]	45.2[b]	14	3.8[b]	14	180.3[b]	18
2	M	59	1.2	58[a]	17.0[b]	4	3.6[b]	19	64.0	53[a]
3	M	46	1.3	58[a]	6.6	33[a]	1.8[b]	44[a]	116.3	25
4	M	50	2.2	43[a]	3.8	23	1.6[b]	91[a]	72.0	67[a]
5	M	70	<0.5	0	<0.5	0	1.5[b]	60[a]	210.0[b]	19
6	M	54	0.8	25	0.8	338[a]	0.6	50[a]	98.0	2
7	M	60	1.8	15	0.6	137[a]	0.7	9	71.7	9
8	M	58	1.4	2	<0.5	0	0.6	94[a]	105.7	20
9	M	52	<0.5	0	<0.5	0	0.8	75[a]	117.7	33[a]
10	M	34	4.3	0	3.6	0	0.7	71[a]	82.0	23
11	M	37	1.9	47[a]	1.6	13	0.5	50[a]	72.0	8
12	M	55	<0.5	0	<0.5	0	1.0	40[a]	72.0	13
13	M	29	3.5	14	4.1	10	0.5	13	79.3	40[a]
14	M	46	8.7	0	1.9	0	0.4	0	73.0	27
15	M	72	1.0	10	1.9	5	0.5	20	130.0	7
16	F	53	1.3	0	56.7[c]	4	6.4[b]	6	125.0	20
17	F	68	2.1	11	33.0[c]	107[a]	1.1	209[a]	168.7	125[a]
18	F	65	4.6	4	13.7	9	0.6	41[a]	40.7	38[a]
19	F	60	1.9	18	2.3	2	0.8	30	73.3	23
20	F	77	3.0	0	10.0	9	1.3	20	138.7	2
21	F	70	10.3	7	18.3	11	0.9	15	81.7	20
22	F[d]	44	<0.5	ND	1.5	ND	0.5	ND	73.0	ND

Basal values are the means of determinations in three serum samples drawn at 15 min intervals. The normal values in men are: LH, 1.9-9.2 U/l; FSH, 1.6-11.1 U/l; α-subunit, 0.4-1.1 µg/l; in premenopausal women: LH, 1.1-49.5 U/l; FSH, 1.8-46.0 U/l; α-subunit, 0.3-2.3 µg/l; in postmenopausal women: LH, 17.5-86.6 U/l; FSH, 26.2-107.7 U/l; α-subunit, 1.3-4.0 µg/l. % increase after TRH = (maximal response to TRH - mean basal value) x 100/mean basal value. A response greater than 30% is considered significant. ND, Not done.

[a] Significant response to TRH.

[b] Elevated basal level.

[c] Inappropiately elevated basal FSH/LH.

[d] Premenopausal woman

Table 2. Serum LH, FSH, α-subunit, and CgA concentrations at baseline and in response to TRH in 9 patients with hormone secreting pituitary adenomas, and in 6 patients with intra- and/or suprasellar tumours of nonendocrine origin.

diagnosis	patient	sex	age	LH		FSH		α-subunit		CgA	
				basal level (U/l)	% increase after TRH	basal level (U/l)	% increase after TRH	basal level (μg/l)	% increase after TRH	basal level (μg/l)	% increase after TRH
acromegaly	1	M	23	6.4	0	4.7	15	2.4[a]	175[b]	82	23
	2	M	43	4.2	62[b]	4.2	2	1.3[a]	0	84	6
	3	M	29	2.0	60[b]	2.3	35[b]	0.7	114[b]	124	5
	4	M	30	8.1	0	9.0	7	0.9	22	80	6
	5	F	43	<0.5	0	0.8	88[b]	0.5	40[b]	76	12
prolactinoma	6	M	66	3.3	24	0.7	43[b]	4.6[a]	7	419[a]	0
	7	M	66	1.6	34[b]	0.8	8	1.2[a]	0	127	0
	8	F	23	3.7	68[b]	3.8	29	0.9	33[b]	65	0
	9	F	26	<0.5	0	<0.5	0	0.8	38[b]	73	1
cranio-	10	M	30	0.7	91[b]	2.1	3	0.8	96[b]	89	21
pharyngioma	11	M	38	1.9	2	1.5	0	0.8	20	94	0
	12	F	40	4.4	0	3.0	0	0.8	0	72	19
	13	F	43	<0.5	0	<0.5	0	0.4	85[b]	109	5
	14	F	81	<0.5	0	<0.5	0	0.9	15	144	8
germinoma	15	M	23	<0.5	0	<0.5	0	0.4	75[b]	143	3
dysgerminoma	16	M	44	1.4	0	3.5	0	0.7	0	104	0
astrocytoma	17	M	16	<0.5	0	2.1	0	1.5	0	75	17
meningioma	18	F	31	2.6	0	3.4	3	0.5	60[b]	102	0
ac. neurinoma	19	M	42	5.9	12	4.0	5	0.9	0	91	12
dermoid cyst	20	M	29	3.6	11	1.5	20	0.5	40[b]	43	19

Basal values are the means of determinations in three serum samples drawn at 15 min intervals. % increase after TRH = (maximal response to TRH - mean basal value) x 100/mean basal value. Ac. neurinoma, neurinoma of acoustic nerve.

[a] elevated basal value

[b] significant response to TRH

In vivo hormonal results in control patients

The characteristics of the control patients are presented in Table 2. There were 13 men and 7 women. Among the 9 patients with hormone-secreting pituitary macroadenomas, gonadotropin levels were within the normal range. Two patients with acromegaly and 2 with prolactinomas demonstrated elevated basal α-subunit concentrations. After the administration of TRH, LH levels significantly increased in 4 (44%), FSH in 3 (33%) and α-subunit in 5 (56%) patients. The baseline CgA concentration was elevated in 1 man with a prolactinoma who also had elevated α-subunit. No significant increases of CgA levels occurred after TRH injection in any of these patients.

Among the 11 patients with nonendocrine tumours in the pituitary region, no elevated basal levels of gonadotropins or α-subunit were detected. Significant LH and α-subunit responses to TRH were present in a patient with a craniopharyngioma. Four additional patients showed a significant α-subunit response. All basal and stimulated CgA levels were within the normal range.

As in the study patients, no relations could be found in these control patients between the presence or absence of a response to TRH and the respective basal serum hormone concentrations (by χ^2-tests).

Hormone release and content of cultured pituitary tumour cells

Culture media and/or cell lysates were available from 19 patients with clinically nonfunctioning adenomas. LH, FSH, α-subunit, or a combination of these were detected in the media and/or cells from 15 patients (Tab. 3). CgA was found in the media and/or cell lysates from 16 of 18 patients. In 3 adenomas, CgA was present in the absence of detectable gonadotropin and α-subunit concentrations.

Immunohistochemistry

Tumour tissue for immunohistochemistry was available from 18 of the 22 clinically nonfunctioning pituitary adenomas (Tab. 3), 6 of the 9 secreting pituitary adenomas (all 5 GH-secreting and 1 of the 4 PRL-secreting adenomas), and 6 of the 11 nonendocrine tumours (3 of 5 craniopharyngiomas, 1 germinoma, 1 astrocytoma, and 1 meningioma).

CgA immunopositivity was detected in 15 of the 18 clinically nonfunctioning pituitary adenomas, none of the secreting adenomas, and none of the nonendocrine pituitary tumours. No relation could be demonstrated between the amount of staining and the concentrations in culture medium or serum (by Wilcoxon's rank sum test).

Table 3. Results of *in vitro* cultures and immunohistochemical staining of adenoma cells of patients with clinically nonfunctioning, α-subunit-secreting or gonadotroph pituitary macroadenomas.

patient	Hormones detected *in vitro*				I H
	LH (U/l)	FSH (U/l)	α-subunit (μg/l)	CgA (μg/l)	CgA
1	-	+	+	+	+
2	+	+	+	ND	+
3	+	+	+	+	+++
4	+	+	+	+	ND
5	-	+	+	+	+++
6	-	-	-	+	++
7	+	+	+	+	++
9	+	-	-	+	+++
10	+	+	+	+	++
11	-	-	-	-	-
13	+	-	-	+	+++
14	-	-	-	+	+++
15	-	-	-	+	-
16	+	+	+	+	+
17	+	+	+	-	+++
18	+	+	+	+	+++
19	+	+	+	+	+++
20	-	+	+	+	+++
22	-	-	+	+	-

Surgically removed tumour tissue from 19 of the 22 patients with clinically nonfunctioning, α-subunit-secreting, or gonadotroph adenomas was brought in dispersed cell culture. FSH, LH, α-subunit, and CgA were determined in medium harvested after 3 - 4 days culture and in cell lysates. The presence of concentrations higher than the detection limit of the assay (LH, 0.5 U/l; FSH, 0.5 U/l; α-subunit, 0.3 μg/l) was considered significant. The fetal calf serum used in the culture media and cell lysates contained small amounts of CgA (< 10 μg/l). Therefore, the detection limit of CgA was 10 μg/l.

Immunohistochemical staining (IH) for CgA was performed on 18 of the 22 adenomas. The extent of immunoreactivity was recorded on the following scale: -, all cells negative (0%); +, occasional cells positive (< 20%); ++, numerous cells positive (20-50%); +++, widespread positivity (> 50%). ND, Not done.

Discussion

Using immunological and molecular biological techniques, several investigators demonstrated that virtually all so-called nonfunctioning pituitary tumours are of gonadotroph origin [1-3]. This is confirmed by our cell culture results, showing readily detectable amounts of LH, FSH, or α-subunit in incubation media or cell lysates from 15 of 19 cultured adenomas. The clinical utility, however, is limited, because this secretion is seldom reflected in serum; only 5 of 15 male and 2 of 7 female patients showed elevated serum concentrations of gonadotropins and/or α-subunit. Other research groups reported elevated levels in 6-67% of their patients [2,3,5-7]. Therefore, alternative markers are required to increase the diagnostic sensitivity.

Deftos and co-workers proposed CgA as a useful tissue and serum marker for endocrine neoplasms [8,9]. Originally discovered in the chromaffin granules of the adrenal medulla [17], it was subsequently detected in many normal and neoplastic tissues of neuroendocrine origin [9,18]. Its exact function is unknown. At the cellular level, CgA is localized in hormone storage granules, suggesting corelease with the resident hormones [9]. Its amino acid sequence contains many proteolytic processing sites, and several cleavage products are present in serum [9]. This suggests that it might serve as a precursor of biologically active peptides, with endo-, para-, or autocrine function [9,19]. In the interpretation of serum concentrations of CgA, one should take into account that all immunoassays for CgA show a variable degree of cross-reaction with these cleavage products [8]. This implies that each immunoassay system should have its own well-defined normal range, so that our normal values cannot be extrapolated to other immunoassays.

Although its biological role has not yet been established, its potential value as a marker of endocrine function is apparent. One of the most promising clinical uses is its ability to identify nonfunctioning endocrine tumours [10-12]. Most hormone-negative neoplasms of endocrine origin retain the capacity to produce CgA. We could demonstrate CgA in the culture media or cell lysates of 16 of 18 cultured clinically nonfunctioning pituitary adenomas. In 3 of these, CgA was present in the absence of detectable amounts of gonadotropins or α-subunit. Immunohisto-

chemistry for CgA confirmed its almost universal presence in this type of adenomas. By contrast, CgA was not detected in the 6 (of 9) secreting adenomas and the 3 (of 6) nonendocrine pituitary tumours that were subjected to CgA immunostaining. Thus, immunohistochemistry for CgA seems an excellent pathological marker for adenomas of gonadotroph origin. This is in accordance with several reports of other research groups, showing immunohistochemical staining for CgA in nearly all null cell adenomas, oncocytomas and gonadotroph adenomas [12,20-24], but absence or only focal staining in prolactinomas and in GH- and ACTH-producing adenomas [12,20,22-24].

As with gonadotropins and α-subunit, the presence of CgA in adenoma tissue is seldomly reflected in elevated serum levels. Elevated serum CgA concentrations were detected in only 2 of our 22 study patients. CgA is produced in a wide variety of normal neuroendocrine tissues throughout the body. The production by the relatively small pituitary tumour bulk is probably lost in the scatter of physiological secretion. One patient with a prolactinoma showed an excessive serum concentration of CgA and α-subunit. The possibility of a plurihormonal adenoma [25], secreting PRL, α-subunit and CgA, is unlikely, because immunohistochemical staining of tumour tissue for CgA was negative. The presence of another extrapituitary endocrine neoplasm that releases CgA in the circulation offers an unconfirmed explanation.

Many groups reported the stimulation of gonadotropin or subunit release by TRH as a useful tool in the workup of nonfunctional pituitary tumours [3,6,7]. By analogy, we used TRH tests in the hope of obtaining a CgA response in these patients. Six of the 21 study subjects (29%) had a significant increase in CgA levels after TRH injection. All responders showed normal basal levels of CgA, and 3 presented elevated basal levels of FSH and/or α-subunit. A response of gonadotropin and/or α-subunit concentrations to TRH occurred in 14 patients (67%). Testing the effect of TRH on CgA release, although less sensitive, seems a more specific diagnostic aid than testing the response of gonadotropins or α-subunit. In the control group of 9 secreting and 11 nonendocrine pituitary tumours, CgA never increased in response to TRH, while gona-

dotropin and especially α-subunit levels increased in 13 patients (65%). In fact, in 4 of the 9 patients with secreting pituitary adenomas, even the basal levels of α-subunit were elevated. The existence of adenoma cells capable of producing more than one hormone has been conclusively proven [25]. α-Subunit is frequently coreleased by GH- and PRL-producing adenomas. This fails to explain, however, the TRH-induced increases in the concentrations of α-subunit in 5 and of LH in 1 of 11 patients with nonendocrine pituitary neoplasms. Therefore, a so-called paradoxical gonadotropin response to TRH and, especially, an increase in α-subunit concentrations cannot be regarded as a specific diagnostic marker of pituitary adenomas of gonadotroph origin. A small increase in LH [26] and α-subunit levels [27,28] after TRH administration can even be demonstrated in normal subjects. It is not clear whether these α-subunit responses originate from gonadotroph or other cell-lines. One possibility is cosecretion with TSH. We could not demonstrate, however, any relation between the TRH-induced increases of the concentrations of α-subunit and those of TSH in our patients. Several patients showed a significant α-subunit response and no TSH response and *vice versa*. Probably, an increase in α-subunit levels after TRH can only be regarded as diagnostic for a pituitary adenoma of gonadotroph origin in the presence of severe hypopituitarism. This hypothesis could not be tested, however, since most of our patients with clinically nonfunctioning adenomas showed severe hypopituitarism, whereas most of our control patients with nonendocrine pituitary tumours showed only partial hypopituitarism (data not shown).

The major difficulty in the diagnosis of clinically nonfunctioning pituitary adenomas is the differentiation from nonendocrine pituitary tumours, such as craniopharyngiomas and meningiomas. Hormone-secreting pituitary tumours, such as somatotropinomas and prolactinomas, can readily be differentiated on clinical grounds and by the demonstration of their principal hormonal product. Using the combination of elevated basal serum concentrations of gonadotropins and/or α-subunit, and/or a positive CgA response to TRH allowed the diagnosis of clinically nonfunctioning, α-subunit secreting, or gonadotroph pituitary adenoma in 10 of 22 patients (45%), without false positive results in patients with nonendocrine pituitary tumours.

Daneshdoost and co-workers [7] presented the LHβ response to TRH as the most sensitive and specific test for the detection of pituitary adenomas of gonadotroph origin, allowing a diagnosis in 69% of their postmenopausal women. The actual clinical usefulness is limited, as good assays for the β-subunit of LH are not widely available. Most assays show a low sensitivity and a high degree of cross-reaction with intact gonadotropins, especially with LH. The specificity of this test in making the differential diagnosis with nonendocrine tumours of the pituitary region has not been evaluated.

We conclude from these data that immunohistochemical staining for CgA is an excellent tool to prove the endocrine origin of clinically nonfunctioning adenomas. Even hormone-negative adenomas retain the capacity to synthesize CgA. *In vivo,* the gonadotroph origin can be recognized in only a minority of patients who have elevated basal levels of LH, FSH, or α-subunit. Examination of the effect of TRH on CgA release is a rather insensitive, but specific diagnostic test, allowing differentiation from nonendocrine pituitary tumours. The responses of gonadotropins and α-subunit to TRH, although more sensitive, are not specific for clinically nonfunctioning pituitary adenomas and probably only reliable in cases of total hypopituitarism. This might not apply to the measurement of LHβ and/or FSHβ, however [7]. In view of the limited experience with CgA in the immunohistochemical and serological diagnosis of tumours of gonadotroph origin, our observations should be extended to larger groups of patients, before firm conclusions can be drawn.

References

1. Snyder PJ. Gonadotroph cell adenomas of the pituitary. Endocr Rev 1985;6:552-563.
2. Black PM, Hsu DW, Klibanski A, *et al.* Hormone production in clinically nonfunctioning pituitary adenomas. J Neurosurg 1987;66:244-250.
3. Kwekkeboom DJ, Jong FH de, 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.
4. Kwekkeboom DJ, Lamberts SWJ. Long-term treatment with the dopamine agonist CV 205-502 of patients with clinically nonfunctioning, gonadotroph, or α-subunit secreting pituitary adenoma. Clin Endocrinol 1992;36:171-176.
5. Oppenheim DS, Kana AR, Sangha JS, Klibanski A. Prevalence of α-subunit hypersecretion in patients with pituitary tumours: clinically nonfunctioning and somatotroph adenomas. J Clin Endocrinol Metab 1990;70:859-864.
6. White MC, Daniels M, Newland P, *et al.* LH and FSH secretion and responses to GnRH and TRH in patients with clinically functionless pituitary adenomas. Clin Endocrinol 1990;32:681-688.
7. Daneshdoost L, Gennarelli TA, Bashey HM, *et al.* Recognition of gonadotroph adenomas in women. N Engl J Med 1991;324:589-594.
8. O'Connor DT, Deftos LJ. Secretion of Chromogranin A by peptide-producing endocrine neoplasms. N Engl J Med 1986;314:1145-1151.
9. Deftos LJ. Chromogranin A: its role in endocrine function and as an endocrine tumour marker. Endocr Rev 1991;12:181-187.
10. Sobol RE, Memoli V, Deftos LJ. Hormone-negative, chromogranin A-positive endocrine tumours. N Engl J Med 1989;320:444-447.
11. Eriksson B, Arnberg H, Öberg K, *et al.* A polyclonal antiserum against chromogranin A and B - a new sensitive marker for neuroendocrine tumours. Acta Endocrinol (Copenh) 1990;122:145-155.
12. Deftos LJ, O'Connor DT, Wilson CB, Fitzgerald PA. Human pituitary tumours secrete chromogranin-A. J Clin Endocrinol Metab 1989;68:869-872.
13. Kwekkeboom DJ, Jong FH de, Hemert AM van, Vandenbroucke JP, Valkenburg HA, Lamberts SWJ. Serum gonadotropins and α-subunit decline in aging normal postmenopausal women. J Clin Endocrinol Metab 1990;70:944-950.
14. Kwekkeboom DJ, Jong FH de, Lamberts SWJ. Confounding factors in the interpretation of gonadotropin and gonadotropin-subunit release from cultured human pituitary adenomas. J Steroid Biochem 1989;33:777-782.
15. Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SWJ. Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. J Endocrinol Invest 1984;7:307-311.

16. Lloyd RV, Wilson BS. Specific endocrine tissue marker defined by a monoclonal antibody. Science 1984;22:628-630.
17. Blaschko H, Comline RS, Schneider FH, Siver M, Smith AD. Secretion of chromaffin granule protein chromogranin from adrenal gland after splachnic stimulation. Nature 1967;215:58-59.
18. O'Connor DT, Burton DW, Deftos LJ. Immunoreactive human chromogranin A in diverse polypeptide hormone producing human tumours and normal endocrine tissues. J Clin Endocrinol Metab 1983;57:1084-1086.
19. Eiden LE. Is chromogranin a prohormone? Nature 1987;325:301.
20. DeStephano DB, Lloyd RV, Pike AM, Wilson BS. An immunohistochemical study of hormone production and chromogranin localization. Am J Pathol 1984;116:464-472.
21. Lloyd RV, Jin L, Fields K, *et al.* Analysis of pituitary hormones and chromogranin A mRNAs in null cell adenomas, oncocytomas and gonadotroph adenomas by in situ hybridization. Am J Pathol 1991;139:553-564.
22. Lloyd RV, Iacangelo A, Eiden LE, Cano M, Jin L, Grimes M. Chromogranin A and B messenger ribonucleic acids in pituitary and other normal and neoplastic human endocrine tissues. Lab Invest 1989;60:548-556.
23. Schmid KW, Kroll M, Hittmair A, Maier H, Totsch M, Gasser R, Finkenstett G, Hogue-Angeletti R, Fischer-Colbrie R. Chromogranin A and B in adenomas of the pituitary. An immunohistochemical study of 42 cases. Am J Surg Pathol 1991;15: 1072-1077.
24. Oda K, Inada K, Osamura RY. Immunohistochemical studies of chromogranins (A and C) in pituitary adenomas. Pathol Res Pract 1991;187:626-628.
25. Kovacs K, Horvath E, Asa SL, Stefaneanu L, Sano T. Pituitary cells producing more than one hormone. Trends Endocrinol Metab 1989;1:104-107.
26. Snyder PJ, Muzyka R, Johnson J, Utiger RD. Thyrotropin-releasing hormone provokes abnormal follicle-stimulating hormone (FSH) and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. J Clin Endocrinol Metab 1980;51:744-748.
27. MacFarlane IA, Beardwell CG, Shalet SM, Ainslie G, Rankin E. Glycoprotein hormone α-subunit secretion in patients with pituitary adenomas: influence of TRH, LRH and bromocriptine. Acta Endocrinol 1982;99:487-492.
28. Winters SJ, Troen P. Pituitary glycoprotein hormone α-subunit secretion after thyrotropin-releasing hormone stimulation in normal men and men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 1990;70:544-547.

Chapter 6

Conclusion: Chromogranin A: its clinical value as marker of neuroendocrine tumours.

F.R.E. Nobels[1], D.J. Kwekkeboom[2], R. Bouillon[3] and S.W.J. Lamberts[1]

Departments of Medicine[1] and Nuclear Medicine[2], University Hospital Dijkzigt, Rotterdam, The Netherlands and Laboratory of Experimental Medicine and Endocrinology[3], University Hospital Gasthuisberg, Leuven, Belgium.

Eur J Clin Invest 1998;28:431-440.

Summary

Chromogranin A (CgA) belongs to a family of secretory proteins that are present in dense-core vesicles of neuroendocrine cells. Owing to its widespread distribution in neuroendocrine tissues, it can be used as an excellent immunohistochemical marker of neoplasms of neuroendocrine origin. It can also serve as serum marker of neuroendocrine activity because it is co-released with the peptide hormone content of the secretory granules. The serum concentration of CgA is elevated in patients with various neuroendocrine tumours. Elevated levels are strongly correlated with tumour volume. Although its sensitivity and specificity cannot compete with that of the specific hormonal secretion products of most of these tumours, it can nevertheless have useful clinical applications. Neuroendocrine tumours for which no peptide marker is available usually retain the capacity to secrete CgA. CgA can thus be used as serum marker for these so-called "nonfunctioning" endocrine tumours. Moreover, in patients with carcinoids and pheochromocytomas, CgA is a more stable and thus more easily manageable marker than plasma levels of respectively serotonin and catecholamines and their urinary metabolites. Its role as an important general neuroendocrine marker may be extended in the future, by the development of immunoscintigraphy of membrane-bound CgA, allowing *in vivo* visualization of neuroendocrine neoplasms.

Introduction

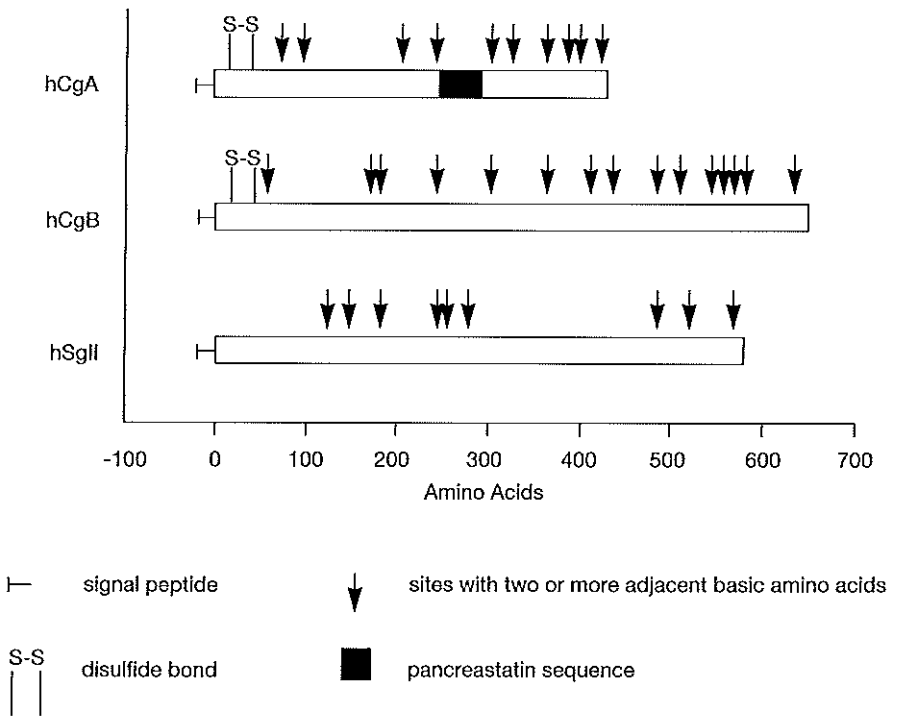
Neuroendocrine cells contain typical secretory granules, called large dense-core vesicles, because of their characteristic appearance on electron microscopy. In addition to the specific peptide hormones or neuropeptides, these granules also contain one or more chromogranin/secretogranin proteins [1-5]. These belong to a unique family of secretory proteins which share many biochemical properties as well as an exclusive presence in neuronal and neuroendocrine secretory granules [6]. The first member of this family to be identified was chromogranin A (CgA). Its name derives from its original discovery in the catecholamine-

containing chromaffin granules of the adrenal medulla [7]. Other well-characterized members of the family are chromogranin B (CgB) and secretogranin II (SgII) [6]. CgA is the best-studied granin in humans. It shows the widest neuroendocrine distribution and is present in some cells that do not express CgB or SgII [1,3]. The physiological functions of CgA are gradually elucidated, although many questions still remain [5]. Its ubiquitous presence in neuroendocrine tissues and its co-secretion with peptide hormones and neuropeptides makes it a suitable tissue and serum marker of neoplasms of neuroendocrine origin [1-4]. The aim of this review is to provide a state of the art of the actual clinical value of CgA as marker of neuroendocrine tumours. Relevant biochemical and clinical data will be summarized and possible future applications will be discussed.

Structure and biochemical properties

The gene encoding for CgA is located on chromosome 14 [8]. Its unique expression in neuroendocrine cells depends on a complex mechanism of transcriptional regulation, which is gradually being unravelled [9]. The primary structure of CgA has been deduced from its corresponding cDNA sequence [10,11]. It is a very hydrophilic protein with an abundance of charged, mostly acidic amino-acids. It contains multiple sites with two or more adjacent basic amino-acids, far exceeding the number observed in other proteins [10-12]. Seven of these dibasic residues are conserved in all mammalian CgA species. They are potential positions for proteolytic processing to smaller peptides, which might be biologically active, as will be discussed below. Another property with possible functional implications is the ability of CgA to bind calcium with moderate affinity at multiple sites, propably due to the excess of negative charges, and to aggregate in the presence of the cation [13,14]. All members of the granin family contain multiple dibasic residues and calcium binding sites, suggesting analogous functions. CgA is most closely related to CgB [15]. Both contain a disulfide-bonded loop structure near their amino termini with a highly homologous amino acid sequence. This structure, which might have a role in directing the chromogranins to the right secretory vesicles, is not present in the secretogranins [16] (Fig. 1).

Figure 1. Diagram of the primary structure of human chromogranin A (hCgA), human chromogranin B (hCgB) and human secretogranin II (hSgII) with indication of the dibasic amino acid sites where proteolytic processing may take place.



CgA-like proteins have been detected in various vertebrate and invertebrate species [3], and related proteins, immunologically cross-reacting with antisera against CgA, have been found in protozoa [17]. There is a considerable structural homology among the CgAs of different mammalian species, implicating conservation during evolution, suggesting important biological functions [2].

Functions

Several hypotheses regarding the biological function of CgA have been proposed. They are summarized in Table 1.

Table 1. Proposed functions of chromogranin A (CgA)

Extracellular
• Precursor of biologically active peptides with auto-, para- and/or endocrine function.
Intracellular
• Modulation of proteolytic processing of peptide hormones and neuropeptides.
• Acts as nucleus for aggregation and precipitation of peptide hormones and neuropeptides to form dense core vesicles.
• Directs peptide hormones and neuropeptides to the regulated pathway of secretion.

Extracellular functions

The best-studied one is that CgA might be processed at its dibasic amino-acid residues, in a tissue-specific manner, to biologically active peptides. Although much evidence is accumulating in favour of this viewpoint, further investigations are needed before definite conclusions can be drawn.

A key finding in this respect was the discovery that the amino acid sequence of pancreastatin is fully contained within the sequence of human CgA corresponding to residues 248-303 [18]. Pancreastatin is a peptide, which, as its name suggests, inhibits insulin and glucagon secretion from the endocrine pancreas [19,20]. It also suppresses parathyroid hormone (PTH) secretion from parathyroid cells [21]. In addition, it has a regulatory effect on secretion from non-endocrine cells, including parietal cells and exocrine pancreas [22,23]. The pancreastatin sequence of the CgA molecule is flanked by sites for proteolytic cleavage, suggesting that the peptide is derived from CgA by proteolytic processing. Its presence has been immunocytochemically documented in many human neuroendocrine cells [23,24].

During the last years several other peptide derivates of CgA have been described. Vasostatin / β-granin is a peptide, derived from the N-terminal part of CgA. In some tissues it is present as a 76-amino-acid chain and in other as a 113-amino-acid chain (called vasostatin I and II respectively) [25,26]. It has been shown to inhibit vasoconstriction of

human veins [27] and to inhibit PTH secretion by bovine parathyroid tissue [28]. Parastatin, a peptide identical to porcine CgA347-419, inhibits PTH secretion [29]. A peptide, homologous to the amino-terminal residues 1-40 of human CgA inhibits the secretion of calcitonin (CT) and PTH-related protein (PTHrP) and stimulates the secretion of CT gene-related peptide (CGRP) [30,31].

Chromostatin, another fragment of CgA corresponding to bovine CgA124-143, has been shown to be able to inhibit the release of catecholamines by adrenal medulla cells [32]. It is liberated from the precursor molecule by cleavage at monobasic sites. A receptor for chromostatin has been described on chromaffin cells that binds the peptide with high affinity and in a saturable way [33]. Recently, however, purifications of chromostatin preparations, with removal from a contaminating non-peptide material, rendered them biologically inactive [34]. Thus, the function of this CgA fragment remains unknown.

All these CgA-derived peptides have been identified *in vivo* [5], with the exception of parastatin and the CgA 1-40 fragments, which have only been generated from CgA by enzymatic digestion *in vitro*. The regions of the CgA molecule that give rise to most of these cleavage products, namely the amino- and carboxy-terminal sites and the pancreastatin sequence, are highly conserved among various species [2].

The proteolytic processing of CgA probably occurs during its stay in the dense-core vesicles [5]. Candidate proteases, able to recognise mono- and dibasic sites, have been found in these vesicles [35-37]. Depending on the type and amount of proteases present, the type and amount of the proteolytic cleavage products may vary in different neuroendocrine tissues [5,38].

Similar proteolytic derivates have been described for CgB and SgII. For CgB two proteolytic peptides have been characterized: CCB, homologous to residues 596-653; and GAWK, homologous to residues 420-483 of the human CgB molecule [39,40]. For SgII a peptide named secretoneurin has been characterized homologous to amino acids 182-204 of human SgII [41].

The above data support the hypothesis that peptides derived from intragranular processing of CgA are co-released with the resident peptide hormones and/or neuropeptides and exert an immediate auto- and paracrine modulatory effect on the secretory activity of the neuroendocrine cells. A classical endocrine effect on distant tissues might also be possible, since these fragments of CgA are also released in the circulation [2].

Intracellular functions

Besides these extracellular functions, CgA might also exert a regulatory role inside the neuroendocrine cell. Obviously, the dibasic sites may serve as competitive substrates for proteolytic enzymes, modulating the proteolytic processing of peptide hormones and neuropeptides during their transport in the neurosecretory vesicles. *In vitro* data supporting this hypothesis have been reported [42].

The granins might also be involved in the packaging of peptides into secretory granules and in directing them to the regulated pathway of secretion. The ability of the granins to bind calcium probably plays a key-role in this process [13,14]. In conditions of high calcium concentration and low pH, as are present in the *trans*-Golgi network, where the dense-core vesicles are formed, CgA avidly binds calcium. This results in a conformational change that induces aggregation of the molecule [13,14]. It has recently been shown that part of the granins firmly bind to the luminal site of the Golgi membrane and form nuclei for aggregation [43]. The peptide hormones and/or neuropeptides are trapped in these aggregates. After budding from the *trans*-Golgi network, typical large dense-core vesicles are formed, containing peptide hormones and granins. *In vitro* experiments suggest that the interaction with CgA occurs only with peptides secreted by the regulatory pathway, whereas constitutive secretory proteins, such as albumin, α1-acid glycoprotein, α1-antitrypsin, immunoglobulins and transferrin, are not precipitated in the aggregates [44,45]. Constitutive secretion refers to the rapid, bulk-flow movement of proteins to the cell surface [46]. The regulated secretory pathway is characterized by the storage of proteins in secretory granules, whose contents are exocytosed when secretion is appropriately stimulated [46]. CgA and the other members of the granin family might thus play a role in the formation of dense-core vesicles that exclude constitutive secretory proteins.

Clinical applications

Although the biological functions of CgA are not established with certainty, several clinical applications as marker for neuroendocrine tumours are already in use or are being developed. CgA is found throughout the neuronal and neuroendocrine system [1-4]. Neuroendocrine cells expressing the protein include endocrine cells of the anterior pituitary, parafollicular C-cells of the thyroid, chief cells of the parathyroid, islet cells of the pancreas and chromaffin cells of the adrenal medulla. It is also present in the widespread neuroendocrine system of the bronchial and gastrointestinal tracts and of the skin (Merkel cells). Endocrine cells that are not of neuroendocrine origin lack CgA. These are the follicular cells of the thyroid gland and the steroid hormone-secreting cells of the adrenal cortex and the gonads.

Use as tissue marker

Immunohistochemical techniques to detect the presence of CgA in tumour tissues are widely used in clinical practice [1,47,48]. A monoclonal antibody LK2H10 against CgA is commercially available for immunohistochemical staining [49]. A list of tumours for which CgA can serve as tissue marker is presented in Table 2. Besides typical neuroendocrine neoplasms, tumours derived from the nervous system, such as ganglioneuroblastoma, ganglioneuroma, and neuroblastoma, also immunostain for CgA [1,3,4]. Even neuroendocrine tumours that lost their ability to produce peptide hormones (*e.g.* null-cell pituitary adenomas) or which produce hormonal products with no apparent clinical effect (*e.g.* clinically non-functioning pituitary adenomas) retain CgA as a constituent of the dense-cored granule matrix [50-52]. Immunostaining for CgA is also positive in neuroendocrine hyperplasia (*e.g.* gastric enterochromaffin-like cell hyperplasia, pulmonary neuroendocrine cell hyperplasia) [53-55].

Immunostaining results should always be interpreted with caution however. Possible reasons for false-negative results are listed in Table 3. Certain neuroendocrine tumours (e.g. small cell lung carcinoma (SCLC)) only weakly express CgA, often below the level of detection of the immunostaining method [56]. The typical characteristic of CgA to undergo cell- and tissue-dependent posttranslational modification can

Table 2. Neuroendocrine tumours for which CgA can serve as tissue marker

Anterior pituitary tumours
Corticotropinoma (Cushing)
Gonadotropinoma
Somatotropinoma (Acromegaly)
Thyrotropinoma
Clinically nonfunctioning neuroendocrine tumours
Null-cell pituitary adenoma
Non-functioning islet cell tumour
GEP (gastro-entero-pancreatic) neuroendocrine tumours
Carcinoid
Gastrinoma
Glucagonoma
Insulinoma
Somatostatinoma
VIPoma
Medullary thyroid carcinoma
Merkel cell tumour
Neural tumours
Ganglioneuroblastoma
Ganglioneuroma
Medulloblastoma
Neuroblastoma
Parathyroid tumours
Pheochromocytoma - paraganglioma
Tumours of the diffuse neuroendocrine system (outside the GEP system)
e.g. in the lungs: Bronchial carcinoid
Small cell lung carcinoma
Large cell neuroendocrine lung carcinoma

also confound the results [5,38]. Moreover, the presence of CgA can be masked for immunodetection by binding to other molecules in the granules. Several neuroendocrine cell types have been found that appear to express only one or two of the chromogranins/secretogranins. For example, prolactin-producing cells of the anterior pituitary are CgA negative but contain CgB and SgII immunoreactivity [50,51]. Likewise, C cells of the thyroid, chief cells of the parathyroid and pancreatic polypeptide cells of the pancreatic islets lack CgB but contain CgA and SgII immunoreactivity [57].

Table 3. Causes for negative immunohistochemistry for CgA in neuroendocrine tissue specimens

• Epitope has been destroyed or rendered inaccessible by fixation or *post-mortem* changes
• Epitope is present, but below the level of detection of the immunostaining method
• Epitope is masked by *in vivo* binding to other molecules (e.g. peptide hormones)
• Epitope has undergone post-translational modification and the antibody used was raised against the unmodified epitope
• CgA is not present (*e.g.* prolactin-producing cells)

Thus, in cases with negative immunoreactivity against CgA, the use of antibodies against CgB or SgII can be helpful. Immunostaining for cleavage products of CgA, such as pancreastatin, does not appear very useful because CgA is not processed in every neuroendocrine cell to immunoreactive pancreastatin [23]. The detection of CgA mRNA, rather than the translation product itself, may have several advantages. First, the techniques of in situ hybridization and/or Northern blot analysis allow detection of very small amounts of mRNA. Second, these techniques are more reliable than immunodetection of protein epitopes.
CgA has also been identified in small amounts in some tumours that are not clearly of neuroendocrine origin [58-62]. This will be discussed below.

Use as serum marker

The fact that CgA is co-released with the peptide hormones present in the secretory granules, opens the possibility to use its serum concentration as a marker of secretion by neuroendocrine neoplasms [2].

This might be of special interest in the following conditions :

1. when no other marker is available, as is the case in so-called "non-functioning" neuroendocrine tumours. Clinically nonfunctioning pituitary adenomas, silent neuroendocrine tumours of the gastro-entero-pancreatic amine precursor uptake and decarboxylation (APUD) system and SCLC might be suitable candidates.
2. when the existing markers are either unstable or rapidly fluctuating or are inconvenient for clinical use. Examples of these are serotonin levels and 24-h urine collections for 5-hydroxy-indole-acetic acid (5-HIAA) in patients with carcinoid tumours and catecholamine levels and 24-h urine collections for catecholamines and their degradation products in patients with pheochromocytomas.
3. when a differentiation should be made between endocrine tumours of neural crest or other origin. The distinction between a pituitary, adrenal or ectopic source of Cushing's syndrome is an example of such a condition.
4. when several neuroendocrine tissues are simultaneously involved in neoplastic disease, as is the case in multiple endocrine neoplasia, CgA could serve as a universal marker of this disease.

Several immunoassay procedures have been developed during recent years [5,63-65]. Both polyclonal- and monoclonal-based techniques are used, with either human or bovine CgA as tracer and standard. The assays all show a variable degree of cross-reaction with the cleavage products of CgA that are present in the circulation. This implies that the normal range of these immunoassay systems is not necessarily similar and should be carefully delineated. Since CgA is a very stable molecule, no special precautions are needed to handle or store the serum samples [64]. The immunoreactivity is stable to repeated freezing and thawing, prolonged incubation at 37°C, and lyophilization [64].

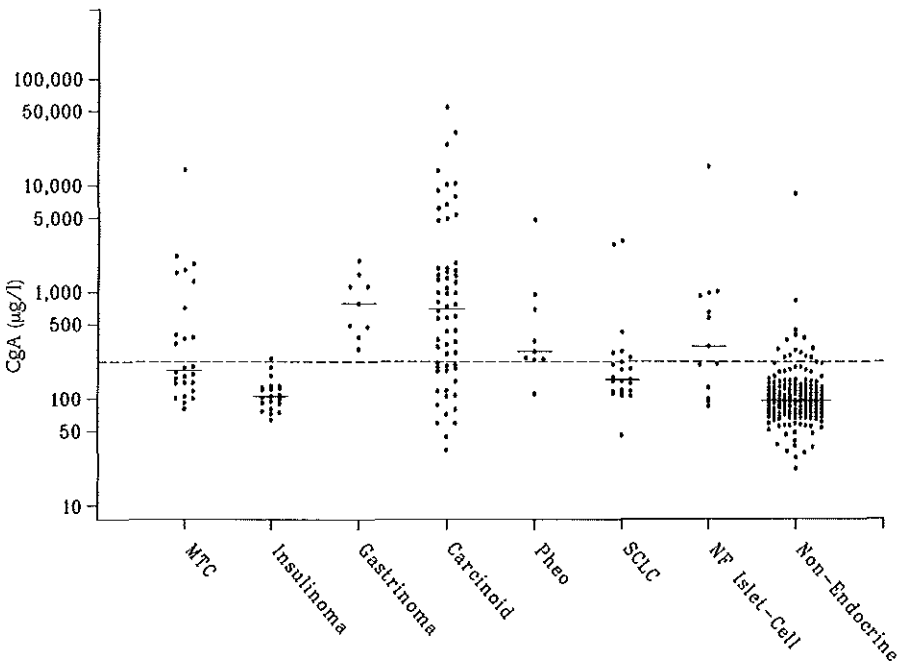
The assays are sufficiently sensitive to measure CgA in normal subjects [52,63,64,66,67]. Levels are slightly higher in postmenopausal women, which might be due to co-secretion with gonadotropins [52]. Spuriously elevated levels can be encountered in patients with renal or hepatic failure. Renal failure, especially as serum creatinine concentrations exceed 400 µmol/l, can firmly increase serum CgA to levels otherwise seen only in neuroendocrine neoplasia [64]. In case of severe hepatic

dysfunction (serum bilirubin exceeding 80 μmol/l), CgA levels are only slightly elevated [64]. As CgA is co-released with catecholamines, one might expect a significant elevation of its serum levels in circumstances of stress, *e.g.* caused by venepuncture. However, even under intense sympatheticoadrenal stimulation, such as during excessive medical stress (*e.g.* cardiac arrest) or during strenuous exercise, CgA levels do not increase more than two-fold [67,68]. As many neuroendocrine tissues contribute to the circulating concentrations of CgA, its plasma pool is substantially greater than that of most peptide hormones. As a consequence, it is more difficult to acutely increase the levels above the physiological background [69].

The serum levels of CgA are elevated in patients with various peptide-producing neuroendocrine tumours [63,64,66,70,71] (Fig. 2). There is a clear correlation between the tumour burden and the serum CgA concentration [63,71-73]. The highest levels are recorded in subjects with metastatic neuroendocrine tumours, with extreme elevations up to 1000 times the upper limit of normal in cases of metastatic carcinoid tumour [66,71]. In contrast, serum concentrations of CgA are rarely elevated in subjects with small neuroendocrine tumours, such as insulinomas, paragangliomas and pituitary adenomas [52,66,71]. These tumours generally induce symptoms at an early stage of oncological evolution because of secretion of peptides with major physiological actions (*e.g.* insulin) or compression of important surrounding tissues. At the moment of their discovery, they are usually too small to be able to elevate the serum CgA levels significantly above the normal steady-state level. However, elevated CgA levels can be encountered in patients with relatively small gastrinomas [71,74]. Chronic hypergastrinaemia induces hyperplasia of the enterochromaffin-like cells of the stomach [75,76]. CgA production by these cells is probably responsible for the elevated serum levels seen in patients with gastrinoma. This is supported by the observation that CgA concentrations can be normalized by gastrectomy alone, leaving the gastrin-producing tumour intact [77].

In general, correlations between serum levels of CgA and of the specific peptide hormones of the tumours are poor [66,71]. Since the plasma pool of these peptides is usually small in normal conditions, even relati-

Figure 2. Serum concentrations of chromogranin A (CgA) in patients with neuroendocrine tumours and in controls with nonendocrine tumours.



Individual levels are presented as dots, median levels as solid lines. The dashed line represents the upper cut-off level of normal values. The results are plotted logarithmically to accomodate extreme values. MTC, medullary thyroid carcinoma; pheo, pheochromocytoma; SCLC, small cell lung carcinoma; NF, non-functioning. Adapted from Ref. 71.

vely small neuroendocrine tumours can induce elevated concentrations. Obviously, the usefulness of CgA as marker for these tumours is limited because it cannot compete with the peptide hormone markers in sensitivity nor specificity. By contrast, CgA can have interesting clinical applications in so-called "nonfunctioning" neuroendocrine tumours that are either not able to secrete hormonal products or release products that cannot be detected using current techniques. These tumours frequently retain the ability to secrete significant amounts of CgA [71,78,79]. These so-called "chromograninomas" were first described by Sobol *et al.* [78]. Unfortunately, serum concentrations of CgA are only rarely increased in patients with "clinically nonfunctioning" pituitary adenomas because of the small volume of these tumours [52,80]. In some cases, injection of TRH can induce significant CgA release by these adenomas [52].

Another possible clinical role for CgA, as outlined above, is its use in tumours lacking stable or convenient markers, as is the case for pheochromocytomas. Plasma concentrations of adrenaline and noradrenaline tend to fluctuate, and 24-h collections of urine for catecholamines and/or their degradation products are cumbersome. Obviously, CgA, which was originally discovered in pheochromocytoma tissue, might serve as a useful serum marker. Patients with pheochromocytoma indeed usually show markedly elevated CgA concentrations [63,64,66, 67,71-73,81]. CgA has a sensitivity and specificity similar to those of the above-mentioned routine markers [72,73]. No substantial elevation of CgA levels is seen in patients with other forms of hypertension [73]. Several drugs, used in the diagnosis or treatment of pheochromocytoma (clonidine, metoprolol, phentolamine, tyramine) have little effect on plasma CgA concentrations, preserving the usefulness of plasma CgA determinations in patients receiving these drugs [69,73].

Another endocrine neoplasm whose hormonal production is difficult to measure in plasma, is a carcinoid tumour. Usually, these tumours are detected late in their oncological evolution, because they generally only induce symptoms in the presence of liver metastases. In these conditions, very high serum levels of CgA are usually seen [66,71,79]. Only sparse data are available on CgA levels in patients with small carcinoid tumours. One study showed normal levels in patients with small bronchial carcinoids with ectopic adrenocorticotrophin hormone (ACTH) secretion [82]. In patients with mid-gut carcinoid tumours only weak correlations are described between the serum CgA concentrations and 24-h urinary 5-HIAA levels or symptoms of flushing or diarrhoea [83]. No correlation exists between CgA and serotonin levels. Octreotide, which is frequently used in the treatment of carcinoids, significantly suppresses serum CgA levels [83]. CgA response to octreotide is associated with clinical improvement. The changes in CgA parallel the corresponding changes in urinary 5-HIAA excretion. Obviously, the correlation between CgA levels and tumour mass is lost during treatment with octreotide, so that in these conditions CgA levels cannot be used as marker of tumour growth.

Another interesting application of CgA is its use in localizing the responsible tumour in Cushing's syndrome. CgA levels usually remain

normal in patients with pituitary corticotroph adenomas because these tumours are generally very small, with diameters of only a few millimeters [82]. Adrenal cortisol-producing tumours obviously do not produce CgA because the adrenal cortex does not originate from the neural crest [82]. In contrast, high serum levels of CgA are frequently encountered in patients with carcinoid tumours and medullary thyroid carcinomas and sometimes also in SCLC [66,71]. Since these tumours can be responsible for ectopic ACTH and/or CRH secretion, CgA might prove to be a serum marker of ectopic Cushing's syndrome. Unfortunately increased CgA levels are only found in cases with extensively metastasized tumours [82]. Thus, the clinical challenge of making an early diagnosis of ectopic Cushing's syndrome caused by a small "occult" neuroendocrine tumour cannot be resolved by measurement of serum CgA.

Theoretically, CgA might be useful as a universal marker of multiple endocrine neoplasia (MEN) syndromes, because it can be produced by the different neuroendocrine tumours characteristic of these diseases [66,71]. It is clear from the previous discussion, however, that CgA levels do not increase early and are less sensitive than the specific peptide hormone markers of neuroendocrine tumours. As a result, CgA is unfortunately no suitable marker for screening for multiple neuroendocrine neoplasia.

CgA in non-endocrine tumours

Elevated CgA levels are not entirely specific for neuroendocrine tumours. Slightly elevated levels have also been identified in patients with nonendocrine tumours [65,71,84-87]. Immunohistochemical studies reveal that many nonendocrine tissues contain neuroendocrine cells belonging to the diffuse APUD system. Recently several studies have shown that these cells are also present in most tumours of nonendocrine origin [58-62]. They are either diffusely scattered throughout the tumour or multifocally located in small nests. They are also present in metastatic tumour tissue [88]. Obviously, these cells might produce peptides that affect the growth of tumour cells. The origin of these cells is not known. They are either cells of the diffuse neuroendocrine system that are attracted by the tumour and are stimulated to proliferate or cells of the tumour itself that undergo neuroendocrine differentiation.

The observation by Aprikian *et al.* [89] that neuroendocrine cells in prostatic adenocarcinoma co-express CgA and prostate-specific antigen (PSA) suggests a common malignant precursor cell. Moreover, these neuroendocrine cells often show morphological changes such as nuclear hyperchromasia and pleomorphism also indicating a malignant origin [90]. Whether the presence of these cells in nonendocrine tumours has prognostic significance demands further investigation. Preliminary data suggest that prostatic adenocarcinomas containing neuroendocrine cells are more resistant to hormonal treatment [60,86,91]. Similarly, patients with colorectal adenocarcinoma, containing numerous neuroendocrine tumour cells, seem to have a worse prognosis [61,85,90]. On the other hand, patients with pancreatic adenocarcinoma [62] or with non-small cell lung carcinoma [58], whose cancer contains many neuroendocrine cells seem to have a better prognosis.

Future prospects

The other members of the chromogranin/secretogranin family are also suitable as immunohistochemical and serum markers for neuroendocrine tumours because they are all widely distributed in neuroendocrine tissues and co-secreted with neuropeptide hormones [3,70]. In fact, using a polyclonal antiserum raised against several chromogranins /secretogranins and against their cleavage products might offer a more powerful tool to differentiate between neuroendocrine and nonneuroendocrine neoplasms. Assays, using such an antiserum, might be used for detection in tissues as well as in serum. Eriksson *et al.* [92] developed a polyclonal antiserum against CgA and CgB and compared it with an antiserum directed against CgA only. The former antiserum appeared to be a much more sensitive marker than the latter one, both immunohistochemically and in the circulation. The concentrations of circulating "granins" were about ten times higher with the CgA + B antiserum than with the CgA antiserum. Assays using such antisera might substantially improve the sensitivity for the detection of small tumours, which could be especially interesting for the early detection of neuroendocrine neoplasms as part of MEN syndromes.

Another future application of chromogranins/secretogranins might be their use in *in vivo* imaging techniques of neuroendocrine tumours. It

has been shown recently that some CgA and CgB molecules are tightly bound to the interior side of the secretory vesicle membrane [43]. After exocytosis, the membrane of these vesicles becomes part of the cell membrane, exposing the chromogranin molecules to the extracellular milieu. This has been proven for CgB in the PC12 neuroendocrine cell line [43]. This provides the opportunity to develop an *in vivo* imaging technique, using immunoscintigraphy with monoclonal antibodies against CgA or CgB [93-96]. Such a "chromogranin-scan" might have the obvious advantage to be specific for neuroendocrine tissues, in contrast with the widely used somatostatin receptor scintigraphy (Octreoscan, Mallinckrodt), that also visualises lymphatic tissues, such as inflammatory infiltrates and lymphomas [97]. Preliminary data have been presented by Magnani *et al.* [93,96], Colombo *et al.* [94] and Siccardi *et al.* [95] about the use of a three-step pretargeted immunoscintigraphy with anti-chromogranin A monoclonal antibody in patients with several neuroendocrine tumours showing a higher diagnostic accuracy than conventional imaging techniques. These data should be confirmed and be compared with a control group of patients with non-neuroendocrine neoplasms. In addition, its clinical value should be compared to that of somatostatin receptor scintigraphy. Furthermore, investigations of the safety and potential adverse effects of the immunoscintigraphic method should be performed.

References

1. Wiedenmann B, Huttner WB. Synaptophysin and chromogranins/secretogranins - widespread constituents of distinct types of neuroendocrine vesicles and new tools in tumour diagnosis. Virchows Archiv B Cell Pathol 1989;58:95-121.
2. Deftos LJ. Chromogranin A : its role in endocrine function and as an endocrine and neuroendocrine tumour marker. Endocrine Reviews 1991;12:181-187.
3. Winkler H, Fischer-Colbrie R. The chromogranins A and B : the first 25 years and future perspectives. Neuroscience 1992;49:497-528.
4. Rosa P, Gerdes HH. The granin family : markers for neuroendocrine cells and tools for the diagnosis of neuroendocrine tumours. J Endocrinol Invest 1994;17:207-225.
5. Iacangelo AL, Eiden LE. Chromogranin A : current status as a precursor for bioactive peptides and a granulogenic/sorting factor in the regulated secretory pathway. Regul Pept 1995;58:65-88.
6. Eiden LE, Huttner WB, Mallet J, O'Connor DT, Winkler H, Zanini A. A nomenclature proposal for the chromogranin/secretogranin proteins. Neuroscience 1987; 21:1019-1021.
7. Blaschko H, Comline RS, Schneider FH, Silver M, Smith AD. Secretion of a chromaffin granule protein, chromogranin from the adrenal gland after splanchnic stimulation. Nature 1967;215:58-59.
8. Wu HJ, Rozansky DJ, Parmer RJ, Gill BM, O'Connor DT. Structure and function of the chromogranin A gene. Clues to evolution and tissue specific expression. J Biol Chem 1991;266:13130-13134.
9. Nolan EM, Cheung TC, Burton DW, Deftos LJ. Identification and characterization of a neuroendocrine-specific 5'-regulatory region of the human chromogranin A gene. Endocrinology 1995;136:5632-5642.
10. Benedum UM, Baeuerle PA, Konecki DS, Frank R, Powell J, Mallet J, Huttner WB. The primary structure of bovine chromogranin A. EMBO J 1986;5:1495-1502.
11. Helman LJ, Ahn TG, Levine MA, Allison A, Cohen PS, Cooper MJ, Cohn DV, Israel MA. Molecular cloning and primary structure of human chromogranin A (secretory protein I) cDNA. J Biol Chem 1988;263:11559-11563.
12. Grimes M, Iacangelo A, Eiden LE, Godfrey B, Herber E. Chromogranin A : the primary structure deduced from cDNA clones reveals the presence of pairs of basic amino acids. Ann NY Acad Sci 1987;493:351-378.
13. Gorr SU, Dean WL, Radley TL, Cohn DV. Calcium-binding and aggregation properties of parathyroid secretory protein-I (chromogranin A). Bone and Mineral 1988;4:17-25.
14. Yoo SH, Albanesi JP. Ca^{2+}-induced conformational change and aggregation of chromogranin A. J Biol Chem 1990;265:14414-14421.
15. Benedum UM, Lamouroux A, Konecki DS, Rosa P, Hille A, Baeuerle PA, Frank R, Lottspeich F, Mallet J, Huttner WB. The primary structure of human secretogranin I (chromogranin B) : comparison with chromogranin A reveals homologous terminal domains and a large intervening variable region. EMBO J 1987;6:1203-1211.

16. Chanat E, Weiss U, Huttner WB, Tooze SA. Reduction of the disulfide bond of chromogranin B (secretogranin I) in the trans-Golgi network causes its missorting to the constitutive secretory pathways. EMBO J 1993;12:2159-2168.
17. Peterson JB, Nelson DL, Ling E, Hogue-Angeletti R. Chromogranin A-like proteins in the secretory granules of a protozoan, Paramecium tetraurelia. J Biol Chem 1987;262:264-267.
18. Iacangelo A, Fischer-Colbrie R, Koller KJ, Brownstein MJ, Eiden LE. The sequence of porcine chromogranin A messenger RNA demonstrates chromogranin can serve as the precursor for the biologically active hormone pancreastatin. Endocrinology 1988;122:2339-2341.
19. Tatemoto K, Efendic S, Mutt V, Makk G, Feistner GJ. Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. Nature 1986;324:476-478
20. Ishizuka J, Asada I, Poston GJ, Lluis F, Tatemoto K, Greely GH, Thompson JC. Effect of pancreastatin on pancreatic endocrine and exocrine secretion. Pancreas 1989;4:277-281.
21. Fasciotto BH, Gorr SU, DeFranco DJ, Levine MA, Cohn DV. Pancreastatin, a presumed product of chromogranin-A (secretory protein-I) processing, inhibits secretion from porcine parathyroid cells in culture. Endocrinology 1989;125:1617-1622.
22. Lewis JJ, Goldering JR, Asher VA, Modlin IM. Pancreastatin : a novel peptide inhibitor of parietal cell signal transduction. Biochem Biophys Res Commun 1989; 163:667-673.
23. Schmidt WE, Siegel EG, Lamberts R, Gallwitz B, Creutzfeldt W. Pancreastatin: molecular and immunocytochemical characterization of a novel peptide in porcine and human tissues. Endocrinology 1988;123:1395-1404.
24. Sekiya K, Ghatei MA, Minamino N, Bretherton-Watt D, Matsuo H, Bloom SR. Isolation of human pancreastatin fragment containing the active sequence form a glucagonoma. FEBS Lett 1988;228:153-156.
25. Hutton JC, Hansen F, Peshavaria M. Proteolytic processing of chromogranin A in purified insulin granules. Formation of a 20 kDa N-terminal fragment (betagranin) by the concerted action of a Ca^{2+}-dependent endopeptidase and carboxypeptidase. Biochem J 1987;244:457-464.
26. Drees BM, Hamilton JW. Processing of chromogranin A by bovine parathyroid secretory granules : production and secretion of N-terminal fragments. Endocrinology 1994;134:2057-2063.
27. Aardal S, Helle KB. The vasoinhibitory activity of bovine chromogranin A fragment (vasostatin) and its independence of extracellular calcium in isolated segments of human blood vessels. Regul Pept 1992;41:9-18.
28. Russell J, Gee P, Liu SM, Angeletti RH. Inhibition of parathyroid hormone secretion by amino-terminal chromogranin peptides. Endocrinology 1994;135:337-342.
29. Fasciotto BH, Trauss CA, Greeley GH, Cohn DV. Parastatin (porcine chromogranin A347-419), a novel chromogranin A-derived peptide, inhibits parathyroid cell secretion. Endocrinology 1993;133:461-466.

30. Deftos LJ, Hogue-Angeletti R, Chalberg C, Tu S. A chromogranin A-derived peptide differentially regulates the secretion of calcitonin gene products. J Bone Miner Res 1990;5:989-991.
31. Deftos LJ, Hogue-Angeletti R, Chalberg C, Tu S. PTHrP secretion is stimulated by calcitonin and inhibited by chromogranin A peptides. Endocrinology 1989; 125:563-565.
32. Galindo E, Rill A, Bader MF, Aunis D. Chromostatin, a 20-amino acid chromogranin A-derived peptide, inhibits chromaffin cell secretion. Proc Natl Acad Sci USA 1991;88:1426-1430.
33. Galindo E, Mendez M, Calvo S, Gonzales-Garcia C, Cena V, Hubert P, Bader MF, Aunis D. Chromostatin receptors control calcium channel activity in adrenal chromaffin cells. J Biol Chem 1992;267:407-412.
34. Galindo E, Zwiller J, Bader MF, Aunis D. Correction. Proc Natl Acad Sci USA 1991;88:1426-1430.
35. Laslop A, Fischer-Colbrie R, Kirschke H, Hogue-Angeletti R, Winkler H. Chromogranin A-processing proteinases in purified chromaffin granules: contaminants or endogenous enzymes? Biochem Biophys Acta 1990;1033:65-72.
36. Eskeland NL, Zhou A, Dinh TQ, Wu H, Parmer RJ, Mains RE, O'Connor DT. Chromogranin A processing and secretion : specific role of endogenous and exogenous prohormone convertases in the regulated secretory pathway. J Clin Invest 1996;98:148-156.
37. Christie DL, Batchelor DC, Plamer DJ. Identification of kex2-related proteases in chromaffin granules by partial amino acid sequence analysis. J Biol Chem 1991;266:679-693.
38. Deftos LJ, Gazdar AF, Hogue-Angeletti R, Mullen PS, Burton DW. Distinct patterns of chromogranin A-related species can be demonstrated in endocrine cells. Bone Mineral 1990;9:169-178.
39. Benjannet S, Leduc R, Adrouche N, Falgueyret JP, Marcinkiewicz M, Seidah NG, Mbikay M, Lazure C, Chretien M. Chromogranin B (secretogranin I), a putative precursor of two novel pituitary peptides through processing at paired basic residues. FEBS Lett 1987;224:142-148.
40. Sekiya K, Ghatei MA, Salahuddin MJ, Bishop AE, Hamid QA, Ibayashi H, Polak JM, Bloom SR. Production of GAWK (chromogranin-B 420-493)-like immunoreactivity by endocrine tumours and its possible diagnostic value. J Clin Invest 1989; 83:1834-1842
41. Vaudry H, Conlon JM. Identification of a peptide arising from the specific post-translational processing of secretogranin II. FEBS Lett 1991;284:31-33.
42. Seidah NG, Hendy GN, Hamelin J, Paquin J, Lazure C, Metters KM, Rossier J, Chrétien M. Chromogranin A can act as a reversible processing enzyme inhibitor. Evidence from the inhibition of the IRCM-serine protease I cleavage of pro-enkephalin and ACTH at pairs of basic amino acids. FEBS Lett 1987;211:144-150.
43. Pimplikar SW, Huttner WB. Chromogranin B (secretogranin I), a secretory protein of the regulated patway, is also present in a tightly membrane-asociated form in PC12 cells. J Biol Chem 1992;267:4110-4118.

44. Tooze SA, Huttner WB. Cell-free protein sorting to the regulated and constitutive secretory pathways. Cell 1990;60:837-847.
45. Chanat E, Huttner WB. Milieu-induced, selective aggregation of regulated secretory proteins in the trans-Golgi network. J Cell Biol 1991;115:1505-1519.
46. Kelly RB. Pathways of protein secretion in eukaryotes. Science 1985; 230:25-32.
47. O'Connor DT, Burton D, Deftos LJ. Immunoreactive chromogranin A in diverse polypeptide hormone producing tumours and normal endocrine tissues. J Clin Endocrinol Metab 1983;57:1084-1086.
48. Weiler R, Fischer-Colbrie R, Schmid KW, Feichtinger H, Bussolati G, Grimelius L, Krisch K, Kerl H, O'Connor D, Winkler H. Immunological studies on the occurrence and properties of chromogranin A and B and secretogranin II in endocrine tumours. Am J Surg Pathol 1988;12:877-884.
49. Lloyd RV, Wilson BS. Specific endocrine tissue marker defined by a monoclonal antibody. Science 1983;222:628-630.
50. DeStephano DB, Lloyd RV, Pike AM, Wilson BS. Pituitary adenomas : an immunohistochemical study of hormone production and chromogranin localization. Am J Pathol 1984;116:464-472.
51. Lloyd RV, Iacangelo A, Eiden LE, Cano M, Jin L, Grimes M. Chromogranin A and B messenger ribonucleic acids in pituitary and other normal and neoplastic human endocrine tissues. Lab Invest 1989;60:548-556.
52. Nobels FRE, Kwekkeboom DJ, Coopmans W, Hoekstra R, Bouillon R, Lamberts SWJ. A comparison between the diagnostic value of the measurement of gonadotropins, α-subunits and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. J Clin Endocrinol Metab 1993;77:784-789.
53. Gould VE, Lee I, Warren WH. Immunohistochemical evaluation of neuroendocrine cells and neoplasms of the lung. Pathol Res Pract 1988;183:200-213.
54. Schmid KW, Hittmair A, Ladurner D, Sandbichler P, Gasser R, Totsch M. Chromogranin A and B in parathyroid tissue of cases of primary hyperparathyroidism : an immunohistochemical study. Virchows Arch A Pathol Anat Histopathol 1991;418:295-299.
55. Belaiche J, Delwaide J, Vivario M, Gast P, Louis E, Boniver J. Fundic argyrophil cell hyperplasia in atrophic gastritis : a search for a sensitive diagnostic method. Acta Gastroenterol Belg 1993;56:11-7.
56. Lloyd RV, Cano M, Rosa P, Hille A, Huttner WB. Distribution of chromogranin A and secretogranin I (chromogranin B) in neuroendocrine cells and tumours. Am J Pathol 1988;130:296-304.
57. Rindi G, Buffa R, Sessa F, Tortora O, Solcia E. Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Distribution, distinction from costored hormones / prohormones and relationship with the argyrophil component of secretory granules. Histochemistry 1986;85:19-28.

58. Sundaresan V, Reeve JG, Stenning S, Stewart S, Bleehen NM. Neuroendocrine differentiation and clinical behaviour in non-small cell lung tumours. Br J Cancer 1991;64:333-338.
59. Scopsi L, Andreola S, Pilotti S, Testori A, Baldini MT, Leoni F, Lombardi L, Hutton JC, Shimizu F, Rosa P. Argyrophylia and granin (chromogranin/secretogranin) expression in female breast carcinomas. Their relationship to survival and other disease parameters. Am J Surg Pathol 1992;16:561-576.
60. di Sant'Agnese PA. Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic and therapeutic implications. Cancer 1992;70:254-268.
61. De Bruine AP, Wiggers T, Beek C, Volovics A, von Meyenfeldt M, Arends JW, Bosman FT. Endocrine cells in colorectal adenocarcinomas : incidence, hormone profile and prognostic relevance. Int J Cancer 1993;54:765-771.
62. Pour PM, Permert J, Mogaki M, Fujii H, Kazakoff K. Endocrine aspects of exocrine cancer of the pancreas. Their patterns and suggested biologic significance. Am J Clin Pathol 1993;100:223-230.
63. O'Connor DT, Bernstein KN. Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. N Engl J Med 1984;311:764-770.
64. O'Connor DT, Pandlan MR, Carlton E, Cervenka JH, Hsiao RJ. Rapid radioimmunoassay of circulating chromogranin A : in vitro stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. Clin Chem 1989;35:1631-1637.
65. Sobol RE, O'Connor DT, Addison J, Suchocki K, Royston I, Deftos LJ. Elevated serum chromogranin A concentrations in small cell lung carcinoma. Ann Intern Med 1986;105:698-700.
66. O'Connor DT, Deftos LJ. Secretion of chromogranin A by peptide-producing endocrine neoplasms. N Engl J Med 1986;314:1145-1151.
67. Cryer PE, Wortsman J, Shah SD, Nowak RM, Deftos LJ. Plasma chromogranin A as marker of sympathochromaffin activity in humans. Am J Physiol 1991;260:E243-246.
68. Elias AN, Wilson AF, Pandian MR, Kayaleh R. Chromogranin A concentrations in plasma of physically active men after acute exercise. Clin Chem 1992;38:2348-2349.
69. Takiyyuddin MA, Cervenka JH, Pandian MR, Stuenkel CA, Neumann HPH, O'Connor DT. Neuroendocrine sources of chromogranin-A in normal man : clues from selective stimulation of endocrine glands. J Clin Endocrinol Metab 1990; 71:360-369.
70. Stridsberg M, Öberg K, Li Q, Engström U, Lundqvist G. Measurements of chromogranin A, chromogranin B (secretogranin I), chromogranin C (secretogranin II) and pancreastatin in plasma and urine from patients with carcinoid tumours and endocrine pancreatic tumours. J Endocrinol 1995;144:49-59.

71. Nobels FRE, Kwekkeboom DJ, Coopmans W, Schoenmakers CHH, Lindemans J, De Herder WW, Krenning EP, Bouillon R and Lamberts SWJ. Chromogranin A as serum marker for neuroendocrine neoplasia : comparison with neuron-specific enolase and the α-subunit of glycoprotein hormones. J Clin Endocrinol Metab 1997;82:2622-2628.
72. Hsiao RJ, Neumann HPH, Parmer RJ, Barbosa JA, O'Connor DT. Chromogranin A in familial pheochromocytoma : diagnostic screening value, prediction of tumour mass, and post-resection kinetics indicating two-compartment distribution. Am J Med 1990;88:607-613.
73. Hsiao RJ, Parmer RJ, Takiyyuddin MA, O'Connor DT. Chromogranin A storage and secretion : sensitivity and specificity for the diagnosis of pheochromocytoma. Medicine 1991;70:33-45.
74. Syversen U, Mignon M, Bonfils S, Kristensen A, Waldum HL. Chromogranin A and pancreastatin-like immunoreactivity in serum of gastrinoma patients. Acta Oncol 1993;32:161-165.
75. D'Adda T, Corleto V, Pilato FP, Baggi MT, Robutti F, Delle Fave G. Quantitative ultrastructure of endocrine cells of oxyntic mucosa in Zollinger-Ellison syndrome. Gastroenterology 1990;99:17-26.
76. Waldum HL, Arnestad JS, Brenna E, Eide I, Syversen U, Sandvik AK. Marked increase in gastric acid secretory capacity after omeprazole treatment. Gut 1996; 39:649-653.
77. Stabile BE, Howard TJ, Passaro E, O'Connor DT. Source of plasma chromogranin A elevation in gastrinoma patients. Arch Surg 1990;125:451-453.
78. Sobol RE, Memoli V, Deftos LJ. Hormone-negative, chromogranin A-positive endocrine tumours. N Engl J Med 1989;320:444-447.
79. Schürmann G, Raeth U, Wiedenmann B, Buhr H, Herfarth C. Serum chromogranin A in the diagnosis and follow-up of neuroendocrine tumours of the gastroenteropancreatic tract. World J Surg 1992;16:697-702.
80. Deftos LJ, O'Connor DT, Wilson CB, Fitzgerald PA. Human pituitary tumours secrete chromogranin-A. J Clin Endocrinol Metab 1989;68:869-872.
81. Stridsberg M, Husebye ES. Chromogranin A and chromogranin B are sensitive circulating markers for pheochromocytoma. Eur J Endocrinol 1997;136:29-29.
82. Nobels FRE, de Herder WW, Kwekkeboom DJ, Coopmans W, Mulder A, Bouillon R, Lamberts SWJ. Serum chromogranin A in the differential diagnosis of Cushing's syndrome. Eur J Endocrinol 1994;131:589-93.
83. Moattari AR, Deftos LJ, Vinik AI. Effects of sandostatin on plasma chromogranin-A levels in neuroendocrine tumours. J Clin Endocrinol Metab 1989;69:902-905.
84. Kadmon D, Thompson TC, Lynch GR, Scardino PT. Elevated plasma chromogranin-A concentrations in prostatic carcinoma. J Urol. 1991;146:358-61.
85. Syversen U, Halvorsen T, Marvik R, Waldum HL. Neuroendocrine differentiation in colorectal carcinomas. Eur J Gastroenterol Hepatol 1995;7:667-674.

86. Cussenot O, Villette JM, Valeri A, Cariou G, Desgrandchamps F, Cortesse A, Meria P, Teillac P, Fiet J, Le Duc A. Plasma neuroendocrine markers in patients with benign prostatic hyperplasia and prostatic carcinoma. J Urol 1996;155:1340-1343.
87. Deftos LJ, Nakada S, Burton DW, di Sant'Agnese PA, Cockett AT, Abrahamsson PA. Immunoassay and immunohistology studies of chromogranin A as a neuroendocrine marker in patients with carcinoma of the prostate. Urology 1996;48:58-62.
88. Aprikian AG, Cordon-Cardo C, Fair WR, Zhang ZF, Bazinet M, Hamdy SM, Reuter VE. Neuroendocrine differentiation in metastatic prostatic adenocarcinoma. J Urol 1994;151:914-919.
89. Aprikian AG, Cordon-Cardo C, Fair WR, Reuter VE. Characterization of neuroendocrine differentiation in human benign prostate and prostatic adenocarcinoma. Cancer 1993;71:3952-3965.
90. Hamada Y, Oishi A, Shoji T, Takada H, Yamamura M, Hioki K, Yamamoto M. Endocrine cells and prognosis in patients with colorectal carcinoma. Cancer 1992;69:2641-2646.
91. Krijnen JL, Bogdanowicz JF, Seldenrijk CA, Mulder PG, van der Kwast TH. The prognostic value of neuroendocrine differentiation in adenocarcinoma of the prostate in relation to progression of disease after endocrine therapy. J Urol 1997;158:171-174.
92. Eriksson B, Arnberg H, Öberg K, Hellman U, Lundqvist G, Wernstedt C, Wilander E. A polyclonal antiserum against chromogranin A and B - a new sensitive marker for neuroendocrine tumours. Acta Endocrinologica (Copenh) 1990;122:145-155.
93. Magnani P, Paganelli G, Siccardi AG, Songini C, Colombo P, Faglia G, Fazio F. Combined use of 111In-labeled pentetreotide and three-step immunoscintigraphy with antichromogranin A monoclonal antibody in the diagnosis of pituitary adenomas. Cell Biophys 1994;24-25P:307-313.
94. Colombo P, Siccardi AG, Paganelli G, Magnani P, Songini C, Buffa R, Faglia G, Fazio F. Three-step immunoscintigraphy with anti-chromogranin A monoclonal antibody in tumours of the pituitary region. Eur J Endocrinol 96;135:216-21.
95. Siccardi AG, Paganelli G, Pontiroli AE, Pelagi M, Magnani P, Viale G, Faglia G, Fazio F. In vivo imaging of chromogranin A-positive endocrine tumours by three-step monoclonal antibody targeting. Eur J Nucl Med 1996;23:1455-1459.
96. Magnani P, Paganelli G, Songini C, Samuel A, Sudati F, Siccardi AG, Fazio F. Pretargeted immunoscintigraphy in patients with medullary thyroid carcinoma. Br J Cancer 1996;74:825-831.
97. Lamberts SWJ, Krenning EP, Reubi J-C. The role of somatostatin and its analogs in the diagnosis and treatment of tumours. Endocr Rev 1991;12:450-482.

Chapter 7

Long-term treatment with the dopamine agonist quinagolide (CV205-502) of patients with a clinically non-functioning pituitary adenoma.

F.R.E. Nobels[1], W.W. de Herder[1], W.M. van den Brink[2], D.J. Kwekkeboom[3], L.J. Hofland[1], J. Zuyderwijk[1], F.H.de Jong[1], and S.W.J. Lamberts[1]

Departments of Medicine[1], Neurosurgery[2] and Nuclear Medicine[3], University Hospital Dijkzigt, Rotterdam, The Netherlands.

Summary

This study was performed to evaluate the effect of prolonged treatment with the dopamine agonist quinagolide on serum gonadotropin and α-subunit concentrations and tumour volume in patients with clinically non-functioning pituitary adenomas (CNPA). Ten patients with a CNPA were treated with quinagolide in a final daily dose of 0.3 mg. The median duration of treatment was 57 months (range 36 to 93 months). Blood samples for measurement of serum gonadotropin and α-subunit concentrations were drawn before treatment, after 5 days, and at each outpatient visit. Computerized tomography or magnetic resonance imaging of the pituitary region and Goldmann perimetry were done before and at regular intervals during treatment. Tumour volume was calculated by a surface to volume summation method and by a method based on the 3 axes of the tumor. A significant decrease of serum follicle stimulating hormone (FSH), luteinizing hormone (LH) or α-subunit concentrations was found in 9 patients. Response was shown within the first year in nearly all patients, except in one patient whose FSH levels decreased after more than 12 months. No escape to treatment occurred; the levels remained low during the entire treatment period. In 2 of 3 patients with pre-existing visual field defects a slight improvement was shown during the first months of treatment, but eventually deterioration occurred in all 3 patients. A fourth patient developed unilateral ophthalmoplegia during treatment. During the first year tumour volume decreased in 3 patients, but in 2 of them regrowth occurred after a few months. In 6 patients progressive tumour growth occurred despite sustained suppression of gonadotropin or α-subunit levels. At the end of follow-up tumor volume remained significantly below pretreatment volume in 2 patients, remained at pretreatment volume in 2 patients and was significantly increased in 6 patients. We conclude that in patients with CNPA long-term treatment with high doses of the dopamine agonist quinagolide induced a prolonged decrease in serum concentrations of gonadotropins and/or α-subunits. Progressive increase in tumour size occurred in most patients, however, and no favourable long-term effects on visual function were shown.

Introduction

Approximately 25 to 30% of pituitary adenomas are classified as clinically non-functioning [1-5]. Because patients harbouring these tumours lack clinical signs of hormone excess, the diagnosis is usually made at an advanced stage. Surgical removal is therefore often incomplete and at present there is no established medical treatment available. The majority of these tumours are in fact poorly secreting, rather than completely non-secreting. In cell culture they usually release gonadotropins or their subunits [6-8]. Pharmacological suppression of this hormone production could theoretically induce tumour shrinkage, analogous to the effects of dopamine agonists in patients with prolactinomas and somatostatin analogues in patients with growth hormone (GH)-producing adenomas [9,10].

In general, clinically non-functioning pituitary adenomas (CNPA) express dopamine D2 receptors on their cell membranes [11,12]. This is confirmed by new *in vivo* dopamine receptor scintigraphy techniques with the iodinated benzamides ^{123}I-IBZM or ^{123}I-epidepride [13-16]. The dopamine agonist bromocriptine can suppress the synthesis and secretion of gonadotropins and α-subunits by CNPA cells *in vitro* [17-19]. It also reduces α-subunit mRNA accumulation in tumour tissue removed from patients with pure α-subunit secreting tumours [18]. Therefore several investigators have tried dopamine agonist therapy, usually bromocriptine, in patients with CNPA. The results are rather disappointing, however, showing improvement of visual field defects and/or reduction of tumour mass in only a small number of patients [11,20-24]. Since bromocriptine is generally not well tolerated in high doses, rather low doses were used in these studies. In addition, the treatment periods were generally short.

The density of dopamine receptors on CNPA cells is much lower than on prolactinoma cells. Moreover, prolonged incubation with bromocriptine in high concentrations is needed to obtain a significant reduction of gonadotropin and α-subunit release *in vitro* [19]. This suggests that sustained treatment with high doses of a dopamine agonist is needed to obtain tumour shrinkage.

The new generation of more powerful and more selective dopamine agonists might provide a useful alternative for bromocriptine [25]. These drugs are better tolerated than bromocriptine, allowing the use of higher doses. Our group published preliminary results with the long-acting non-ergot dopamine agonist quinagolide (CV 205-502) in 5 patients with CNPA [26]. At a final daily dose of 0.3 mg during 12 to 18 months a significant decrease in serum follicle stimulating hormone (FSH) and/or α-subunit concentrations was induced in 4 patients, tumour shrinkage in 1, and improvement in visual field defects in 2 patients. The drug was well tolerated.

In order to evaluate the long-term effect of quinagolide *in vivo* we followed serum gonadotropin and α-subunit concentrations and tumour volume in ten patients with a CNPA, who were treated with the dopamine agonist for at least 3 years. Tumour volume was carefully measured using a surface to volume summation method and a mathematical method based on the 3 axes of the tumour.

Subjects and methods

We studied ten patients with a CNPA. Short-term (12-18 months) follow-up data of 4 patients (patients 2,3,7,8) were published previously by Kwekkeboom *et al.* [26]. CNPA was defined as the presence of a solid pituitary tumor without clinical signs of hormonal hypersecretion and lacking clinical and radiological characteristics of non-pituitary tumours (such as craniopharyngiomas, meningiomas, metastases of distant malignant tumors or aneurysms of the internal carotid artery). Patients with pituitary apoplexia or radiological evidence of bleeding, necrosis or cystic structures in the tumour were excluded. None of the patients had received prior medical, surgical or radiation therapy for the pituitary adenoma.

All patients gave informed consent to participate in the study, which was approved by the hospital's ethical committee. Medical treatment with quinagolide was started at a daily dose of 0.075 mg. The dose was

progressively increased to the final daily dose of 0.3 mg within one month. Reasons for choosing quinagolide treatment were either the presence of relative contraindications to surgery or refusal of surgery by the patient. Patients were regularly followed at the outpatient clinic for at least 3 years.

Pretreatment serum gonadotropin and α-subunit concentrations were determined from samples drawn on a control day hourly for 12 hours. In 6 patients additional hourly sampling for 12 hours was performed 5 days after starting quinagolide treatment. Thereafter blood samples were drawn at each visit to the outpatient department. Prolactin (PRL) levels were measured to assess compliance to the treatment.

PRL, insulin-like growth factor-I (IGF-I), GH, thyroid stimulating hormone (TSH) and adrenocorticotrophic hormone (ACTH) were measured using kits obtained, respectively, from IRE-Medgenix, Brussels, Belgium (PRL, IGF-I); Sorin, Milano, Italy; Behring, Marburg, Germany, and the Radiochemical Centre, Amersham, UK. FSH and luteinizing hormone (LH) were measured using immunoradiometric assay kits supplied by IRE-Medgenix, Brussels, Belgium. The sensitivity of these assays was 0.5 U/l. α-Subunit was measured by RIA, using antibodies purchased from UCB, Brussels, Belgium. The sensitivity of the α-subunit assay was 0.3 μg/l. The intra- and inter-assay coefficients of variation were, respectively, < 5 and < 15 % for LH, < 3 and < 8 % for FSH, and < 6 and < 11 % for α-subunit [27].

Computerized tomographic (CT) scans or magnetic resonance imaging (MRI) of the pituitary region and Goldmann perimetry were done before and at regular intervals during treatment. CT-scan and/or MRI images were used to estimate tumour volume. The scans were digitized using a Hewlett Packard ScanJet II cx/t flatbed scanner (Hewlett Packard Company, U.S.A.). The scanner was operated by a Macintosh LC 475 20/250 mb computer (Apple Computer Inc., Cupertino, Ca, U.S.A.), utilizing Desk scan II software (Hewlett Packard Company U.S.A.). Files were read by the NIH Image 1.27 digital image analysis program (National Institute of Health, shareware, Baltimore, U.S.A.). The scale was calibrated from pixels to millimeters using the scale bar printed on

scans. All measurements were performed by the same observer. Two methods of volume calculation were used: a surface to volume summation method and a method based on the 3 axes of the tumour [28]. In the surface to volume summation method the tumour area was outlined using the computermouse. The surface of the tumour section was calculated and multiplied with the slice thickness. The total volume of the tumor was calculated by adding up these values. Duong *et al.* published an error analysis of this method, showing acceptable measurement deviations from known volumes [29]. In the other method the X, Y and Z radius were measured in the frontal, sagittal and coronal planes, respectively. Assuming a spherical volume, the formula $4/3\ \pi\ R^3$ was used for volume calculation, R being the mean of the X, Y and Z radius.

Individual hormone levels were evaluated using analysis of variance. For the comparison of the means the Newman-Keuls method was applied. P values < 0.05 were considered significant.

Results

Patient data and hormone levels are listed in Table 1. No apparent hypersecretion of gonadotropins or α-subunits was shown, except in one patient (patient 7) who had a slightly elevated serum α-subunit level. Serum levels of PRL were slightly elevated in 4 patients (all < 25 µg/l). Serum levels of GH, IGF-I, TSH and ACTH were not elevated in any of the patients. All patients showed hypopituitarism, with deficient adrenal, thyroidal and gonadal function, except patients 3 and 9, who only had deficient gonadal and thyroidal function, respectively. All patients had pituitary macroadenomas with suprasellar extension.

The median duration of follow-up under quinagolide treatment was 57 months (range 36 to 93 months). PRL levels remained lower than 2 µg/l during the entire treatment in all the patients, indicating good compliance. Tolerance of the drug was excellent. A significant decrease of serum LH, FSH or α-subunit concentrations was found in 9 patients (Tab. 1). No early decrease was seen in the 6 patients whose gonadotro-

Table 1. Patient data and LH, FSH and α-subunit levels before and during treatment with quinagolide.

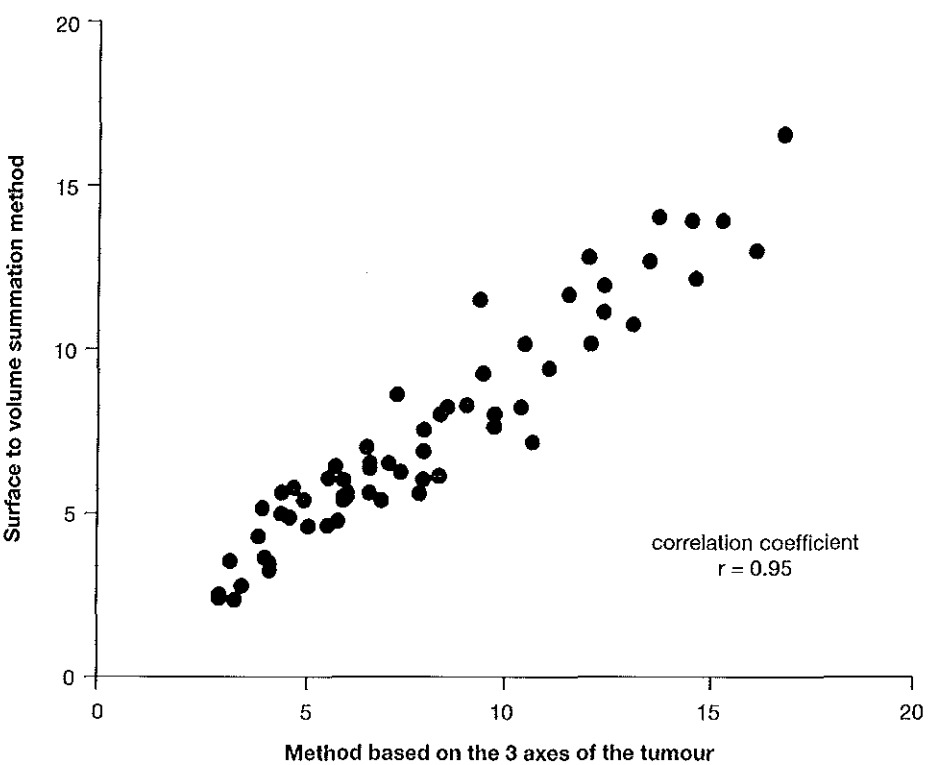
Patient	Sex	Age (years)	Duration of follow-up (months)	PRL (μg/l)	Gonadotropins and α-subunit	quinagolide treatment (months) 0 mean (range)	1-3 mean (range)	3-12 mean (range)	>12 mean (range)
1	M	67	56	22.1	LH (U/l)	1.4 (0.9 - 1.8)	1.0	0.7 (0.4 - 1.0)*	0.5 (0.4 - 0.8)
					FSH (U/l)	1.4 (0.6 - 2.2)	0.9	1.4 (1.0 - 1.9)	1.2 (0.4 - 2.4)
					α-subunit (μg/l)	0.4 (0.3 - 0.6)		0.6 (0.5 - 0.6)	0.5 (0.2 - 0.8)
2	M	62	91	16.5	LH (U/l)	0.6 (0.5 - 0.6)	0.5 (0.4 - 0.6)	0.5 (0.4 - 0.8)	0.5 (0.4 - 0.9)
					FSH (U/l)	8.0 (6.8 - 9.4)	1.5 (0.5 - 2.2)**	2.0 (1.4 - 2.7)	0.9 (0.4 - 1.5)+
					α-subunit (μg/l)	0.5 (0.5 - 0.6)	0.4 (0.2 - 0.5)*	0.5 (0.4 - 0.5)	0.4 (0.2 - 0.6)
3	M	57	93	7.0	LH (U/l)	3.8 (3.4 - 4.7)	2.3 (1.4 - 3.0)**	2.8 (2.3 - 3.2)	1.7 (0.4 - 3.1)++
					FSH (U/l)	6.2 (5.2 - 7.2)	2.6 (1.7 - 3.3)**	2.9 (1.9 - 3.3)	2.7 (1.6 - 4.5)
					α-subunit (μg/l)	1.0 (0.9 - 1.1)	0.5 (0.4 - 0.6)**	0.5 (0.4 - 0.6)	0.5 (0.2 - 0.7)
4	M	62	51	13.2	LH (U/l)	0.6 (0.4 - 1.3)	0.4	0.6 (0.4 - 1.1)	0.5 (0.4 - 1.0)
					FSH (U/l)	2.9 (2.3 - 4.4)	0.4	0.9 (0.4 - 1.3)**	0.7 (0.4 - 1.6)
					α-subunit (μg/l)	1.0 (0.8 - 1.3)	0.3	0.4 (0.3 - 0.4)**	0.4 (0.2 - 0.6)
5	M	62	44	6.0	LH (U/l)	0.5 (0.4 - 0.8)	1.6	0.5 (0.4 - 0.7)	0.4 (0.4 - 0.4)
					FSH (U/l)	2.2 (1.4 - 3.6)		2.8 (0.4 - 6.0)	0.6 (0.4 - 1.0)+
					α-subunit (μg/l)	0.8 (0.6 - 1.0)	0.9	0.6 (0.5 - 0.9)	0.4 (0.2 - 0.5)
6	M	70	41	14.9	LH (U/l)	1.1 (1.0 - 1.2)		0.7 (0.6 - 0.7)**	0.5 (0.4 - 0.8)
					FSH (U/l)	2.3 (1.8 - 3.0)		1.7 (1.5 - 1.9)	1.0 (0.6 - 1.7)
					α-subunit (μg/l)	0.8 (0.6 - 0.9)	0.9	0.8 (0.7 - 0.9)	0.4 (0.2 - 0.5)++
7	M	81	85	8.2	LH (U/l)	1.1 (0.9 - 1.2)	1.5 (1.5 - 1.5)	1.7 (0.6 - 3.0)	0.8 (0.4 - 1.5)
					FSH (U/l)	3.3 (3.3 - 3.3)	1.3 (1.1 - 1.4)*	0.9 (0.4 - 1.9)	1.1 (0.4 - 2.6)
					α-subunit (μg/l)	1.3 (1.2 - 1.4)	0.7 (0.6 - 0.8)**	0.5 (0.5 - 0.6)	0.6 (0.4 - 0.8)
8	M	78	68	17.7	LH (U/l)	3.3 (2.5 - 3.9)	0.9 (0.4 - 1.3)**	0.8 (0.4 - 1.6)	0.8 (0.4 - 2.2)
					FSH (U/l)	5.2 (4.2 - 6.0)	0.5 (0.4 - 0.7)**	1.8 (0.8 - 3.0)	1.9 (0.6 - 2.9)
					α-subunit (μg/l)	0.3 (0.2 - 0.4)	0.2 (0.2 - 0.2)	0.2 (0.2 - 0.2)	0.2 (0.2 - 0.6)
9	V	72	57	21.7	LH (U/l)	0.6 (0.4 - 1.2)		1.2 (0.6 - 1.8)	0.6 (0.4 - 1.1)
					FSH (U/l)	5.1 (4.3 - 5.8)		2.7 (1.0 - 4.3)**	2.6 (1.7 - 3.5)
					α-subunit (μg/l)	0.4 (0.3 - 0.6)	0.7	0.6 (0.5 - 0.7)	0.5 (0.4 - 0.8)
10	M	67	36	13.7	LH (U/l)	0.4 (0.4 - 0.4)	0.4		0.4
					FSH (U/l)	1.1 (0.4 - 1.6)	0.4		1.1
					α-subunit (μg/l)	0.6 (0.4 - 0.7)	0.5		

* P < 0.05 and ** P < 0.01 vs month 0; + P < 0.05 and ++ P < 0.01 vs month 3-12. No statistical evaluation was performed on individual hormone levels. Normal values in men : LH 1.9-9.2 U/l, FSH 1.6-11.1 U/l, α-subunit 0.4-1.1 μg/l; in postmenopausal women : LH 17.5-86.8 U/l, FSH 26.2-107.7 U/l, α-subunit 1.3-4.0 μg/l. Pretreatment levels are means of 13 values on a control day. The levels after 1-3, 3-12 and > 12 months of therapy are means of 2 to 5, 2 to 6 and 4 to 17 serum samples, respectively, except in the few cases when only one value was available.

pin and α-subunit levels were measured 5 days after the beginning of quinagolide treatment (data not shown). Response was shown within the first 12 months of treatment, except in patient 5 whose FSH levels decreased only after more than 12 months. In 3 patients (2, 3 and 6), who responded within the first year, gonadotropin or α-subunit levels further decreased after a treatment duration of more than 12 months. The suppression was most marked in those patients with the highest pretreatment levels. The levels remained low during the entire treatment period. No apparent escape to treatment was seen, not even in patients 2, 3 and 7, who were followed for more than 7 years.

The two methods of tumour volume calculation showed comparable results (correlation coefficient r = 0.95) (Fig. 1). Therefore only the

Figure 1. Comparison between two methods of tumour volume estimation



(axes in cm³)

surface summation method was used for evaluating the effect of quinagolide on tumour volume. A volume change of more than 10% was arbitrarily considered significant (Tab. 2). During the first year tumour volume decreased in 3 patients (patients 3, 5 and 6), remained stable in 3 patients (patients 1, 4, and 7) and increased in 4 patients (patients 2, 8, 9 and 10) (Tab. 2, Fig. 2). In patients 3 and 6, whose tumour initially decreased in volume, regrowth to the pretreatment volume occurred after 10 and 24 months, respectively. Of the 3 patients whose tumour volume initially remained stable, a significant decrease in volume was

Table 2. Tumour volume before and during treatment with quinagolide.

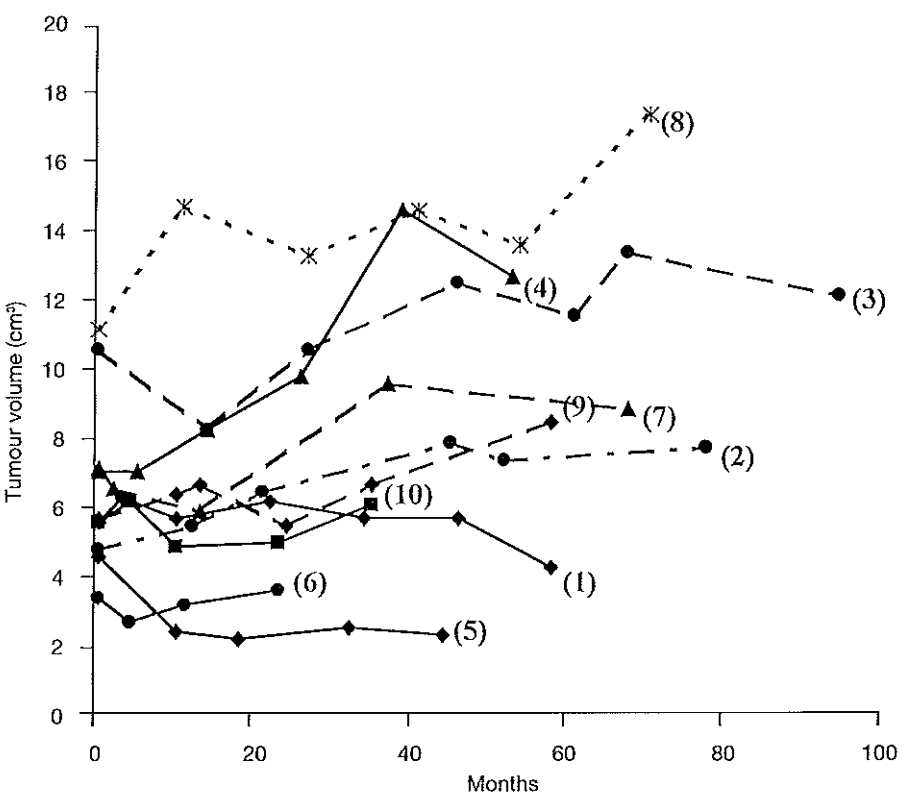
	quinagolide treatment (months)			
	0	1-12	12-48	> 48
Tumour volume (cm^3)	mean	mean (range)	mean (range)	mean (range)
Change from baseline (%)		Δ	Δ	Δ
patient 1	5.5	5.6	5.8 (5.6 - 6.1)	4.2
		Δ 1.8	Δ 5.5	Δ -23.6*
patient 2	4.7	6.9 (5.4 - 8.4)	7.1 (6.4 - 7.8)	7.5 (7.3 - 7.7)
		Δ 46.8*	Δ 51.1*	Δ 59.6*
patient 3	10.5	8.2	11.5 (10.5 - 12.4)	12.3 (11.5 - 13.3)
		Δ -21.9*	Δ 9.5	Δ 17.1*
patient 4	7.0	7.6 (7.0 - 8.2)	12.1 (9.7 - 14.5)	12.6
		Δ 8.6	Δ 72.9*	Δ 80.0*
patient 5	4.5	2.3	2.2 (2.1 - 2.4)	
		Δ -48.9*	Δ -51.1*	
patient 6	3.3	2.9 (2.6 - 3.1)	3.5	3.4
		Δ -12.1*	Δ 6.1	Δ 3.0
patient 7	7.1	6.5	7.7 (5.8 - 9.5)	8.8
		Δ -8.5	Δ 8.5	Δ 23.9*
patient 8	11.1	14.6	13.9 (13.2 - 14.5)	15.4 (13.5 - 17.3)
		Δ 31.5*	Δ 25.2*	Δ 38.7*
patient 9	5.6	6.5 (6.3 - 6.6)	6.0 (5.4 - 6.6)	8.4
		Δ 16.1*	Δ 7.1	Δ 50.0*
patient 10	5.5	6.1	6.0	
		Δ 10.9*	Δ 9.1	

Δ = tumour volume change from baseline. * a change of more than 10 % is arbitrarely considered significant

shown in patient 1 after 56 months of treatment, but significant growth occurred in patients 4 and 7 after 24 and 37 months, respectively. At the end of follow-up (range 36 to 93 months) tumour volume remained significantly below pretreatment volume in 2 patients (patient 1 and 5), remained at pretreatment volume in 2 patients (patient 6 and 10) and was significantly increased in 6 patients (patients 2, 3, 4, 7, 8 and 9). No relationship between changes in tumour volume and changes in gonadotropin and/or α-subunit levels could be demonstrated.

Patients 1, 2, 3, 4, 5, 9 and 10 had no visual field defects before or during the treatment. In patient 6 a pre-existing discrete left superior temporal quadrantanopsia slightly deteriorated during treatment with development of bitemporal superior quadrantanopsia, although no

Figure 2. Evolution of tumour volume



( ) = patient number

significant increase in tumour volume could be detected on MRI. In patients 7 and 8, who had bitemporal superior quadrantanopsia and bitemporal hemianopsia, respectively, visual field defects improved in the first 4 months of treatment. When tested after 59 and 35 months of treatment, respectively, a slight deterioration was shown in both patients, correlating with a significant increase in tumour volume. Patient 2, whose tumour volume was progressively increasing on successive CT- and MRI-scans, developed unilateral ophthalmoplegia (6th cranial nerve) after 83 months of quinagolide treatment, that responded favourably to treatment with dexamethasone.

Patients 6 and 10 were eventually operated on. Pathological examination of the resection specimens revealed chromophobic adenoma, with negative immunohistochemistry for PRL and ACTH.

Discussion

The dopamine agonist bromocriptine induces an increasing time-dependent inhibitory effect on the synthesis and release of gonadotropins and α-subunits *in vitro*, when added to cultures of tumour cells of gonadotroph origin [17-19]. Previously, we reported that it takes prolonged incubation of 2 to 6 weeks before maximal inhibition is attained [19]. In this *in vivo* study, in which we report on the efficacy of treatment with quinagolide in ten patients with CNPA, we found a significant decrease of serum concentrations of LH, FSH or α-subunit in nearly all patients. In concordance with our *in vitro* studies, no early decrease was seen a few days after the beginning of quinagolide treatment. Response was shown within the first year in nearly all patients, except in one patient whose FSH levels decreased only after more than 12 months. In 3 patients, who responded within the first year, gonadotropin or α-subunit levels further decreased after more than 1 year treatment. The levels remained low during the entire follow-up period. No escape to treatment was seen, not even in patients followed for more than 7 years.

In patients with PRL- or GH-producing pituitary adenomas, drugs that suppress hormonal activity induce tumour shrinkage in a substantial proportion of tumours [9,10]. One might expect similar effects in patients with CNPA. The reported *in vivo* effects of bromocriptine treatment on tumour size have been variable, however [11, 20-24]. In general no reduction in tumour size has been observed, but occasional patients respond to dopaminergic therapy with improvement in visual field defects and/or reduction of tumour mass. In many reports the administered doses were low and the treatment period short. Based on the *in vitro* data, showing suppression of hormone production only when high concentrations of dopaminergic drugs are used for a long time, we hypothesized that long-term treatment with high doses would be needed to obtain tumour shrinkage. For this reason we chose the potent selective dopamine D2 agonist quinagolide (CV 205-502), in a final daily dose of 0.3 mg, comparable to 30 mg bromocriptine. In a preliminary study in five patients, treated for 12 to 18 months, promising results were obtained with tumour shrinkage in 1, and improvement in visual field defects in 2 patients [26]. Our long-term results in 10 patients do not confirm these observations. During the first year tumour volume decreased in 3 patients, but in 2 of them regrowth occurred after a few months. In 6 patients progressive increase in tumour size occurred despite sustained suppression of gonadotropin or α-subunit levels. In 2 of 3 patients with pre-existing visual field defects a slight improvement was shown during the first months of treatment, but eventually deterioration occurred in all 3 patients. One patient developed unilateral ophthalmoplegia during treatment. At the end of follow-up (median 56 months, range 36 to 92 months) tumour volume remained significantly below pretreatment volume in 2 patients, remained at pretreatment volume in 2 patients and was significantly increased in 6 patients.

In the absence of a control group it is difficult to determine whether quinagolide was effective in slowing down tumour growth or not. CNPA are usually slowly growing tumours. Recurrence rates after transsphenoidal surgery without radiation for CNPA have been reported to be between 12 and 22 % [30-32]. This is probably an underestimation, however, because of selection bias induced by the tendency to use radiotherapy in those patients with the most aggressive tumours and

because of loss to follow-up due to death to unrelated causes in this elderly patient-group. Moreover, the recurrence rates have been retrospectively determined on a clinical basis, by detection of new visual field defects or the presence of a clear increase in tumour size on CT or MRI imaging. No prospective studies, using meticulous measurement techniques of tumour volume, such as used in our study, have been reported.

A possible explanation for the discrepancy between the efficient suppression of hormone production by quinagolide and the disappointing effects on tumour volume could be the inclusion of tumors with a very low dopamine receptor density. This problem can now be approached by dopamine receptor scintigraphy with ^{123}I-IBZM or preferably with ^{123}I-epidepride [13-16]. These nuclear medical techniques can predict the suppressive effect of dopamine agonists on the growth of prolactinomas. In a recent study we demonstrated tumour shrinkage in 2 and tumour stabilisation in 1 of 3 ^{123}I-IBZM SPECT-positive CNPA patients treated with quinagolide, although in one of these patients an additional effect of octreotide could not be excluded [14]. In 1 of 4 patients without ^{123}I-IBZM uptake in the pituitary fossa tumour growth occurred under quinagolide therapy. Future long-term studies are needed to validate whether SPECT with ^{123}I-IBZM or ^{123}I-epidepride might be of assistance in selecting patients, who may potentially benefit from dopaminergic therapy.

Another explanation for the limited effects of quinagolide on tumour volume in our study could be that dopamine receptors on CNPA have only a small effect on hormone production. Dopamine receptors on normal pituitary tissue are coupled to the inhibition of both adenyl cyclase and the reduction of cytosolic free Ca^{2+} levels. Lania *et al.* showed that, while dopamine receptors in prolactinomas display these characteristics, in CNPA dopamine in pharmacological concentrations had no effect on cAMP production and was only able to reduce cytosolic free Ca^{2+} levels [33]. These data indicate that dopamine receptors of CNPA show a defective transduction mechanism, which could explain the weak inhibitory response to dopamine agonists. Our observation that quinagolide was able to induce a significant and permanent suppression of the serum concentrations of LH, FSH and α-subunit, without a favourable effect on tumour volume in most patients, makes this explanation less likely, however.

Another reason for the relatively low success rate of dopamine agonist therapy to induce tumour shrinkage might be that most CNPA only secrete very low amounts of glycoprotein hormones or subunits. Drugs that suppress hormone production only induce significant shrinkage of active endocrine cells containing a well-developed secretory apparatus [9,10]. Organelles involved in the synthesis and storage of hormones, such as the endoplasmatic reticulum and the secretory vesicles, make a substantially smaller contribution to the tumour volume in CNPA than in prolactinomas. In our study group no patients with adenomas hypersecreting gonadotropins or α-subunits were included, except one, in whom serum α-subunit levels were only moderately elevated. An improvement of visual field defects or a tumour size reduction during dopamine agonist treatment has been reported in a greater percentage of patients with pituitary adenomas that hypersecrete gonadotropins or α-subunits than in patients without apparent overproduction [11,20-24].

In conclusion: in patients with CNPA, long-term treatment with high doses of the dopamine agonist quinagolide induces a prolonged decrease in serum concentrations of gonadotropins and/or α-subunits. While this is accompanied by tumour shrinkage in some patients, progressive increase in tumour size occurs in most patients, despite persistent suppression of hormone secretion. Additional studies are needed to investigate whether subgroups of patients, e.g. those with positive dopamine receptor scintigraphy or those with marked hypersecretion of intact gonadotropins or subunits, will respond more favourably to treatment with dopamine agonists.

Acknowledgement

We are grateful to Miss K. Van Wessem who performed the tumour volume measurements.

References

1. Klibanski A. Nonsecreting pituitary tumors. Endocrinol Metab Clin North Am 1987;16:793-804.
2. Snyder PJ. Clinically nonfunctioning pituitary adenomas. Endocrinol Metab Clin North Am 1993;22:163-175.
3. Katznelson L, Alexander JM, Klibanski A. Clinical review 45. Clinically nonfunctioning pituitary adenomas. J Clin Endocrinol Metab 1993;76:1089-1094.
4. Lamberts SWJL, de Herder WW, van der Lely AJ, Hofland LJ. Imaging and medical management of clinically nonfunctioning pituitary tumors. The Endocrinologist 1995;5:3-6.
5. Snyder PJ. Extensive personal experience: gonadotroph adenomas. J Clin Endocrinol Metab 1995;80:1059-1061.
6. Snyder PJ, Bashey HM, Phillips JL, Gennarelli TA. Comparison of hormonal secretory behavior of gonadotroph cell adenomas in vivo and in culture. J Clin Endocrinol Metab 1985;61:1061-1065.
7. 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;62:1011-1019.
8. Kwekkeboom DJ, de Jong FH, Lamberts SWJ. Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas in vivo and in vitro: relation to sex and effects of TRH, GnRH and bromocriptine. J Clin Endocrinol Metab 1989;68:1128-1135.
9. 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. Endocrine Reviews 1988;9:417-436.
10. Lamberts SWJ, MacLeod RM. Regulation of prolactin secretion at the level of the lactotroph. Physiological Reviews 1990;70:279-318.
11. Bevan JS, Burke CW. Non-functioning pituitary adenomas do not regress during bromocriptine therapy but possess membrane-bound dopamine receptors which bind bromocriptine. Clin Endocrinol 1986;25:561-572.
12. Koya M, Nakao H, Arao M, Sato B, Noma K, Morimoto Y, Kishimoto S, Mori S, Uozumi T. Demonstration of specific dopamine receptors on human pituitary adenomas. Acta Endocrinol 1987;114:595-602.
13. Pirker W, Brucke T, Riedl M, Clodi M, Luger A, Asenbaum S, Podreka I, Deecke L. Iodine-123-IBZM-SPECT: studies in 15 patients with pituitary tumors. J Neural Transm Gen Sect 1994;97:235-244.
14. De Herder WW, Reijs AEM, Kwekkeboom DJ, Hofland LJ, Nobels FRE, Oei HY, Krenning EP, Lamberts SWJ. In vivo imaging of pituitary tumours using a radiolabelled dopamine D2 receptor radioligand. Clin Endocrinol 1996;45:755-767.

15. Pirker W, Riedl M, Luger A, Czech T, Rossler K, Adsenbaum S, Angelberger P, Kornhuber J, Deecke L, Podreka I, Brucke T. Dopamine D2 receptor imaging in pituitary adenomas using iodine-123-epidepride and SPECT. Eur J Nucl Med 1995;22:833.
16. de Herder WW, Reijs AEM, de Swart J, Kaandorp Y, Lamberts SWJ, Krenning EP, Kwekkeboom DJ. Comparison of iodine-123-epidepride and iodine-123-IBZM for dopamine D2 receptor imaging in clinically nonfunctioning pituitary macroadenomas and macroprolactinomas. Eur J Nucl Med 1999;26:46-50.
17. Lamberts SWJ, Verleun T, Oosterom R, Hofland L, van Ginkel LA, van Loeber JG, van Vroonhoven CCJ, Stefanko SZ, de Jong FH. The effects of bromocriptine, thyrotropin-releasing hormone, and gonadotropin-releasing hormone on hormone secretion by gonadotropin-secreting pituitary adenomas in vivo and in vitro. J Clin Endocrinol Metab 1987;64:524-530.
18. Klibanski A, Shupnik MA, Bikkal HA, Black PMcL, Kliman B, Zervas NT. Dopaminergic regulation of α-subunit secretion and messenger ribonucleic acid levels in α-secreting pituitary tumours. J Clin Endocrinol Metab 1988;65:96-102.
19. Kwekkeboom DJ, Hofland LJ, van Koetsveld PM, Singh R, van den Berge JH, Lamberts SWJ. Bromocriptine increasingly suppresses the in vitro gonadotropin and α-subunit release from pituitary adenomas during long term culture. J Clin Endocrinol Metab 1990;71:718-725.
20. Renner U, Mojto J, Lange M, Muller OA, von Werder K, Stalla GK. Effect of bromocriptine and SMS 201-995 on growth of human somatotrophic and non-functioning pituitary adenoma cells in vitro. Eur J Endocrinol 1994;130;80-91.
21. Klibanski A, Deutsch PJ, Jameson JL, Ridgway EC, Growley WF, Hsu DW, Habener JF, Black PMcL. Luteinizing hormone-secreting pituitary tumor: biosynthetic characterization and clinical studies. J Clin Endocrinol Metab 1987;64:536-542.
22. Vance ML, Ridgway BC, Thorner MO. Follicle-stimulating hormone- and α-subunit secreting pituitary tumor treated with bromocriptine. J Clin Endocrinol Metab 1985;61:580-584.
23. Wood DF, Johnston JM, Johnston DG. Dopamine, the dopamine D2 receptor and pituitary tumours. Clin Endocrinol 1991;35:455-466.
24. Bevan JS, Webster J, Burke CW, Scanlon WF. Dopamine agonists and pituitary tumor shrinkage. Endocr Rev 1992;13:220-240.
25. 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 hyperprolactinaemia. Clin Endocrinol 1990;32:565-571.
26. Kwekkeboom DJ, Lamberts SWJ. Long-term treatment with the dopamine agonist CV 205-502 of patients with a clinically nonfunctioning, gonadotroph, or α-subunit secreting pituitary adenoma. Clin Endocrinol 1992;36:171-176.
27. 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;33:777-782.

28. Petersen OF, Espersen JO. Extradural hematomas: measurement or size by volume summation on CT scanning. Neuroradiology 1984;26:363-367.
29. Duong DH, Rostomily RC, Haynor DR *et al.* Measurement of tumor resection volumes from computerized images. J Neurosurg 1992;77:151-154.
30. Ciric I, Mikhael M, Stafford T, Lawson L, Garces R. Transsphenoidal microsurgery of pituitary macroadenomas with long-term follow-up results. J Neurosurg 1983;59:395-401.
31. Ebersold MJ, Quast LM, Laws ER, Scheithauer B, Randall RV. Long-term results in transsphenoidal removal of nonfunctioning pituitary adenomas. J Neurosurg 1986;64:713-719.
32. Comtois R, Beauregard H, Somma M, Serri O, Aris-Jilwan N, Hardy J. The clinical and endocrine outcome to transsphenoidal microsurgery of nonsecreting pituitary adenomas. Cancer 1991;68:860-866.
33. Lania A, Reza-Elahi F, Gil-del-Alamo P, Saccomanno K, Mantovani S, Spada A. Abnormal transduction of dopamine signal in human nonfunctioning pituitary adenomas. J Endocrinol Invest 1995;18:265-70.

Chapter 8

In vivo imaging of pituitary tumours using a radiolabeled dopamine D2 receptor radioligand.

W.W. de Herder[1], A.E.M. Reijs[2], D.J. Kwekkeboom[2], L.J. Hofland[1], F.R.E. Nobels[1], H.Y. Oei[2], E.P. Krenning[2], S.W.J. Lamberts[1]

Departments of Medicine[1] and Nuclear Medicine[2], University Hospital Dijkzigt, Rotterdam, The Netherlands.

Clin Endocrinol 1996;45:755-767.

Summary

Knowledge of the dopamine D2 receptor status of pituitary tumours may play a predictive role in differential diagnosis and therapeutic decisions. This study was performed to evaluate the value of pituitary dopamine D2 receptor scintigraphy with (S)-2-hydroxy-3-^{123}I-iodo-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (^{123}I-IBZM) in the diagnostic evaluation of patients with pituitary tumours.

Scintigraphy using ^{123}I-IBZM was performed in 5 patients with PRL-secreting macroadenomas, 2 patients with PRL-secreting microadenomas, 17 patients with clinically nonfunctioning pituitary adenomas (NFPAs), 12 patients with GH-secreting adenomas and 1 patient with a TSH-secreting macroadenoma.

Single photon emission tomography (SPECT) showed significant uptake of ^{123}I-IBZM in the pituitary region in 3/5 macroprolactinoma patients. These results closely correlated with the response of plasma PRL levels to the dopamine D2 receptor agonist quinagolide. In two scan-positive prolactinoma patients, repeated SPECTs during therapy with quinagolide showed a reduction in the pituitary uptake of ^{123}I-IBZM. Pituitary SPECT was negative in the 2 microprolactinoma patients, who responded to quinagolide administration. In 4/17 patients with NFPA, significant uptake of the radioligand in the pituitary region was observed. In 2/3 scan-positive NFPA patients, who were treated with quinagolide, shrinkage of the pituitary tumours was observed. Treatment with quinagolide resulted in stabilisation of tumour growth in the other scan-positive patients. Four out of 17 patients with NFPA and a negative SPECT were treated with quinagolide. Tumour growth was observed in 1 patient, and tumour size did not change in the other 3 patients. The pituitary region of none of the 12 acromegaly patients showed significant uptake of ^{123}I-IBZM. Sensitivity of the GH-secreting adenomas to quinagolide was demonstrated in 8/12 patients *in vivo* by an acute test, and in 6/9 of the tumours *in vitro*. Pituitary SPECT was negative in the patient with the TSH-secreting macroadenoma and this tumour also showed no sensitivity to quinagolide *in vivo* and *in vitro*.

We conclude that ^{123}I-IBZM is a ligand for *in vivo* imaging of dopamine agonist-sensitive macroprolactinomas, but not microprolactinomas or GH-secreting adenomas. The technique potentially provides a means of predicting dopamine agonist-responses of NFPAs *in vivo*.

Introduction

Pituitary PRL secretion is under the tonic inhibitory control of dopamine secreted from the hypothalamus via stimulation of the membrane-bound dopamine D2 receptors on the lactotrophs [1]. Several dopamine D2 receptor agonists are available for first-line medical therapy of prolactinomas. These drugs successfully control pathological tumoral PRL secretion in the majority of patients with pituitary micro- and macroprolactinomas. This is accompanied by tumour shrinkage in approximately 90% of patients [1]. Resistance of prolactinomas to dopamine D2 agonists may result from a reduced dopamine D2 receptor density on the tumours, or from post-receptor defects [2,3]. Persistently elevated PRL levels in prolactinoma patients on dopaminergic therapy are caused by dopamine resistance or are the consequence of lack of compliance with medical therapy. The clinical differentiation between these two conditions is often difficult.

A substantial number of clinically nonfunctioning pituitary adenomas (NFPA) and gonadotroph pituitary adenomas also possess dopamine D2 receptors [4,5,6]. Preliminary evidence demonstrates that dopamine D2 agonists suppress *in vivo* and *in vitro* the secretion by these tumours of gonadotrophins or their subunits while in exceptional cases the growth of these tumours may also be restrained [7,8].

In most patients with GH-secreting pituitary adenomas, D2 agonist therapy successfully lowers plasma GH levels. However, long-term normalization of plasma IGF-I concentrations and significant tumour shrinkage with this therapy have been achieved in only a few cases [9].

Variable effects of dopamine agonists in thyrotrophin TSH-secreting tumours have been reported, and suppression of the inappropriate TSH secretion by these agents has been demonstrated in a few cases [10,11].

(*S*)-2-Hydroxy-3-^{123}I-iodo-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl] benzamide (^{123}I-IBZM) is a currently available radioligand for the imaging of dopamine D2 receptors *in vivo* by single-photon emission tomography (SPECT). The purpose of the present study was to evaluate the

value of ^{123}I-IBZM SPECT in the diagnostic evaluation and treatment of patients with pituitary tumours. Scintigraphy was also repeated in 4 patients who were treated with the dopamine D2 receptor agonist quinagolide.

Subjects and methods

Patients

Patients' characteristics are shown in Tables 1-3. Five patients with newly diagnosed or recurrent PRL-secreting macroadenomas (tumour diameter > 10 mm), 2 patients with newly diagnosed PRL-secreting microadenomas (tumour diameter < 10 mm), 17 patients with newly diagnosed or recurrent NFPA, 9 patients with newly diagnosed GH-secreting macroadenomas, 3 patients with newly diagnosed GH-secreting microadenomas and 1 patient with a TSH-secreting macroadenoma were studied. All pituitary tumours were documented by nuclear magnetic resonance imaging (MRI, 0.5 T, Philips Gyroscan, Best, The Netherlands). T1-weighted, 3-mm-thick sagital and coronal spin-echo images were obtained native and after gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) enhancement. Tumour volume was calculated according to the formula of Di Chiro and Nelson (volume = 1/2 x height x length x width; range normal sellar volume: 240 - 1092 mm^3, mean sellar volume: 240 mm^3) [12]. Tumours containing > 30% cystic or necrotic areas on MRI were excluded from the study.

The diagnosis of a macroprolactinoma was made in patients with the typical clinical symptoms of galactorrhoea, secondary amenorrhoea and/or loss of libido, together with considerably elevated serum PRL levels (> 200 µg/l (6000 mU/l), normal < 12 µg/l (360 mU/l) in males, < 15 µg/l (450 mU/l) in females), and the presence of a pituitary tumour with a diameter > 10 mm on MRI. The diagnosis of a microprolactinoma was made in patients with the same clinical symptoms with PRL levels < 100 µg/l (3000 mU/l) and the presence of a pituitary hypodensity with a diameter < 10 mm on a Gd-DTPA enhanced MR image. A dopamine agonist-induced reduction in the volume of this hypodense area

Table 1. Patient characteristics of 7 prolactinoma patients, who underwent SPECT with ^{123}I-IBZM.

Patient number	Sex (M/F)	Age (years)	Tumour size	Tumour volume (mm^3)	Pituitary IBZM uptake coronal SPECT*	Previous treatment	Response tumour size to quinagolide *in vivo*†	Long-term hormonal response to quinagolide *in vivo* (µg/l)ǂ PRL off	PRLon	Hormonal response to quinagolide *in vitro*§
1	M	42	Ma	37798	3	none	<	2730	15.1	ND
2	F	57	Ma	4636	3	none	<	2791	3.1	ND
3	F	50	Ma	15933	0	TNH-RT	>	3117	2998	PRL: 32%<
4	F	39	Ma	2775	0	none	>	492	499	ND
5	F	22	Ma	21114	3	TFH-RT	=	291	16	ND
6	F	18	Mi	114	0	none	<	33	9.1	ND
7	F	29	Mi	134	0	none	<	42	8.5	ND

Ma, Macroadenoma; Mi, microadenoma; PRLoff, serum PRL level without therapy; PRLon, serum PRL level during therapy with quinagolide; TNH-RT, trans-nasal hypophysectomy followed by external pituitary irradiation; TFH-RT; trans-frontal hypophysectomy followed by external pituitary irradiation.

* 0, no uptake; 3, isointense with basal ganglia.

† =, unchanged; < decrease; > increase.

ǂ Normal values PRL: M < 12 µg/l, F < 15 µg/l, 1 µg/l = 30 mU/l.

§ Decrease expressed as percentage of controls; ND, not determined.

Table 2. Patient characteristics of 17 patients with clinically nonfunctioning pituitary macroadenomas, who underwent SPECT with ^{123}I-IBZM

Patient number	Sex (M/F)	Age (years)	Tumour volume (mm^3)	Pituitary IBZM uptake coronal SPECT*	Previous treatment	Initial therapy	Follow-up therapy	Immuno-histology	Response tumour size to quinagolide *in vivo*†	Hormonal response to quinagolide *in vitro*‡
8	F	36	14090	0	none	quinagolide	TFH-RT	null cell adenoma	>	ND
9	M	47	7733	2	TNH-RT	none		sparsely granulated PRL cell adenoma	ND	ND
10	F	60	3145	0	none	TNH-RT		gonadotroph cell adenoma	ND	α-SU: 48%< FSH: 30%<
11	M	33	64660	2	none	quinagolide		sparsely granulated ACTH cell adenoma§	<	ND
12	F	71	3254	0	none	none		ND	ND	ND
13	F	54	10296	3	TFH-RT	quinagolide		sparsely granulated GH cell adenoma	=	ND
14	F	34	1908	0	none	quinagolide		ND	=	ND
15	F	77	2775	1	TNH-RT	none		gonadotroph cell adenoma	ND	ND
16	F	60	1580	0	none	quinagolide		ND	ND	ND
17	M	45	9970	0	none	quinagolide		ND	=	ND
18	F	51	1952	0	none	quinagolide		ND	ND	ND
19	M	70	2400	0	none	quinagolide		ND	ND	ND
20	M	46	5339	0	none	TNH-RT		null cell adenoma	ND	No FSH, no LH, no α-SU production
21	M	70	14267	0	TNH (1)-RT	TNH (2)		null cell adenoma	ND	No FSH, no LH, no α-SU production
22	F	27	1715	0	none	none	TNH	gonadotroph cell adenoma	ND	ND
23	M	60	5300	0	TNH (1)-RT	TNH (2)		null cell adenoma	ND	ND
24	M	32	35478	2	none	quinagolide + octreotide		null cell adenoma	<	No FSH, no LH, no αSU production

TNH-RT, trans-nasal hypophysectomy followed by external pituitary irradiation; TFH-RT; trans-frontal hypophysectomy followed by external pituitary irradiation; TNH, trans-nasal hypophysectomy; ND, not determined; α-SU, α-subunit.

* 0, no uptake; 1, less intense than brain; 2, isointense with brain; 3, isointense with basal ganglia.

† =, unchanged; < decrease; > increase.

‡ Decrease expressed as percentage of controls. § Tumour biopsy.

Table 3. Patient characteristics of 12 patients with acromegaly, who underwent SPECT with ^{123}I-IBZM.

Patient number	Sex (M/F)	Age (years)	Tumour size	Tumour volume (mm^3)	Acute GH response to quinagolide *in vivo** (%)	GH response to quinagolide *in vitro** (%)
25	F	58	Ma	4560	50<	80<
26	F	69	Mi	175	20<	ND
27	F	53	Ma	2782	no response	ND
28	M	55	Mi	186	64<	41<
29	M	52	Ma	1308	62<	61<
30	M	63	Mi	194	52<	ND
31	M	58	Ma	1443	10<	No<
32	M	34	Ma	4250	41<	15<
33	F	47	Ma	5270	60<	69<
34	M	43	Ma	4919	78<	56<
35	M	32	Ma	6480	45<	70<
36	F	43	Ma	1545	19<	No<

Ma, Macroadenoma; Mi, microadenoma; ND, not determined.
* Decrease expressed as percentage of controls.

together with a reduction or normalization of serum PRL levels was considered substantial evidence for the diagnosis of microprolactinoma.

Acromegaly was diagnosed in patients with typical clinical signs and symptoms, together with persistently elevated GH levels measured over a 24-h period, elevated plasma IGF-I (> 45 nmol/l) and IGFBP-3 concentrations (> 4.8 mg/l) and a pituitary tumour on MRI. In all patients, immuno-histology on a tumour specimen confirmed the clinical diagnosis.

The diagnosis of a NFPA was based on the detection of a pituitary adenoma on MRI, the absence of a characteristic clinical syndrome apart from hypopituitarism, basal low to normal LH, FSH and glycoprotein hormone α-subunit (α-SU) levels. A slightly elevated PRL concentration (< 100 μg/l (< 3000 mU/l)), which was not in accordance with the tumour size, was interpreted as caused by pituitary stalk compression.

In these patients, no elevation of IGF-I (< 45 nmol/l) or IGFBP-3 (< 4.8 mg/l) levels were found. In 11 patients, the diagnosis was confirmed by (immuno-)histology on tumour specimens, which were obtained at surgery (see Tab. 2).

The diagnosis of a TSH-secreting macroadenoma was made on the basis of detectable TSH levels in the face of high free thyroid hormone concentrations (TSH 2.49 mU/l, T_4 221 nmol/l (normal: 64-132), FT_4 48.7 pmol/l (normal: 11-25), T_3 5.02 nmol/l (normal: 1.35-2.59)), a pituitary lesion at neuroradiology and the clinical picture of hyperthyroidism. In this patient no evidence was found for hypersecretion of PRL, GH, LH, FSH, or ACTH. The TSH response to TRH was absent and the serum levels of SHBG and α-subunit were elevated. In this patient the diagnosis was later confirmed by immuno-histology on a tumour specimen obtained at surgery.

If appropriate, patients received replacement therapy for insufficiencies of the pituitary-adrenal, -thyroidal, or -gonadal axes.

^{123}I-IBZM-SPECT

All patients gave informed consent for the study, which was accepted by the ethics committee of the University Hospital Rotterdam.

Prior to the ^{123}I-IBZM examination, the uptake of free ^{123}I by the thyroid gland was blocked with potassium iodide.

^{123}I-IBZM (Cygne BV, Eindhoven, the Netherlands) (185 MBq) was administered as an i.v. bolus. Gamma-camera images of the head were obtained using single-photon emission computed tomography (SPECT; 64 x 64 matrix) technique using a three-headed gamma-camera (fitted with medium energy collimators) (Picker 3000, Picker Int., Cleveland, Ohio, USA). Peak setting at 159 keV with 20% window. Acquisition parameters for 10 SPECT studies were 60 projections of 10 s each. Images were obtained 90 minutes post injection. Before reconstruction, the 10 SPECT studies were summated. Scintigrams were assessed independently without knowledge of the results of MRI by three observers (WWdeH, AR, DJK). The uptake of radioactivity in the pituitary area was

compared to that in the left and right basal ganglia and in the cerebral cortex. Absence of pituitary uptake of radioactivity was classified as grade 0. Uptake in this region equal to that observed for the basal ganglia was classified as grade 3. Uptake in the pituitary region which equalled that of the cerebral cortex was classified as grade 2. Slight to doubtful uptake in the pituitary region was classified as grade 1. Examples of grade 3, 2 and 0 scans are given in the Figures 1a and 2a, 1c and 2c. The uptake of radioactivity in the pituitary area was also established as an uptake index (UI). For this purpose, circular regions of interest were placed on the pituitary area and on the basal ganglia areas (see Fig. 3). The radioactivities were expressed as counts per pixel. The UI was determined as the ratio of the pituitary activity over the total of the activities in the basal ganglia. Grade 3 scans corresponded to an UI > 0.80, grade 2 scans corresponded to an UI ranging from 0.75 to 0.80, grade 1 scans corresponded to an UI ranging from 0.65 to 0.75, and grade 0 scans corresponded to an UI < 0.65. The inter- and intraobserver variability in the UI was less than 5%.

^{123}I-IBZM SPECT was repeated during therapy with the dopamine D2 receptor agonist quinagolide (Norprolac, Sandoz, Basel, Switzerland) in 4 patients with initially positive scintigraphic findings (patients 1, 2, 11 and 13, Tab. 1 and 2).

In vitro studies

The clinical diagnosis was histologically confirmed in 23 tumour specimens.

Single-cell suspensions of the adenomas were prepared by enzymatic dissociation with dispase, as has been described in detail previously [13]. The cells were plated at a density of 10^5 cells per well (GH-secreting adenomas and prolactinomas), or 2 x 10^5 cells per well (NFPAs) in 1 ml of culture medium. After 3 days of culture, the medium was changed and 72-h incubations with or without 10 nM quinagolide were performed in quadruplicate. Hormone concentrations in the media were determined as described in detail previously [7,13]. The culture medium consisted of Minimal Essential Medium supplemented with non-essential amino acids, sodium pyruvate (1 mmol/l), 10% fetal calf serum, L-glutamate (2 mmol/l), sodium bicarbonate (2.2 g/l) and antibiotics.

Figure 1. 42-year-old male patient with a macroprolactinoma (patient 1, Table 1), before therapy with quinagolide.
a. ^{123}I-IBZM SPECT of the brain, coronal section showing the intense (grade 3) uptake of radiolabel in the pituitary tumour and in the basal ganglia.
b. Contrast-enhanced coronal magnetic resonance image of the sella, showing the macroadenoma.
The same patient, being treated with 0.3 mg quinagolide for 1 year.
c. ^{123}I-IBZM SPECT of the brain, coronal section showing the reduced (grade 2) uptake of radiolabel in the pituitary tumour as compared to figure 1a.
d. Contrast-enhanced coronal magnetic resonance image of the sella, showing reduction in tumour size as compared to figure 1b.

Figure 1a.

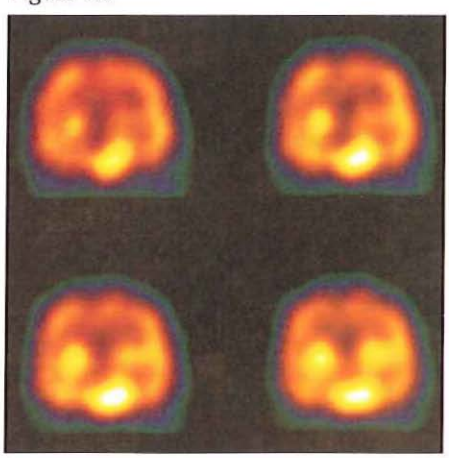


Figure 1b.

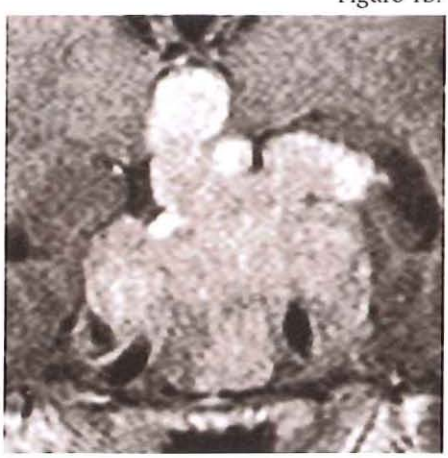


Figure 1c.

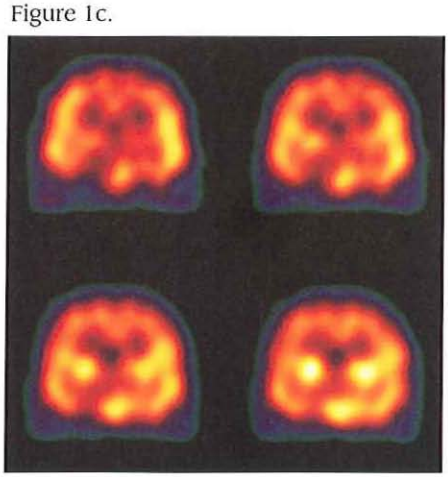


Figure 1d.

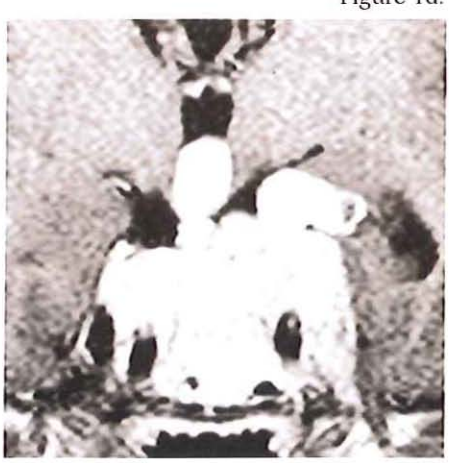


Figure 2. 57-year-old female patient with a macroprolactinoma (patient 2, Table 1), before therapy with quinagolide.

a. ^{123}I-IBZM SPECT of the brain, coronal section showing the intense (grade 3) uptake of radiolabel in the pituitary tumour and in the basal ganglia.

b. Contrast-enhanced coronal magnetic resonance image of the sella, showing the macroadenoma.

The same patient, being treated with 0.3 mg quinagolide for 12 months.

c. ^{123}I-IBZM SPECT of the brain, coronal section showing that the uptake of the radiolabel in the pituitary tumour has completely disappeared (grade 0)

d. Contrast-enhanced coronal magnetic resonance image of the pituitary, showing reduction in tumour size as compared to figure 1b.

Figure 2a.

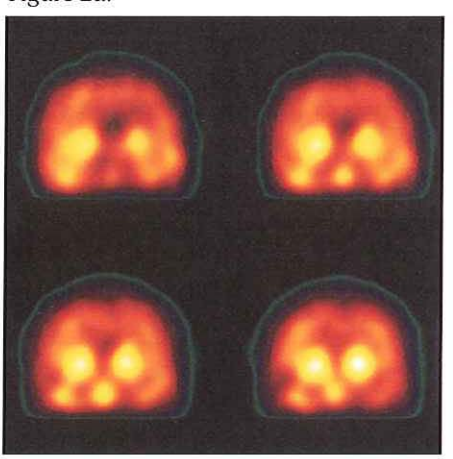


Figure 2b.

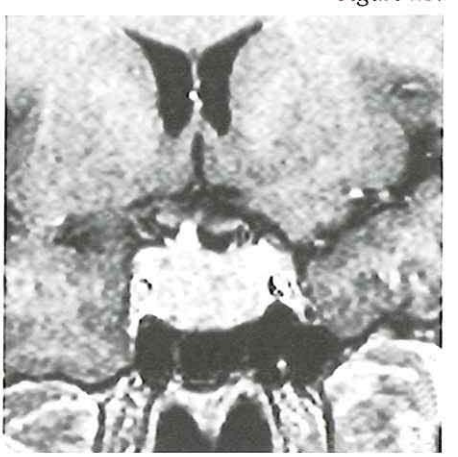


Figure 2c.

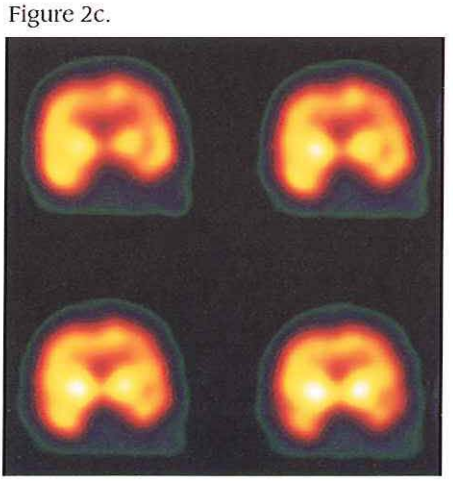
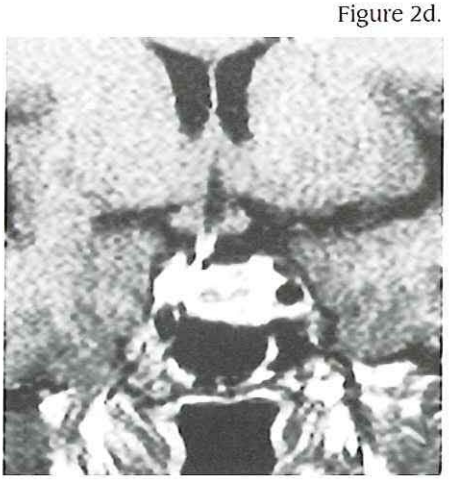


Figure 2d.

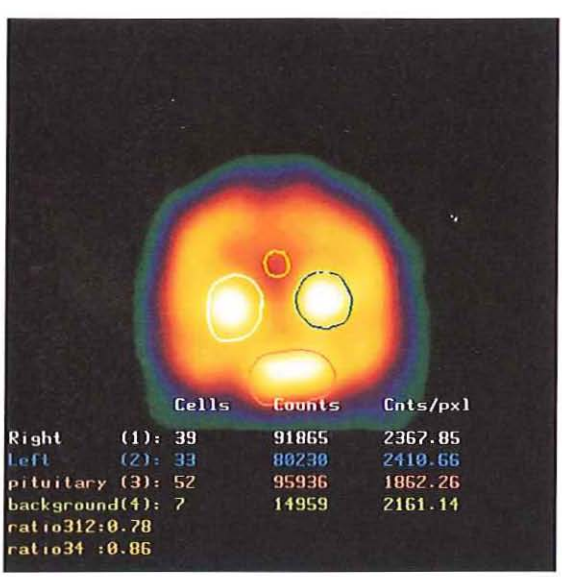


In vivo studies

All prolactinoma patients were treated with quinagolide in a final dosage of 0.3 mg daily. Serum PRL levels were evaluated every 3 months and the evolution of tumour size was evaluated by MRI. One patient (patient 4, Tab. 1) was operated upon because of resistance to quinagolide, which was demonstrated by persistently elevated PRL levels during therapy and tumour growth as demonstrated on MRI. Two other patients (patients 3 and 5, Tab. 1) had already been operated upon in the past.

Nine out of 17 NFPA patients underwent pituitary surgery and 8 of them subsequently received external pituitary radiotherapy. Two patients were reoperated. SPECT studies were performed before surgery in 4 patients and after surgery in 5 patients (Tab. 2). Nine NFPA patients were treated with quinagolide in a final dosage of 0.3 mg daily (Tab. 2). Plasma concentrations of LH, FSH and α-SU were evaluated every 3 months and the evolution of tumour size was measured by MRI.

Figure 3. Example of an uptake index calculation in a patient with a grade 2 IBZM SPECT (patient 24, Table 2).



(1) = right basal ganglia, (2) = left basal ganglia, (3) = pituitary, (4) = background

All patients with acromegaly were primarily operated upon. None of these patients was additionally treated with quinagolide, but the acute reaction of GH to a single dose of 0.075 mg of this drug was investigated preoperatively. On the first day, serum was sampled for GH from an indwelling venous catheter at hourly intervals from 08.00 to 20.00 h. On the second day, 0.075 mg quinagolide was given orally at 08.00 h and the sampling protocol was repeated. GH levels were analyzed as area under the curve (AUC) values, and the percentage decrease of GH after quinagolide was calculated from the AUCs obtained on the control and test days.
In the patient with the TSH-secreting macroadenoma, the acute reaction of TSH to a single dose of 0.075 mg of quinagolide was investigated following the same procedure as in the patients with acromegaly. After transsphenoidal debulking of the tumour, this patient was treated with octreotide and external pituitary radiotherapy.

The clinical decisions concerning surgery, radiotherapy and/or medical therapy in all 37 patients had not been influenced at any time by the results of the ^{123}I-IBZM SPECT studies, which were not known to the attending physician(s).

Results

Prolactinomas

^{123}I-IBZM SPECT showed significant (grades 2 and 3) uptake in the pituitary region in 3 out of 5 macroprolactinoma patients. In these patients, a significant positive correlation ($P < 0.001$, Fisher's exact test) was found between the results of the *in vivo* scintigraphy and the *in vivo* response of PRL release by the tumours to quinagolide (Tab. 1).

In two ^{123}I-IBZM-positive patients (patients 1 and 2, Tab. 1), scintigraphy was repeated during treatment with 0.3 mg quinagolide. In patient 1, the uptake of radioactivity in the basal ganglia and cerebral cortex had remained unchanged, while the pituitary uptake had been reduced from grade 3 to 2 after 10 months therapy. MRI demonstrated shrinkage of

the macroprolactinoma by 33% after 8 months therapy. Tumour size stabilized in the following 12 months. In patient 2, no change in the uptake of ^{123}I-IBZM was observed for the basal ganglia and cerebral cortex, while the uptake in the pituitary region (grade 3 before the start of quinagolide) had disappeared (grade 0) after 12 months therapy. MRI demonstrated shrinkage of the macroprolactinoma by 56% after a 6 months therapy and by 78% after 18 months. Neither of the 2 microprolactinoma patients showed uptake of the radioactivity in the pituitary region, although suppression of the pathological PRL levels to < 10 ug/l and disappearance of the tumours followed treatment with quinagolide in these patients.

NFPA

In 4 of the 17 patients with NFPA, significant uptake (grades 2 and 3) of the radioligand in the pituitary region was observed. In one ^{123}I-IBZM-positive patient (patient 11, Tab. 2), treatment consisted of quinagolide in a final dosage of 0.3 mg. In another scan-positive patient (patient 13, Tab. 2), quinagolide therapy (final dosage of 0.3 mg) was started 24 years after transfrontal surgery and external pituitary radiotherapy, because MRI indicated progressive growth of a perisellar tumour remnant to a large macroadenoma with extensive invasion in the surrounding tissues. In both patients, scintigraphy was repeated during treatment with 0.3 mg quinagolide. In patient 11, the uptake of radioactivity in the basal ganglia and cerebral cortex had remained unchanged after 6 months therapy, while the uptake in the pituitary region was slightly reduced, without change in the grade (grade 2). MRI demonstrated shrinkage of the pituitary tumour by 20% after 6 months therapy. Tumour size stabilized in the following 10 months. In patient 13, the uptake in the basal ganglia, cerebral cortex and the pituitary region had remained unchanged (grade 3) after 6 months therapy. MRI demonstrated stabilisation of tumour growth. In patient 24, follow-up MRI examination after 3 months therapy with quinagolide (0.3 mg per day) and octreotide (0.3 mg per day) demonstrated shrinkage of the pituitary tumour by 20%, which was accompanied by the almost complete disappearance of visual field defects. In three other patients with NFPA (patients 14, 17 and 18, Tab. 2), long-term therapy with quinagolide (0.3 mg per day for 12, 18 and 12 months, respectively) did not result in a

change in the pituitary tumour size. SPECT with ^{123}I-IBZM did not show significant (> grade 1) uptake of radioactivity in the pituitary fossa of these three patients. In one patient with NFPA and a negative SPECT (patient 8, Tab. 2), tumour growth occurred after 9 months of quinagolide therapy (0.3 mg per day), and the patient subsequently had to undergo pituitary surgery.

Acromegaly

None of the pituitary regions of the 12 acromegaly patients investigated showed significant uptake of ^{123}I-IBZM. In 8 of these patients, however, a more than 20% reduction of the areas under the curves for GH was observed after a single dose of 0.075 mg quinagolide in comparison with the levels on the control day (Tab. 3). In 6 of 9 patients whose tumour cells were studied *in vitro*, sensitivity of GH release to quinagolide was confirmed (Tab. 3). There was a statistically significant relation between the response of GH secretion to quinagolide *in vivo* and *in vitro* in these 9 acromegalic tumours ($P < 0.05$, Fisher's exact test).

TSH-secreting macroadenoma

Pituitary SPECT was negative in the patient with the TSH-secreting macroadenoma. In this patient no reduction of the areas under the curves for TSH was observed after a single dose of 0.075 mg quinagolide in comparison with the levels after placebo administration. Also, tumour cells *in vitro* showed no sensitivity to quinagolide of TSH release.

Discussion

Dopamine D2 receptors can be successfully visualized on prolactinomas and somatotroph adenomas using positron emission tomography (PET) with ^{11}C labelled dopamine D2 antagonists and agonists [14-17]. However, the installation and maintenance of PET equipment is expensive and this technique is available in only a limited number of centres throughout the world.

Experience with SPECT with ^{123}I-IBZM in patients with pituitary tumours is also limited, although this technique in principle can be performed in a larger number of centres. In a study by Assies *et al.*, SPECT with ^{123}I-IBZM failed to visualize a primary malignant pituitary macroprolactinoma, whereas low uptake of this radioligand was found in one intracranial metastasis [18]. This metastatic tumour showed only a partial clinical response to dopaminergic therapy *in vivo* [18]. Pirker *et al.* performed SPECT with ^{123}I-IBZM in 15 patients with a variety of pituitary tumours [19]. Only one of two dopamine D2 agonist-responsive macroprolactinomas could be visualized and three dopamine D2 agonist-resistant macroprolactinomas were negative on the scan. Nomikos *et al.* compared the results of ^{123}I-IBZM SPECT in dopamine (D2) agonist-resistant patients and in responsive prolactinoma patients [20]. In 5 of 12 dopamine (D2) agonist-resistant patients ^{123}I-IBZM SPECT was negative. All 5 dopamine (D2)-responsive patients showed uptake of the radioligand in the pituitary region [20]. Scillitani *et al.* have performed ^{123}I-IBZM SPECT in one bromocriptine-resistant macroprolactinoma and in one bromocriptine-responsive macroprolactinoma [21]. They showed that the uptake of the radioligand was consistent with the *in vivo* response of the tumour to bromocriptine.

In our study, 3 of 5 patients with macroprolactinomas showed high (grade 3) uptake of ^{123}I-IBZM. Two patients showed no pituitary uptake of the radioligand and also failed to show a significant PRL-lowering effect in response to high doses (0.3 mg per day) of quinagolide. Resistance of the tumour to quinagolide was also demonstrated *in vitro* in one of these patients. Pellegrini *et al.* have also shown that the density of dopamine D2 receptors was significantly reduced in bromocriptine-resistant prolactinomas as compared to dopamine-agonist-sensitive prolactinomas [3]. However, most physicians will generally start dopaminergic therapy, as resistance of prolactinomas to dopamine agonists is rare. In our studies, ^{123}I-IBZM SPECT of the pituitary region was negative in both dopamine agonist-sensitive microprolactinomas. Therefore, the sensitivity of ^{123}I-IBZM SPECT is probably too low to visualize adenomas with a diameter < 10 mm. We have repeated the SPECT studies in two patients with dopamine agonist-sensitive macroprolactinomas. The uptake of radioactivity in the pituitary region had been reduced in

one patient, whereas it had disappeared in the other. This might be explained by therapy-induced tumour shrinkage and fibrosis, or decreased viability [22].

NFPAs represent about one-quarter of all pituitary adenomas. The surgical removal of these tumours is often incomplete, and at present there is no established medical treatment [4,5,6]. In some cases, the clinical distinction of these tumours from other nonendocrine tumours in the pituitary region is difficult. The effect of long-term dopaminergic therapy on the size of NFPA has so far been investigated only in small series [7,8]. Clinical evaluation of the efficacy of dopaminergic therapy is difficult because of the absence of reliable hormonal markers and the fact that it is impossible to select those NFPA patients with dopamine D2 receptor-positive tumours. In general, NFPA show a lower dopamine D2 receptor density than PRL-secreting adenomas [23-26]. Future studies are needed to validate whether SPECT with ^{123}I-IBZM might be of assistance in the differential diagnosis of tumours in the pituitary region. Ideally, a positive SPECT would then point to the presence of a pituitary adenoma. In patients with NFPA, *in vivo* scintigraphy with ^{123}I-IBZM may be used for the selection of patients, who may potentially benefit from dopaminergic therapy. Three of our SPECT-positive NFPA patients responded to dopaminergic therapy with tumour shrinkage, although in one of these patients an (additional) effect of octreotide treatment can not be excluded.

In our series, none of the GH-secreting pituitary tumours could be visualized on ^{123}I-IBZM SPECT, although some of these tumours showed a significant acute GH response to quinagolide *in vivo* and *in vitro*. The long-term response to dopaminergic treatment has not been studied in these patients. Although dopaminergic treatment successfully lowers plasma GH in acromegalic patients, normalization of plasma IGF-I and a significant reduction in tumour size is achieved in only a few cases [9]. As in NFPA, lower dopamine D2 receptor densities have been demonstrated on GH-secreting adenomas as compared to PRL-secreting adenomas [23,24,28,29]. As most tumours studied were macroadenomas, the results are obviously not hampered by the small size of the tumours or the limited spatial resolution of SPECT. However, tumour size can not

be excluded as a determinant for uptake, as all SPECT-positive NFPA's were larger than the GH-secreting macroadenomas. The findings implicate that in our patients with acromegaly ^{123}I-IBZM SPECT was not sensitive enough to visualize GH-secreting pituitary tumours.

Verhoef *et al.* reported on the successful visualization of a mixed TSH-, α-SU- and PRL-secreting pituitary macroadenoma by SPECT with ^{123}I-IBZM [11]. An acute response to the dopamine agonist bromocriptine could be demonstrated in this patient, but long-term treatment with this drug did not induce significant tumourshrinkage. However, in our patient with the TSH- (and α-SU-) secreting tumour, the SPECT studies were negative and we could not demonstrate an acute *in vivo*, or *in vitro* response of the tumour to quinagolide.

From our studies it can not be certainly concluded that dopamine D2 receptor binding has been determined by SPECT with ^{123}I-IBZM, because repeat studies with adequate receptor blocking to determine specific binding were not feasible. Therefore, variability in the pituitary uptake might have been to a variable degree related to non-specific binding. It is also not possible to use the uptake in the cortex as an indication of non-specific binding, because this can be influenced by variations in tissue composition, flow and other parameters. However, the close correlation between the results of the SPECT studies and the PRL response to quinagolide in the macroprolactinoma patients suggests that we were indeed looking at specific tracer uptake.

In conclusion, ^{123}I-IBZM is a ligand for *in vivo* imaging of dopamine agonist-sensitive macroprolactinomas. Future studies are needed to determine the usefulness of this technique in the differential diagnosis of tumours in the pituitary region and for the prediction of dopamine D2 agonist-responses of NFPA *in vivo*. In our hands, the technique is not sensitive enough for the visualization of microprolactinomas and somatotroph adenomas. However, higher sensitivities might be obtained with other recently developed ligands like [^{123}I]-epidepride [30].

References

1. Molitch ME. Pathologic hyperprolactinemia. Endocrinol Metab Clin N Am 1992;21:877-901.
2. Muhr C, Lundberg PO, Antoni G, Bergstrom K, Hartvig P, Lundqvist H, Langstrom B, Stalnacke C-G. Assies J, Verhoeff NP, Bosch DA, Hofland LJ. Intracranial dissemination of a macroprolactinoma. Clin Endocrinol 1993;38:539-546.
3. Pellegrini I, Rasolonjanahary R, Gunz G, Bertrand P, Delivet S, Jedynak CP, Kordon C, Peillon F, Jaquet P, Enjalbert A. Resistance to bromocriptine in prolactinomas. J Clin Endocrinol Metab 1989;69:500-509.
4. Brue T, Pellegrini I, Gunz G, Morange I, Dewailly D, Brownell J, Enjalbert A, Jaquet P. Effects of the dopamine agonist CV 205-502 in human prolactinomas resistant to bromocriptine. J Clin Endocrinol Metab 1992;74:577-584.
5. Snyder PJ. Clinically nonfunctioning pituitary adenomas. Endocrinol Metab Clin N Am 1993;22:163-175.
6. Snyder PJ Extensive personal experience: gonadotroph adenomas. J Clin Endocrinol Metab 1995;80:1059-1061.
7. Samuels MH. Gonadotroph adenomas. Curr Ther Endocrinol Metab 1994;5:52-56.
8. Kwekkeboom DJ, de Jong FH, Lamberts SW. Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas in vivo and in vitro: relation to sex and effects of thyrotropin-releasing hormone, gonadotropin-releasing hormone, and bromocriptine. J Clin Endocrinol Metab 1989;68: 1128-1135.
9. Kwekkeboom DJ, Lamberts SW. Long-term treatment with the dopamine agonist CV 205-502 of patients with a clinically non-functioning, gonadotroph, or alpha-subunit secreting pituitary adenoma. Clin Endocrinol 1992;36:171-176.
10. Jaffe CA, Barkan AL. Treatment of acromegaly with dopamine agonists. Endocrinol Metab Clin N Am 1992;21:713-735.
11. Karlsson FA, Burman P, Kampe O, Westlin JE, Wide L. Large somatostatin-insensitive thyrotrophin-secreting pituitary tumour responsive to D-thyroxine and dopamine agonists. Acta Endocrinol Copenh 1993;129:291-295.
12. Verhoeff NP, Bemelman FJ, Wiersinga WM, van Royen EA. Imaging of dopamine D2 and somatostatin receptors in vivo using single-photon emission tomography in a patient with a TSH/PRL-producing pituitary macroadenoma. Eur J Nucl Med 1993;20:555-561.
13. Di Chiro G, Nelson KB. The volume of the sella turcica. Am J Roentgenol 1962;87:989-1008.
14. Hofland LJ, van Koetsveld PM, Verleun TM, Lamberts SW. Glycoprotein hormone alpha-subunit and prolactin release by cultured pituitary adenoma cells from acromegalic patients: correlation with GH release. Clin Endocrinol 1989;30:601-611.

15. Muhr C, Lundberg PO, Antoni G, Bergstrom K, Hartvig P, Lundqvist H, Langstrom B, Stalnacke C-G. The uptake of 11C-labelled bromocriptine and methionine in pituitary tumors studied by positron emission tomography. In Trends in the diagnosis and treatment of pituitary adenomas (eds SWJ Lamberts, FJH Tilders, EA van der Veen, J Assies), 1984;151-155. Free university press, Amsterdam.
16. Muhr C, Bergstrom M, Lundbewg PO, Bergstrom K, Hartvig P, Lundqvist H, Antoni G, Langstrom B. Dopamine receptors in pituitary adenomas: PET visualization with 11C-N-methylspiperone. J Comput Ass Tomogr 1986;10:175-180.
17. Muhr C, Bergstrom M, Lundberg PO, Bergstrom K, Langstrom B. In vivo measurement of dopamine receptors in pituitary adenomas using positron emission tomography. Acta Radiol Suppl. 1986;369: 406-408.
18. Bergstrom M, Muhr C, Lundberg PO, Langstrom B. PET as a tool in the clinical evaluation of pituitary adenomas. J Nucl Med 1991;32:610-615.
19. Pirker W, Brucke T, Riedl M, Clodi M, Luger A, Asenbaum S, Podreka I, Deecke L. Iodine-123-IBZM-SPECT: studies in 15 patients with pituitary tumors. J Neur Transm Gen Sect 1994;97:235-244.
20. Nomikos P, Honegger J, Becker W, Platsch G, Feistel H, Schott W, Buchfelder M, Schrell UMH. Receptor-scintigraphy and in vitro study of dopamine receptor activity in prolactinomas non-responsive to therapy with dopamine-agonists. Exper Clin Endocrinol 1995;103 (suppl 1):103 (Abstr).
21. Scillitani A, Dicembrino F, Di Fazio P, Vettori PP, D'Angelo V, Scarabino T, Liuzzi A. In vivo visualization of pituitary dopaminergic receptors by iodine-123 methoxybenzamide (IBZM) correlates with sensitivity to dopamine agonists in two patients with macroprolactinomas. J Clin Endocrinol Metab 1995;80: 2523-2525.
22. Esiri MM, Bevan JS, Burke CW, Adams CB. Effect of bromocriptine treatment on the fibrous tissue content of prolactin-secreting and nonfunctioning macroadenomas of the pituitary gland. J Clin Endocrinol Metab 1986;63:383-388.
23. Serri O, Marchisio AM, Collu R, Hardy J, Somma M. Dopaminergic binding sites in human pituitary adenomas other than prolactinomas. Horm Res 1984;19:97-102.
24. Koga M, Nakao H, Arao M, Sato B, Noma K, Morimoto Y, Kishimoto S, Mori S, Uozumi T. Demonstration of specific dopamine receptors on human pituitary adenomas. Acta Endocrinol Copenh 1987;114:595-602.
25. Muhr C, Bergstrom M, Lundberg PO, Bergstrom K, Langstrom B. Positron emission tomography for the in vivo characterization and follow-up of treatment in pituitary adenomas. In Advances in pituitary adenoma research (eds AM Landolt, PU Heitz, J Zapf, J Girard, E Del Pozo), 1988:163-170. Pergamon Press, Oxford.
26. Peillon F, Le Dafniet M, Garnier P, Brandi AM, Moyse E, Birman P, Blumberg-Tick J, Grouselle D, Joubert-Bression D. Receptors and neurohormones in human pituitary adenomas. Horm Res 1989;31:13-18.
27. Jaffe CA, Barkan AL. Treatment of acromegaly with dopamine agonists. Endocrinol Metab Clin N Am 1992;21:713-735.

28. Cronin MJ, Cheung CY, Wilson CB, Jaffe RB, Weiner RI. [3H]Spiperone binding to human anterior pituitaries and pituitary adenomas secreting prolactin, growth hormone, and adrenocorticotropic hormone. J Clin Endocrinol Metab 1980; 50:387-391.
29. Bression D, Brandi AM, Nousbaum A, Le Dafniet M, Racadot J, Peillon F. Evidence of dopamine receptors in human growth hormone (GH)-secreting adenomas with concomitant study of dopamine inhibition of GH secretion in a perifusion system. J Clin Endocrinol Metab 1982;55:589-593.
30. Pirker W, Riedl M, Luger A, Czech T, Rossler K, Asenbaum S, Angelberger P, Deecke L, Podreka I, Brucke T. SPECT imaging of dopamine D2 receptors in pituitary adenomas using [123I]-epidepride. Eur J Nucl Med 1995;22:833 (Abstr).

Chapter 9

Conclusion: Clinically non-functioning pituitary adenomas: new diagnostic and therapeutic options.

F.R.E. Nobels[1], D.J. Kwekkeboom[2], W.W. de Herder[1], and S.W.J. Lamberts[1]

Departments of Medicine[1] and Nuclear Medicine[2], University Hospital Dijkzigt, Rotterdam, The Netherlands.

Shorter Dutch version in:
Ned Tijdschr Geneesk 1992;136:1148-1152.

Introduction

The majority of pituitary adenomas produce typical clinical syndromes as a result of hypersecretion of one or more anterior pituitary hormones. Identifiable syndromes include acromegaly, caused by overproduction of growth hormone (GH), Cushing's disease, caused by overproduction of adrenocorticotrophic hormone (ACTH) and the amenorrhea-galactorrhea syndrome in women, caused by overproduction of prolactin (PRL). Approximately 25-30 % of patients with pituitary tumours do not present with symptoms related to pituitary hormone hypersecretion [1-5]. As a result they escape early detection and usually present with symptoms related to compression of important surrounding tissues by the expanding tumour mass, or are found incidentally on radiographic studies [6]. Unlike for "secreting" pituitary adenomas, no accepted medical treatment yet exists for these "non-secreting" tumours. The treatment of choice is surgical debulking, often followed by external radiation therapy.

Due to advances in immunological and molecular biological techniques to detect hormonal secretion *in vitro*, it has been demonstrated in recent years that the majority of these tumors are in fact poorly secreting, rather than completely non-secreting [6-7]. In cell culture they usually synthesize and secrete the gonadotropic hormones follicle stimulating hormone (FSH) and/or luteinizing hormone (LH), and/or their free α- or β-subunits. Even adenomas that lost all neuropeptide synthetizing activity (so-called null-cell adenomas) demonstrate typical dense core neurosecretory granules on electron microscopy, while the secretory granule-specific protein chromogranin A (CgA) can be identified in virtually all cases [8]. In a subset of tumours clinically inapparent production of ACTH or GH, or even of a combination of several anterior pituitary homones, is present [9,10]. These silent corticotroph or somatotroph adenomas may be detected in up to 8 and 3 % of cases, respectively.

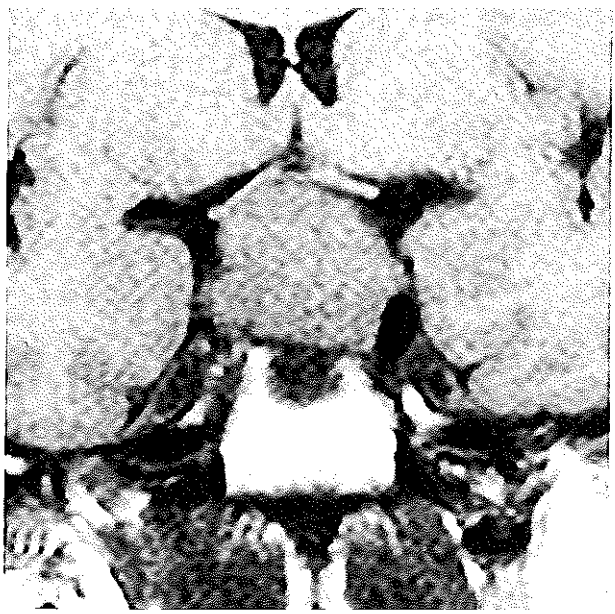
The term "clinically non-functioning" pituitary adenomas (CNPA) appears thus to be preferable to the terms "inactive" or "non-secreting" adenomas. Tumors of gonadotroph origin, secreting gonadotropic hormones or their subunits are also called gonadotropinomas. Research on diagnostic techniques and pharmacological treatment of these CNPA is rapidly progressing and will be discussed in this review.

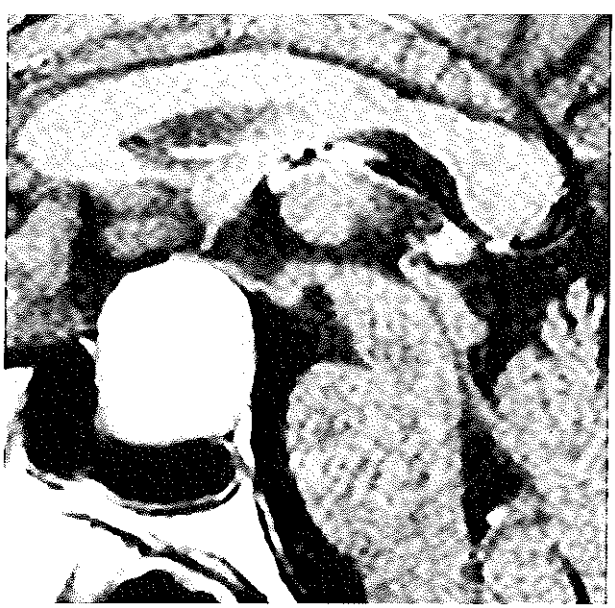
Clinical presentation

CNPA are diagnosed in middle-aged or elderly patients [1,2,5]. This not only reflects the slow rate of growth but also the lack of clinical manifestations of excessive hormone secretion. Gonadotropinomas occur more frequently in men than in women [1,2,5]. This may be biased, however, by the fact that the diagnosis is more difficult in postmenopausal women. Making the distinction between an adenoma hypersecreting gonadotropins, and a nonsecreting adenoma associated with normal postmenopausal elevations of serum levels of FSH and LH, is not easy. By the time most CNPA are recognized clinically, they are sufficiently large (diameter > 10 mm) so as to extend outside the sella turcica and produce symptoms related to the mass of the tumour (Fig. 1) [1,2,5,6]. Headache due to traction on the parasellar meninges is a frequent complaint. Neuro-ophthalmological complications include visual field defects (usually bitemporal hemianopsia) caused by pressure on the optic chiasm and ophthalmoplegia caused by pressure on the oculomotor nerves in the cavernosal sinus [11].
Mechanical compression of the normal anterior pituitary gland can result in partial or complete hypopituitarism. GH- and gonadotropin-axes are most frequently affected, while TSH and ACTH deficiencies are less common [12,13]. Compression of the pituitary stalk may impair the dopaminergic inhibition of PRL secretion. The resulting mild degree of hyperprolactinemia (usually < 100 µg/L) can be differentiated from the firmly increased PRL levels in patients with macroprolactinomas [14]. Clinically symptomatic diabetes insipidus is an uncommon finding at the time of initial presentation. Its presence should stimulate a thorough investigation to exclude a non-endocrine tumour of the pituitary region (*e.g.* craniopharyngioma).

Hypopituitarism often goes undetected. After menopause secondary hypogonadism obviously does not cause symptoms, while men frequently do not seek medical attention, because decreased libido and impotence are usually ascribed to the effect of aging. The symptoms related to GH-, TSH- and/or ACTH-deficiency are vague. The specific clinical signs and symptoms caused by GH deficiency in adulthood have only recently been recognized [15,16]. The predominant features of this

Figure 1. Clinically nonfunctioning pituitary adenoma causing compression of the optic chiasm.





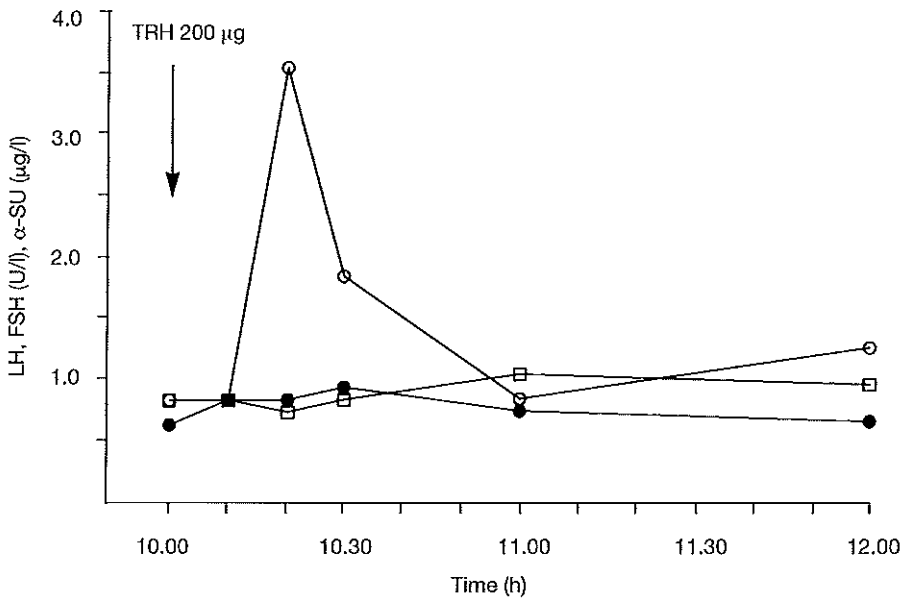
syndrome are diminished quality of life, as perceived by the patients and as indicated by standardized psychological tests, diminished muscle strength and increase of body fat. Premature mortality from cardiovascular disease may also be part of this syndrome.

In a small subgroup of male patients with glycoprotein hormone producing tumors, overproduction of biologically active intact FSH results in hypertrophy of the seminiferous tubules and bilateral testicular enlargement [2]. Intact LH secretion, causing elevated serum testosterone levels in men, occurs even much rarer [17]. A case of ovarian hyperstimulation due to hypersecretion of intact FSH by a gonadotropinoma has been described [18]. There are no clinical effects from excess secretion of uncombined glycoprotein-subunits, since they are biologically inactive.

Hormonal characteristics *in vivo*

Most CNPA secrete gonadotropins and/or their subunits. Demonstration of elevated serum levels of these markers, most frequently of α-subunit and/or FSH, suggests the presence of an adenoma of gonadotroph origin [19,20]. Differentiation of normal from abnormal gonadotropin and subunit secretion may be difficult, however, especially in postmenopausal women [1,2]. Many groups report the response of gonadotropins and/or their subunits to the injection of TRH as a useful addition in the workup of CNPA [7,21-23]. Normal gonadotrophs do not respond to TRH. Therefore any observed increased secretion derives from tumorous gonadotrophs. Responses of intact gonadotropins and α-subunits are most frequently used in clinical practice (Fig. 2). TRH stimulated β-LH secretion is reported to be more sensitive, being elevated in about one third of men and two thirds of women harboring CNPA [21,22]. The measurement of subunit levels is limited by several factors, including assay specificity, sensitivity and commercial availability [3]. The sensitivities and specificities of subunit assays are highly variable. Polyclonal subunit assays for instance may show considerable cross-reactivity with intact hormones [24]. Moreover, the specificity of the TRH-test is doubtful. TRH-stimulated gonadotropin and/or subunit release has also been observed in patients with PRL-[25,26] and GH-[25] producing pituitary adenomas and in patients with nonendocrine tumours of the pituitary region [25].

Figure 2. TRH stimulation test in a patient with a clinically nonfunctioning pituitary adenoma, showing a marked response of α-subunit.



CgA might also be a suitable candidate marker for CNPA, since CNPA almost invariably show immunostaining for CgA. Unfortunately, due to the relatively small volume of these tumours, serum concentrations of CgA are only rarely increased [25]. Indeed, CgA is produced in a wide variety of normal neuroendocrine tissues throughout the body. Usually, only large tumours succeed in elevating serum CgA levels.

In those cases where a preoperative hormonal diagnosis is not possible, the differential diagnosis should include a large spectrum of non-hormone producing neoplasms of the hypothalamo-pituitary region (Tab. 1) [27]. The most frequent space-occupying lesions encountered in that area are craniopharyngiomas, meningiomas, metastases of distant malignant tumors, and aneurysms of the internal carotid artery. Failing to recognise the last option before neurosurgery, may obviously cause huge problems during the operation. Fortunately, the present MRI-imaging techniques can reliably indicate the presence of such an aneurysm.

Table 1: Nonhormone producing space-occupying lesions of the hypothalamo-pituitary region

cell rest tumours	**primitive germ cell tumours**	**gliomas**
cholesteatoma	teratoma	astrocytoma
chordoma	germinoma	ependymoma
colloid cyst	dysgerminoma	microglioma
craniopharyngioma	ectopic pinealoma	oligodendroglioma
infundibuloma	dermoid	Rathke's cleft cyst
sphenoid mucocele		
infectious, inflammatory	**cerebrospinal fluid related**	**miscellaneous**
abcess (bacterial, fungal)	arachnoid cyst	enchondroma
echinococcal cyst	empty sella syndrome	lipoma
giant cell granuloma	pseudotumor cerebri	meningioma
histiocytosis		metastatic tumours
lymphocytic hypophysitis		vascular lesions
sarcoidosis		
tuberculosis		

Invasive treatment

Transsphenoidal surgery is the preferred treatment of CNPA. The procedure is primarily meant to improve visual field defects and loss of visual acuity, and to remove as much tumor tissue as possible. It is also important to obtain tissue for pathologic diagnosis. In experienced hands serious complications are uncommon, but appear to be greater when the adenoma is very large. The group of J. Hardy, one of the pioneers of transsphenoidal surgery, reports a perioperative mortality of 1.6 % in their series of 125 macroadenomas [28]. Significant operative blood loss, requiring transfusion occurs in 12.7 %, nasal liquorrhoea in 4.8%, meningitis in 1.6% and cranial nerve palsy in 1.6% of cases. Transient diabetes insipidus or inappropriate antidiuretic hormone (ADH) secretion are frequently seen. Diabetes insipidus remains permanent in 5.6% of cases.

In most instances substantial improvement of visual defects occurs after surgery. Several large series report recovery or improvement of visual fields in 60-70 % of cases and stabilisation in 25-35 % [6,29-31]. Even severe preoperative field defects often strikingly improve, with noticeable visual recovery within 12 to 24 hours after surgery. A seriously compromised visual acuity has a worse prognosis, however, although surgery can still induce improvement. Optic disc pallor is an ominous prognostic sign : visual outcome never significantly ameliorates in the event of frankly atrophic discs. For both acuity and fields there is an inverse correlation between duration of symptoms and final outcome [31]. However, even when symptoms last longer than 6 months, visual improvement still occurs in more than 50 % of cases. Ophthalmoplegia, caused by pressure on the ocular motoric nerves in the cavernous sinus, usually also improves within a few weeks to months after surgery.

Panhypopituitarism with lack of response to pituitary stimulation tests is not likely to improve after surgical decompression [12]. In patients with a normal or mildly elevated PRL level, and a rise in TSH levels in response to TRH stimulation, however, significant postoperative improvement may occur [12,13,32]. This suggests that pituitary failure is caused by compression of the portal circulation in this setting, and not by destruction of the normal anterior pituitary gland. Secondary hypothyroidism, adrenal failure and hypogonadism can rather easily be treated by hormone replacement therapy [33]. In patients with GH deficiency, GH replacement therapy has been reported to be of benefit for their quality of life, as well as for normalizing body composition, improving muscle strength and increasing cardiac performance [15,16]. Preliminary data suggest that an increase in HDL- and a decrease in LDL-cholesterol, induced by GH treatment, may contribute to the prevention of early atherosclerosis. GH replacement therapy is expensive however, with total yearly costs amounting to 10,000 - 12,000 dollars.

Because most CNPA are already very large at the time of diagnosis, they are often not completely resectable. Adjunctive radiation therapy to prevent further tumour growth may be considered for patients with sub-

stantial residual tumour tissue or in those with recurrent adenomas [6,34,35]. The completeness of tumour removal by transsphenoidal surgery is often difficult to evaluate, however. On early postoperative MRI-scans the distinction between adenomatous tissue, hematoma, fibrosis or muscle used by the neurosurgeon to pack the tumour bed after resection, can hardly be made. One has to wait for a period of more than 6 months before proper neuroradiological investigations can be carried out to demonstrate the size and localizations of tumour remnants [36].

Recurrence rates after transsphenoidal surgery without radiation for CNPA between 12 and 22 % have been reported [6,28,30,37-39]. In patients who received postoperative radiotherapy tumour regrowth rates between 2 and 18 % have been reported [6,30,37-42]. Because of selection bias in these studies it is not possible to directly compare the results of surgery alone with those of surgery followed by radiation therapy. No prospective randomised trials have been published yet.

Side effects of radiotherapy include nausea and fatigue, which last up to 1 or 2 months after treatment, diminished taste and olfaction, which may last for 6 months, and loss of hair in the temporal region, which may last for a year [2]. Partial or complete hypopituitarism, which usually not begins until a year or more after the procedure, occurs eventually in over 50 % of the patients 10 years after radiation [33,43]. Severe complications, such as radionecrosis of the optic nerve or of parts of the brain (usually the temporal lobe), are seldomly reported. However, when specifically looked for with meticulous imaging studies, parenchymal changes of the brain can be found in 25 to 30 % of the patients in the years following radiation therapy [44,45]. Moreover, patients who undergo radiotherapy are more likely to have difficulties with memory on neurocognitive function testing than those who undergo surgery alone [46].
Taking these data into account, one might recommend careful post-surgical follow-up MRI scans. If there is evidence of tumour (re)growth, radiation therapy is promptly instituted, or repeat transsphenoidal surgery is considered, depending on the size of the residual tumour, the importance of preserving pituitary function and the experience of the neurosurgeon.

Medical treatment

In analogy with the use of dopamine agonists and somatostatin analogs in the treatment of respectively GH- and PRL-producing pituitary tumors, the possibility of medical treatment in patients with CNPA may be considered. Several pharmacological options might be used.

GnRH agonists and antagonists

The release of gonadotropins by normal anterior pituitary cells is regulated by pulsatile secretion of gonadotropin releasing hormone (GnRH) by the hypothalamus. The chronic administration of GnRH to normal individuals produces an initial rise in gonadotropin levels, followed by gonadotroph desensitization, leading to efficient suppression of gonadotropin release. Long acting agonist analogs of GnRH have been used therapeutically to decrease gonadotropin secretion in patients with precocious puberty, polycystic ovary syndrome and metastatic carcinoma of the prostate gland [47]. Obviously, they might also be of therapeutical benefit in patients with gonadotropinomas. Investigations in patients with prostatic carcinoma, treated with GnRH-analogs, indicate that, although the production of LH by the pituitary gland is suppressed, the production of FSH and α-subunits remains intact or is even stimulated [48]. Since FSH and α-subunit are the predominant secretion products of gonadotropinomas, doubt rises whether GnRH-analogs could be safely used as treatment. Indeed, when cells of gonadotropinomas are brought in culture in the presence of GnRH-agonists, no desensitization phenomenon is observed. Instead, a stimulating effect on gonadotropin release frequently occurs [49]. Several groups have tried the medication in a small number of patients, bearing gonadotropinomas [50-54]. They either induce an agonist effect, no effect, or rarely, a decrease of gonadotropin levels. It is unclear why some tumours respond to chronic GnRH analog administration with a persistent agonist effect and others with desensitization. Thus, GnRH-agonists have not been proven of consistent benefit in the treatment of CNPA, and should not be used. Whether they could be used to prevent regrowth after surgical resection in patients whose tumours respond favorably to chronic GnRH-stimulation *in vitro* has not been evaluated yet.

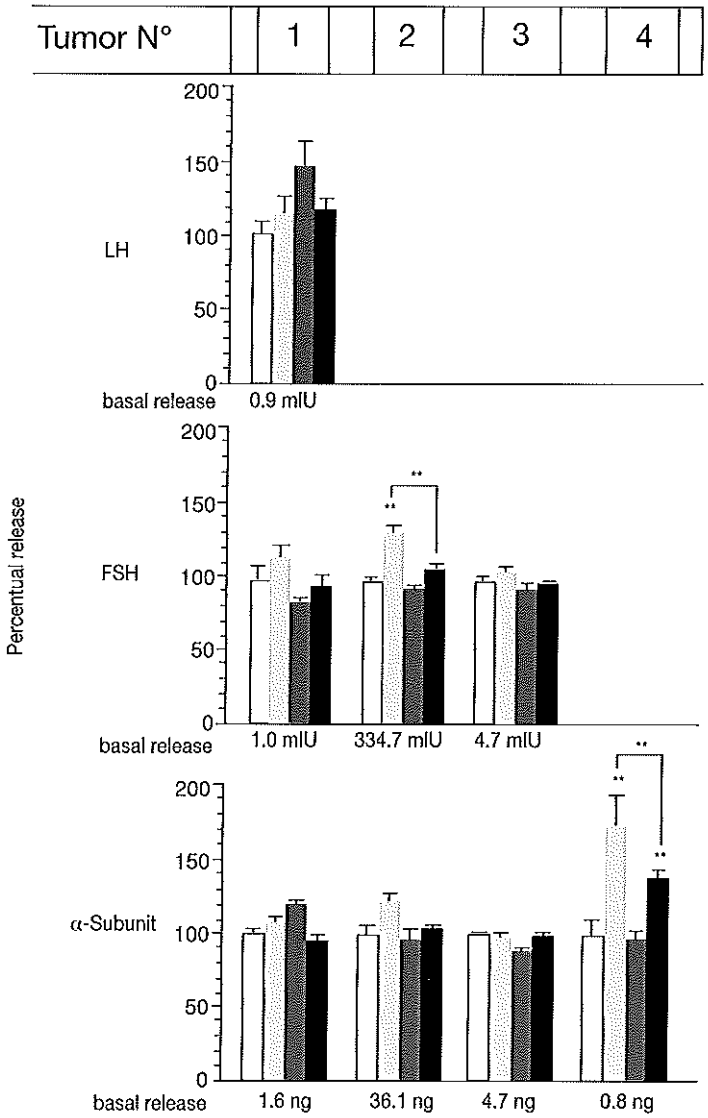
The use of GnRH antagonists has a theoretical advantage, because they lack the initial gonadotropin stimulating effect of GnRH agonists. Incubation of CNPA cells with a GnRH antagonist can diminish the GnRH-stimulated gonadotropin and subunit release, but has no effect in unstimulated conditions (Fig. 3). Long-term treatment with the GnRH antagonist Nal-Glu GnRH reduced elevated FSH levels to normal in five patients but had no effect on tumour size [55,56]. More long-term studies are needed before conclusions can be drawn.

Somatostatin analogs

The development of octreotide, a long-acting agonist of somatostatin, was an important advancement in the medical treatment of acromegaly [57]. Octreotide inhibits GH release and induces a modest degree of tumour shrinkage via specific somatostatin receptors on most GH-secreting pituitary tumours. Using *in vitro* autoradiography, these receptors are not only detected on somatotropinomas, but also on some CNPA [58]. Twelve of 16 macroadenomas of gonadotroph origin could be visualised *in vivo* by ^{111}In-DTPA-octreotide scintigraphy [59]. In another study, using ^{123}I-Tyr^{3}-octreotide scintigraphy, only 2 of 8 CNPA could be visualized [60]. In cell cultures no or only modest effect of octreotide on hormone secretion by these tumours is shown (Fig. 4) [61], but, when used in high unphysiological concentrations, a significant reduction in cell growth can be induced [62]. To date only sparse data are available on the chronic use of octreotide in the treatment of these patients. Preliminary results are rather disappointing, showing only modest reductions of gonadotropin levels or tumour size in a low percentage of patients [61,63-67]. Interestingly, a clear improvement of visual fields and acuity can be seen in some patients, within the first hours after starting the drug [67,68]. This seems to be unrelated to the effect on tumour volume, suggesting either a direct action on the optic nerve and/or the retina, or an effect on their vascularization.

Somatostatin receptor scintigraphy cannot be used in the differential diagnosis of a pituitary mass, as other tumours of that region (*e.g.* meningiomas, gliomas) may also possess somatostatin receptors [69]. Whether somatostatin receptor scintigraphy can predict the effect of treatment with octreotide remains a point of discussion. In a recent

Figure 3. Effects of GnRH and a GnRH-antagonist on mean (± SE) glycoprotein hormone release by 4 clinically nonfunctioning pituitary adenomas *in vitro*.



Basal release is per 200,000 cells and per 72 h.

□ Control

▨ GnRH 100 nmol/l

▨ GnRH-antagonist 100 nmol/l

■ GnRH 100 nmol/l plus GnRH-antagonist 100 nmol/l

** $p < 0.01$ vs. group indicated

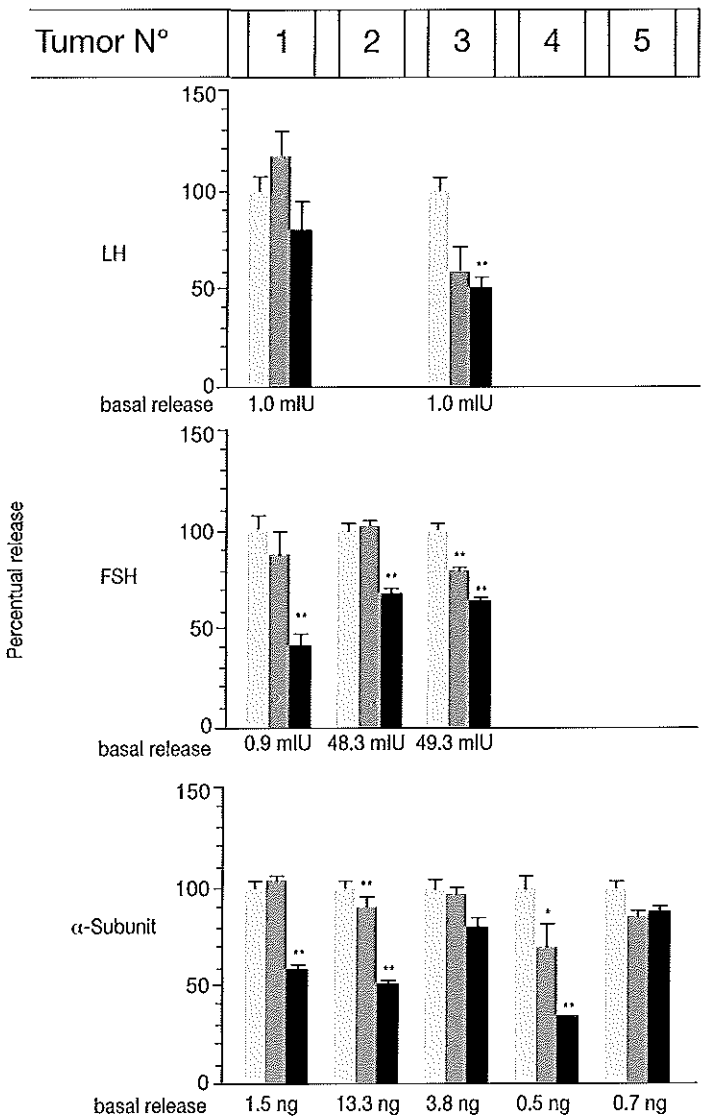
report in 14 patients no close relation was observed between the results of the scan and the effect of octreotide therapy on tumour shrinkage [66], while in another smaller study in 5 patients such a relation could indeed be shown [70].

Recent studies indicate the presence of a variety of somatostatin receptor subtypes on CNPA [71-73]. Octreotide binds with a high affinity to somatostatin receptor subtypes 2 and 5 only. Using autoradiography Hofland *et al.* found specific binding of octreotide in only 2 of 5 CNPA, while binding of natural somatostatin occurred in 4 adenomas [73]. The 2 CNPA that only interacted with natural somatostatin and not with octreotide, lacked mRNA expression for somatostatin receptor subtype 2. Using natural somatostatin, a significant inhibition in the production of glycoprotein hormones and/or their free subunits was reported in 7 of 11 cultured tumours, while octapeptide somatostatin-analogs (including octreotide) were only effective in 3 of 10 [73]. Klibanski *et al.* showed significant suppression of tumoural secretion products by natural somatostatin in 10 of 15 cultured tumours [74]. This suggests that other subtype-specific somatostatin analogs might exert different actions on these tumours. However, dopamine agonists are generally equally or more effective than natural somatostatin in the suppression of gonadotropin and/or subunit secretion by CNPA [61, 73].

Dopamine agonists

Finally, the use of dopamine agonists for treating CNPA can be considered. CNPA express dopamine receptors on their cell membranes, but their number and affinity are smaller than in prolactinomas [75,76]. Addition of the dopamine agonist bromocriptine, in high pharmacological concentrations, to cultures of tumour cells of gonadotroph origin suppresses the release and synthesis of gonadotropins and α-subunits [61,77-79]. Maximal effects are only attained after prolonged incubation during several days to weeks. These *in vitro* data suggest that prolonged treatment with high doses of bromocriptine might exert an inhibitory effect on tumour growth *in vivo*. The clinical data are disappointing, however, showing improvement of visual field defects and/or reduction of tumour mass in 4 of 6 patients with gonadotroph or α-subunit secreting pituitary adenomas and in 7 of 84 patients with CNPA [17,78,80,81].

Figure 4. Effects of octreotide and bromocriptine on mean (± SE) glycoprotein hormone release by 5 clinically nonfunctioning pituitary adenomas *in vitro*.



Basal release is per 200,000 cells and per 72 h.

In tumor 4 α-subunit values during incubation with bromocriptine were undetectable.

Control

Octreotide 10 nmol/l

Bromocriptine 10 nmol/l

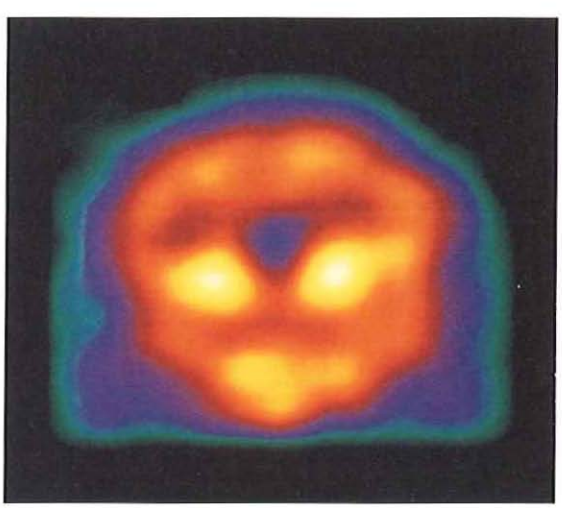
* $p < 0.05$, ** $p < 0.01$ vs. control

adapted from ref. 61

Since the use of bromocriptine in high doses is generally not well tolerated, however, these studies have been performed with rather small doses. The new generation of more powerful and more selective dopamine agonists, might provide a useful alternative for bromocriptine. In a preliminary study of 5 patients with CNPA promising results were shown with the long-acting dopamine agonist quinagolide (CV205-502) in a dosis of 300 µg/day for 12-18 months [82]. In a long-term study in 10 patients, however, progressive increase in tumour size was shown in most patients, despite efficient suppression of gonadotropin and α-subunit secretion [83]. At the end of follow-up (36 to 93 months) tumour volume remained significantly below pretreatment volume in 2 patients, remained at pretreatment volume in 2 patients and was significantly increased in 6 patients [83].

Reasons for the relatively low success rate of dopamine agonist therapy include the inclusion of dopamine-receptor negative tumours in these studies. This problem can now be approached by dopamine receptor scintigraphy with the iodinated benzamides ^{123}I-IBZM or ^{123}I-epidepride (Fig. 5) [84-87]. With this technique one can predict the suppressive

Figure 5: ^{123}I-IBZM SPECT of the brain of a patient with a clinically non-functioning pituitary adenoma, showing slight uptake in the adenoma and intense uptake in the basal ganglia.



effect of dopamine agonists on the tumour volume of prolactinomas [84,85]. In a recent study in patients with CNPA de Herder *et al.* demonstrated tumour shrinkage in 2 and tumour stabilisation in 1 of 3 ^{123}I-IBZM SPECT-positive patients treated with quinagolide [85]. In one of these patients an additional effect of octreotide could not be excluded. In 1 of 4 patients without pituitary ^{123}I-IBZM uptake tumour expansion occurred under quinagolide therapy. The recently developed dopamine receptor ligand ^{123}I-epidepride shows a higher affinity for dopamine D2 receptors than ^{123}I-IBZM, and thus a higher sensitivity for detecting dopamine receptor-positive pituitary adenomas [86, 87]. Future long-term studies are needed to investigate whether *in vivo* imaging of dopamine receptors with these ligands is useful in selecting patients, who may benefit from dopaminergic therapy.

Conclusions

The detection of hormonal activity in most CNPA has allowed a thorough examination of the behaviour of these tumours and their response to medical manipulation *in vitro* as well as *in vivo*. Demonstration in serum of elevated levels of intact glycoprotein or subunit secretion products, in basal conditions or after stimulation by TRH, can be used for diagnostic purposes. Transsphenoidal surgery is still the treatment of choice. Postoperative external irradiation might be omitted in most patients, providing that repeated MRI-imaging is possible, to allow early detection of recurrence. Medical treatment with dopamine agonists and/or somatostatin analogs can be recommended only in patients without an important "mass effect" of the tumour. New nuclear techniques revealing the presence of dopamine and/or somatostatin receptors on these tumours may help to select patients who might benefit from medical therapy.

References

1. Klibanski A. Nonsecreting pituitary tumors. Endocrinol Metab Clin North Am 1987;16:793-804.
2. Snyder PJ. Clinically nonfunctioning pituitary adenomas. Endocrinol Metab Clin North Am 1993;22:163-175.
3. Katznelson L, Alexander JM, Klibanski A. Clinical review 45. Clinically nonfunctioning pituitary adenomas. J Clin Endocrinol Metab 1993;76:1089-1094.
4. Lamberts SWJL, de Herder WW, van der Lely AJ, Hofland LJ. Imaging and medical management of clinically nonfunctioning pituitary tumors. The Endocrinologist 1995;5:3-6.
5. Snyder PJ. Extensive personal experience : gonadotroph adenomas. J Clin Endocrinol Metab 1995;80:1059-1061.
6. Ebersold MJ, Quast LM, Laws ER, Scheithauer B, Randall RV. Long-term results in transsphenoidal removal of nonfunctioning pituitary adenomas. J Neurosurg 1986;64:713-719.
7. Kwekkeboom DJ, de Jong FH, Lamberts SWJ. Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas in vivo and in vitro : relation to sex and effects of TRH, GnRH and bromocriptine. J Clin Endocrinol Metab 1989;68:1128-1135.
8. Lloyd RV, Jin L, Fields K, Chandler WF, Horvath E, Stefaneanu L, Kovacs K. Analysis of pituitary hormones and chromogranin A mRNAs in null cell adenomas, oncocytomas, and gonadotroph adenomas by in situ hybridization. Am J Pathol 1991;139:553-564.
9. Horvath E, Kovacs K, Killinger DW, Smyth HS, Platts ME, Singer W. Silent corticotropic adenomas of the human pituitary gland. Am J Pathol 1989;98:617-635.
10. Kovacs K, Lloyd R, Horvath E, Asa SL, Stefaneanu L, Killinger DW, Smyth HS. Silent somatotroph adenomas of the human pituitary. A morphologic study of three cases including immunocytochemistry, electron microscopy, in vitro examination and in situ hybridization. Am J Pathol 1989;134:345-353.
11. Melen O. Neuro-ophthalmologic features of pituitary tumors. Endocrinol Metab Clin North Am 1987;16:585-608.
12. Nelson AT, St George Tucker Jr H, Becker DP. Residual anterior pituitary function following transsphenoidal resection of pituitary macroadenomas. J Neurosurg 1984;61:577-580.
13. Arafah BM. Reversible hypopituitarism in patients with large non-functioning pituitary adenomas. J Clin Endocrinol Metab 1986; 62:1173-1179.
14. Bevan JS, Burke CW, Esiri MM, Adams CBT. Misinterpretation of prolactin levels leading to management errors in patients with sellar enlargement. Am J Med 1987;82:29-32.
15. Salomon F, Cuneao RC, Hesp R, Sönksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth homone deficiency. New Engl J Med 1989;321:1797-1803.

16. de Boer H, Blok GJ, Van der Veen EA. Clinical aspects of growth hormone deficiency in adults. Endocr Rev 1995;16:63-86.
17. Klibanski A, Deutsch PJ, Jameson JL, Ridgway EC, Growley WF, Hsu DW, Habener JF, Black PMcL. Luteinizing hormone-secreting pituitary tumour: biosynthetic characterization and clinical studies. J Clin Endocrinol Metab 1987;64:536-542.
18. Djerassi A, Coutifaris C, West VA, Asa SL, Kapoor SC, Pavlou SN, Snyder PJ. Gonadotroph adenoma in a premenopausal women secreting follicle-stimulating hormone and causing ovarian hyperstimulation. J Clin Endo Metab 1995;80:591-594.
19. Oppenheim DS, Kana AR, Sangha JS, Klibanski A. Prevalence of α-subunit hypersecretion in patients with clinically nonfunctioning and somatotroph adenomas. J Clin Endocrinol Metab 1990;70:859-864.
20. Warnet A, Porsova-Dutoit I, Lahlou N, Seret-Begue D, Lajeunie E, Chanson P, Woimant F, Lot G, Guillausseau PJ, Roger M. Glycoprotein hormone alpha-subunit secretion in prolactinomas and in non-functioning adenomas: relation with the tumor size. Clin Endocrinol (Oxf) 1994;41:177-184.
21. Daneshdoost L, Gennarelli TA, Bashey HM, Savino PJ, Sergott RC, Bosley TM, Snyder PJ. Recognition of gonadotroph adenomas in women. N Engl J Med 1991;324:589-594.
22. Daneshdoost L, Gennarelli TA, Bashey HM, Savino PJ, Sergott RC, Bosley TM, Snyder PJ. Identification of gonadotroph adenomas in men with clinically nonfunctioning pituitary adenomas by the luteinizing hormone β-subunit response to thyrotropin-releasing hormone. J Clin Endocrinol Metab 1993;77:1352-1355.
23. Gil-del-Alamo P, Pettersson KS, Saccomanno K, Spada A, Faglia G, Beck-Peccoz P. Abnormal response of luteinizing hormone beta subunit to thyrotrophin-releasing hormone in patients with non-functioning pituitary adenoma. Clin Endocrinol (Oxf) 1994;41:661-666.
24. 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;33:777-782.
25. Nobels FRE, Kwekkeboom DJ, Coopmans W, Hoekstra R, Bouillon R, Lamberts SWJ. A comparison between the diagnostic value of the measurement of gonadotropins, α-subunits and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. J Clin Endocrinol Metab 1993;77:784-789.
26. Chanson P, Pantel J, Young J, Couzinet B, Bidart JM, Schaison G. Free luteinizing-hormone beta-subunit in normal subjects and patients with pituitary adenomas. J Clin Endocrinol Metab 1997;82:1397-1402.
27. Post KD, McCormick PC, Bello JA. Differential diagnosis of pituitary tumors. Endocrinol Metab Clin North Am 1987;16:609-645.
28. Comtois R, Beauregard H, Somma M, Serri O, Aris-Jilwan N, Hardy J. The clinical and endocrine outcome to transsphenoidal microsurgery of nonsecreting pituitary adenomas. Cancer 1991;68:860-866.

29. Trautmann JC, Laws ER. Visual status after transsphenoidal surgery at the Mayo Clinic. Am J Ophthalmol 1983;96:200-208.
30. Ciric I, Mikhael M, Stafford T, Lawson L, Garces R. Transsphenoidal microsurgery of pituitary macroadenomas with long-term follow-up results. J Neurosurg 1983;59:395-401.
31. Cohen AR, Cooper PR, Kupersmith MJ, Flamm ES, Ransohoff J. Visual recovery after transsphenoidal removal of pituitary adenomas. Neurosurgery 1985;17:446-452.
32. Marazuela M, Astigarraga B, Vicente A, Estrada J, Cuerda C, Garcia-Uria J, Lucas T. Recovery of visual and endocrine function following transsphenoidal surgery of large nonfunctioning pituitary adenomas. J Endocrinol Invest 1994;17:703-707.
33. Vance ML. Hypopituitarism. N Engl J Med 1994;330:1651-1662.
34. Flickinger JC, Nelson PB, Martinez AJ, Deutsch M, Taylor F. Radiotherapy of nonfunctional adenomas of the pituitary gland. Results with long-term follow-up. Cancer 1989;63:2409-2414.
35. Tran LM, Blount L, Horton D, Sadeghi A, Parker R. Radiation therapy of pituitary tumors : results in 95 cases. Am J Clin Oncol 1991;14: 25-29.
36. Nelson PB, Robinson AG, Hirsch W. Postoperative computed tomographic evaluation of patients with large pituitary tumors treated with operative decompression and radiation therapy. Neurosurgery 1991;28:238-241.
37. Chun M, Masko GB, Hetelekidis S. Radiotherapy in the treatment of pituitary adenomas. Int J Radiat Oncol Biol Phys 1988;15:305-309.
38. Vlahovitch B, Reynaud C, Rhiati J, Mansour H, Hammoud F. Treatment and recurrences in 135 pituitary adenomas. Acta Neurochir Suppl (Wien) 1988;42:120-123.
39. Brada M, Rajan B, Traish D, Ashley S, Holmes-Sellors PJ, Nussey S, Uttley D. The long-term efficacy of conservative surgery and radiotherapy in the control of pituitary adenomas. Clin Endocrinol (Oxf) 1993;38:571-578.
40. Grigsby PW, Stokes S, Marks JE, Simpson JR. Prognostic factors and results of radiotherapy alone in the management of pituitary adenomas. Int J Radiat Oncol Biol Phys 1988;15:1103-1110.
41. McCollough WM, Marcus RB Jr, Rhoton AL Jr, Ballinger WE, Million RR. Long-term follow-up of radiotherapy for pituitary adenoma : the absence of late recurrence after greater than or equal to 4500 cGy. Int J Radiat Oncol Biol Phys 1991;21:607-614.
42. Sassolas G, Trouillas J, Treluyer C, Perrin G. Management of nonfunctioning pituitary adenomas. Acta Endocrinol 1993;129(Suppl1):21-26.
43. Snyder PJ, Fowble BF, Schatz NJ, Savino PJ, Gennarelli TA. Hypopituitarism following radiation therapy of pituitary adenomas. Am J Med 1986;81:457-462.
44. al-Mefty O, Kersh JE, Routh A, Smith RR. The long-term side effects of radiation therapy for benign brain tumors in adults. J Neurosurg 1990;73:502-512.
45. Rauhut F, Stolke D. Combined surgery and radiotherapy of invasive pituitary adenomas - problems of radiogenic encephalopathy. Acta Neurochir Suppl 1996; 65:37-40.

46. McCord MW, Buatti JM, Fennell EM, Mendenhall WM, Marcus RB Jr, Rhoton AL, Grant MB, Friedman WA. Radiotherapy for pituitary adenoma : long-term outcome and sequelae. Int J Radiat Oncol Biol Phys 1997;39:437-444.
47. Mansfield JM, Beardsworth DE, Loughlin JS, Crawford JD, Bode HH, Rivier J, Vale W, Kushner DC, Crigler JF Jr, Crowley JF Jr. Long-term treatment of central precocious puberty with a long-acting analogue of luteinizing hormone-releasing hormone: effects on somatic growth and skeletal maturation. N Engl J Med 1983; 309:1286-1290.
48. Kwekkeboom DJ, Lamberts SWJ, Blom JHN, Schroeder FH, de Jong FH. Prolonged treatment with the GnRH analog busurelin suppresses LH-β production by the pituitary gonadotroph, while α-subunit production does not change. Clin Endocrinol (Ocf) 1990;32:443-451.
49. Daniels M, Newland P, Dunn J, Kendall-Taylor P, White MC. Long-term effects of a gonadotrophin-releasing hormone agonist ([D-Ser(But)^6] GnRH (1-9) nonapeptide-ethylamide) on gonadotrophin secretion from human pituitary gonadotroph cell adenomas in vitro. J Endocrinol 1988;118:491-496.
50. Oppenheim DS, Klibanski A. Medical therapy of glycoprotein hormone-secreting pituitary tumours. Endocrinol Metab Clin North Am 1989;18:339-358.
51. Colombo P, Ambrosi B, Saccomanno K, Bassetti M, Cortelazzi D, Faglia G. Effects of long-term treatment with the gonadotropin-releasing hormone analog nafarelin in patients with non-functioning pituitary adenomas. Eur J Endocrinol 1994;130:339-345.
52. Roman SH, Goldstein M, Kourides IA, Comite F, Bardin CW, Kriefer DT. The luteinizing hormone-releasing hormone (LHRH) agonist [D-TRP^6-Pro^9-NEt]LHRH increased rather than lowered LH and α-subunit levels in a patient with an LH-secreting pituitary tumor. J Clin Endocrinol Metab 1984;58:313-319.
53. Sassolas G, Lejeune H, Trouillas J, Forest MG, Claustrat B, Lahlou N, Loras B. Gonadotropin-releasing hormone agonists are unsuccessful in reducing tumoural gonadotropin secretion in two patients with gonadotropin-secreting pituitary adenomas. J Clin Endocrinol Metab 1988;67:180-185.
54. Klibanski A, Jameson JL, Biller BMK, Crowley WF Jr, Zervas NT, Rivier J, Vale WW, Bikkal H. Gonadotropin and α-subunit responses to chronic gonadotropin-releasing hormone analog administration in patients with glycoprotein hormone-secreting pituitary tumors. J Clin Endocrinol Metab1989;68:81-86.
55. Daneshdoost L, Pavlou S, Molitch ME, Gennarelli TA, Savino PJ, Sergott RC, Bosley TM, Rivier JE, Vale WW, Snyder PJ. Inhibition of follicle-stimulating hormone secretion of gonadotroph adenomas by repetitive administration of a gonadotropin-releasing hormone antagonist. J Clin Endocrinol Metab 1990;71:92-97.
56. McGrath GA, Goncalves RJ, Udupa JK, Grossman RT, Pavlou SN, Molitch ME, Rivier J, Vale WW, Snyder PJ. New technique for quantitation of pituitary adenoma size: use in evaluating treatment of gonadotroph adenomas with a gonadotropin-releasing hormone antagonist. J Clin Endocrinol Metab 1993;76:1363-1368.

57. 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 Engl J Med 1985;313:1576-80.
58. 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;65:65-73.
59. Kwekkeboom DJ, Krenning EP, Lamberts SWJ. Clinically nonfunctioning pituitary adenomas: *in vivo* receptor imaging and *in vitro* hormone responses. In: Faglia G, Beck-Peccoz P, Ambrosi B, Travaglini P, Spada A, eds. Pituitary adenomas: new trends in basic and clinical research. Amsterdam: Excerpta Medica, 1991;359-364.
60. Faglia G, Bazzoni N, Spada A, Arosio M, Ambrosi B, Spinelli F, Sara R, Bonino C, Lunghi F. *In vivo* detection of somatostatin receptors in patients with functionless pituitary adenomas by means of a radioiodinated analog of somatostatin ([^{123}I]SDZ 204-090). J Clin Endocrinol Metab 1991;73:850-856.
61. de Bruin TWA, Kwekkeboom DJ, van 't Verlaat JW, Reubi JC, Krenning EP, Lamberts SWJ, Croughs RJM. Clinically nonfunctioning pituitary adenomas and octreotide response to long term high dose treatment, and studies *in vitro*. J Clin Endocrinol Metab 1992;75:1310-1317.
62. Renner U, Mojto J, Lange M, Muller OA, von Werder K, Stalla GK. Effect of bromocriptine and SMS 201-995 on growth of human somatotrophic and non-functioning pituitary adenoma cells *in vitro*. Eur J Endocrinol 1994;130;80-91.
63. Liuzzi A, Dallabonzana D, Oppizzi G, Arrigoni GL, Cozzi R, Strada S, Benini Z, Chiodini J, Chiodini PG. Is there a real medical treatment for "non secreting pituitary adenomas? In: Faglia G, Beck-Peccoz P, Ambrosi B, Travaglini P, Spada A, eds. Pituitary adenomas: new trends in basic and clinical research. Amsterdam: Excerpta Medica, 1991:383-390.
64. Katznelson L, Oppenheim DS, Coughlin JF, Kliman B, Schoenfeld DA, Klibanski A. Chronic somatostatin analog administration in patients with α-subunit-secreting pituitary tumours. J Clin Endocrinol Metab 1992;75:1318-1325.
65. Gasperi M, Petrini L, Pilosu R, Nardi M, Marcello A, Mastio F, Bartalena L, Martino E. Octreotide treatment does not affect the size of most non-functioning pituitary adenomas. J Endocrinol Invest 1993;16:541-543.
66. Plockinger U, Reichel M, Fett U, Saeger W, Quabbe HJ. Preoperative octreotide treatment of growth hormone-secreting and clinically nonfunctioning pituitary macroadenomas: effect on tumour volume and lack of correlation with immunohistochemistry and somatostatin receptor scintigraphy. J Clin Endocrinol Metab 1994;79:1416-1423.
67. Warnet A, Harris AG, Renard E, Martin D, James-Deidier A, Chaumet-Riffaud P. A prospective multicenter trial of octreotide in 24 patients with visual field defects caused by nonfunctioning and gonadotropin-secreting pituitary adenomas. Neurosurgery 1997;41:786-795.

68. Warnet A, Timsit J, Chanson P, Guillausseau PJ, Zamfirescu F, Harris AG, Derome P, Cophignon J, Lubetzki J. The effect of somatostatin analogue on chiasmal dysfunction from pituitary macroadenomas. J Neurosurg 1989;71:687-690.
69. Lamberts SWJ, Hofland LJ, De Herder WW, Kwekkeboom DJ, Reubi JC, Krenning EP. Octreotide and related somatostatin analogs in the diagnosis and treatment of pituitary disease and somatostatin receptor scintigraphy. Front Neuroendocrinol 1993;14:27-55.
70. Duet M, Mundler O, Ajzenberg C, Berolatti B, Chedin P, Duranteau L, Warnet A. Somatostatin receptor imaging in non-functioning pituitary adenomas: value of an uptake index. Eur J Nucl Med 1994;21:647-650.
71. Greenman Y, Melmed S. Heterogeneous expression of two somatostatin receptor subtypes in pituitary tumours. J Clin Endocrinol Metab 1994;78:398-403.
72. Miller OM, Alexander JM, Bikkal HA, Katznelson L, Zervas NT, Klibanski A. Somatostatin receptor subtype gene expression in pituitary adenomas. J Clin Endocrinol Metab 1995;80:1386-1392.
73. Hofland LJ, de Herder WW, Visser-Wisselaar HA, Van Uffelen C, Waaijers M, Zuyderwijk J, Uitterlinden P, Kros MJ, van Koetsveld PM, Lamberts SW. Dissociation between the effects of somatostatin (SS) and octapeptide SS-analogs on hormone release in a small subgroup of pituitary and islet cell tumours. J Clin Endocrinol Metab 1997;82:3011-3018.
74. Klibanski A, Alexander JM, Bikkal HA, Hsu DW, Swearingen B, Zervas NT. Somatostatin regulation of glycoprotein hormone and free subunit secretion in clinically non-functioning and somatotroph adenomas *in vitro*. J Clin Endocrinol Metab 1991;73:1248-1255.
75. Bevan JS, Burke CW. Non-functioning pituitary adenomas do not regress during bromocriptine therapy but possess membrane-bound dopamine receptors which bind bromocriptine. Clin Endocrinol 1986;25:561-572.
76. Koya M, Nakao H, Arao M, Sato B, Noma K, Morimoto Y, Kishimoto S, Mori S, Uozumi T. Demonstration of specific dopamine receptors on human pituitary adenomas. Acta Endocrinol 1987;114:595-602.
77. Lamberts SWJ, Verleun T, Oosterom R, Hofland L, van Ginkel LA, van Loeber JG, van Vroonhoven CCJ, Stefanko SZ, de Jong FH. The effects of bromocriptine, thyrotropin-releasing hormone, and gonadotropin-releasing hormone on hormone secretion by gonadotropin-secreting pituitary adenomas *in vivo* and *in vitro*. J Clin Endocrinol Metab 1987;64:524-530.
78. Klibanski A, Shupnik MA, Bikkal HA, Black PMcL, Kliman B, Zervas NT. Dopaminergic regulation of α-subunit secretion and messenger ribonucleic acid levels in α-secreting pituitary tumours. J Clin Endocrinol Metab 1988;65:96-102.
79. Kwekkeboom DJ, Hofland LJ, van Koetsveld PM, Singh R, van den Berge JH, Lamberts SWJ. Bromocriptine increasingly suppresses the *in vitro* gonadotropin and α-subunit release from pituitary adenomas during long term culture. J Clin Endocrinol Metab 1990;71:718-725.

80. Vance ML, Ridgway BC, Thorner MO. Follicle-stimulating hormone- and α-subunit secreting pituitary tumour treated with bromocriptine. J Clin Endocrinol Metab 1985;61:580-584.
81. Bevan JS, Webster J, Burke CW, Scanlon WF. Dopamine agonists and pituitary tumor shrinkage. Endocr Rev 1992;13:220-240.
82. Kwekkeboom DJ, Lamberts SWJ. Long-term treatment with the dopamine agonist CV 205-502 of patients with a clinically nonfunctioning, gonadotroph, or α-subunit secreting pituitary adenoma. Clin Endocrinol 1992;36:171-176.
83. Nobels FRE, de Herder WW, van den Brink WM, Kwekkeboom DJ, Hofland LJ, Zuyderwijk J, de Jong FH, Lamberts SWJ. Long-term treatment with the dopamine agonist quinagolide (CV 205-502) of patients with a clinically non-functioning pituitary adenoma. (submitted for publication).
84. Pirker W, Brucke T, Riedl M, Clodi M, Luger A, Asenbaum S, Podreka I, Deccke L. Iodine-123-IBZM-SPECT: studies in 15 patients with pituitary tumours. J Neural Transm Gen Sect 1994;97:235-244.
85. de Herder WW, Reijs AEM, Kwekkeboom DJ, Hofland LJ, Nobels FRE, Oei HY, Krenning EP, Lamberts SWJ. In vivo imaging of pituitary tumours using a radiolabelled dopamine D2 receptor radioligand. Clin Endocrinol 1996;45:755-767.
86. Pirker W, Riedl M, Luger A, Czech T, Rossler K, Adsenbaum S, Angelberger P, Deecke L, Podreka I, Brucke T. SPECT imaging of dopamine D2 receptors in pituitary adenomas using [123I]-epidepride. Eur J Nucl Med 1995;22:833.
87. de Herder WW, Reijs AEM, de Swart J, Kaandorp Y, Lamberts SWJ, Krenning EP, Kwekkeboom DJ. Comparison of iodine-123-epidepride and iodine-123-IBZM for dopamine D2 receptor imaging in clinically nonfunctioning pituitary macroadenomas and macroprolactinomas. Eur J Nucl Med 1999;26:46-50.

Summary

Chromogranin A (CgA) as serum marker of neuroendocrine tumours.

CgA is a protein localised in the secretory granules of neuroendocrine cells. Its physiological functions are gradually elucidated, although many questions still remain (chapter 6). It is co-secreted with the hormonal content of the secretory granules. Therefore, its concentrations in serum are a reflection of neuroendocrine secretory activity. Since neuroendocrine tumours retain the capacity to secrete CgA, it can be used as tumour marker. It is a general marker of neuroendocrine neoplastic growth. Endocrine cells, that are not of neuroendocrine origin, such as the follicular cells of the thyroid gland and the steroid hormone secreting cells of the adrenal cortex and the gonads, lack CgA production.

CgA is more specific for neuro-endocrine tumours, than other general neuroendocrine markers, such as neuron-specific enolase and the α-subunit of glycoprotein hormones (chapter 3).

Obviously, the usefulness of CgA as marker of tumours, that secrete specific peptide hormones, is limited, since it cannot compete with these neuropeptide markers in sensitivity nor specificity. It can nevertheless have interesting clincal applications for so-called "non-functioning" neuroendocrine tumours for which no marker is available, or for tumours, such as carcinoids or pheochromocytomas, where the available markers are either unstable or inconvenient for clinical use (chapter 3).

"Non-functioning" neuroendocrine tumours, that are either not able to secrete hormonal products or release products that cannot be detected by current techniques, frequently retain the ability to secrete significant amounts of CgA. This is very useful for "silent" gastro-entero-pancreatic neuroendocrine tumours, facilitating their differentiation from neoplasms of the exocrine pancreas with higher malignant potential and worse prognosis (chapter 3).

Very high serum concentrations of CgA can be encountered in patients with carcinoids or pheochromocytomas. Since CgA is a very stable molecule, no special precautions are needed to handle or store the

serum samples, in contrast to samples for measurement of serotonin in the case of carcinoids or catecholamines in the case of pheochromocytomas. Moreabove, serum determinations are more convenient and better reproducable than 24-h collections of urine for 5-hydroxy-indoleacetic acid (5-HIAA) in patients with carcinoid tumours and for catecholamines and their degradation products in patients with pheochromocytomas (chapter 3).

CgA cannot be considered as an early marker of neuroendocrine neoplasms. Only extensive tumours are able to elevate the serum concentrations above the large physiological back-ground, produced by the vast amount of normal neuroendocrine cells throughout the body. Elevated levels are strongly correlated with tumour volume and thus small tumours may escape detection (chapter 3).

Therefore, CgA is not suitable for screening neoplastic growth in syndromes of multiple endocrine neoplasias, where it could serve as a universal marker of the different tumours involved. Indeed, CgA levels do not increase early and are less sensitive than the specific peptide hormone markers of these neuroendocrine neoplasms (chapter 3).

CgA can be used as marker of Cushing's syndrome, caused by ectopic secretion of adrenocorticotropic hormone (ACTH) or corticotropin releasing hormone (CRH) by non-pituitary neuroendocrine tumours, such as carcinoid tumours, medullary thyroid carcinomas and small cell lung carcinomas (chapter 4). CgA levels are generally not elevated in pituitary Cushing's disease, since corticotroph microadenomas are usually very small. In addition, adrenal cortisol-producing tumours do not secrete CgA, because they are not from neuroendocrine origin. Unfortunately, CgA does not provide help in the difficult cases of so-called occult ACTH-secreting neuroendocrine tumours, usually bronchial carcinoids, since these tumours are very small and therefore don't produce enough CgA.

Slightly elevated levels of CgA are, unexpectedly, also found in some patients with nonendocrine tumours (chapter 3). Many nonendocrine

tissues contain cells, belonging to the diffuse neuroendocrine system. Recently several immuno-histochemical studies have shown that these cells are also present in most tumours of non-endocrine origin. They are either diffusely scattered throughout the tumour or multifocally located in small nests. Whether the presence of elevated serum levels of CgA in patients with these tumours has prognostic significance demands further investigation.

Clinically nonfunctioning pituitary adenomas (CNPA), as a paradigm of neuroendocrine tumours for which no classical markers are available.

CNPA represent about one quarter of all pituitary adenomas. Since they do not present symptoms related to pituitary hormone hypersecretion, they usually escape early detection. Surgical treatment of these tumours is therefore often incomplete, and at present no established medical treatment is available yet (chapter 9).

In recent years it has been demonstrated that the majority of these tumours are in fact poorly secreting, rather than completely nonsecreting. In cell culture they usually secrete the gonadotropic hormones follicle stimulating hormone (FSH) and/or luteinizing hormone (LH) and/or their free α- or β-subunits (chapter 5).

Unfortunately, most CNPA secrete very low amounts of glycoprotein hormones and/or subunits, making detection in serum difficult. Therefore provocative testing with thyrotropin releasing hormone (TRH) is frequently used, since paradoxical secretion of gonadotropins and/or their subunits frequently occurs in CNPA, while normal gonadotrophs do not respond. In patients with nonendocrine, and thus not hormonally active tumours of the pituitary region, release of LH or α-subunits in response to TRH can also occur, however, casting doubt on the ability of the test to distinguish endocrine from nonendocrine tumours (chapter 5).

In vitro, CgA immunostaining is almost invariably present in CNPA, even in so-called null-cell adenomas, that have lost all neuropeptide synthe-

tizing activity. However, due to the relatively small volume of these tumours, serum concentrations of CgA are only rarely increased. Release of CgA can also be induced by injection of TRH. This seems to be more specific for CNPA than the release of gonadotropins and/or their subunits in response to TRH. The sensitivity of this test is rather low, however (chapter 5).

CNPA express dopamine receptors on their cell membranes, but their number and affinity are smaller than in prolactinomas. *In vitro* data suggest that prolonged treatment with high doses of dopamine agonists might be able to exert an inhibitory effect on tumour growth *in vivo*. Since the use of bromocriptine in high doses is generally not well tolerated, however, the clinical data are disappointing. The new generation of more powerful and more selective dopamine agonists, might provide a useful alternative. Long-term medical treatment of subjects with CNPA with the dopamine agonist quinagolide (CV205-502), however, generally fails to induce a significant reduction in tumour volume. Although it is able to cause a persistent suppression of hormone secretion by CNPA, progressive increase in tumour size occurs in most patients (chapter 7).

The reason why some CNPA respond and others not could be due to differences in expression of dopamine-receptors on these tumours. The density of dopamine receptors on tumour tissue can be examined *in vivo* by scintigraphy with ^{123}I-IBZM or with ^{123}I-epidepride. Positive scans, suggesting a high density of dopamine receptors, are found in about a quarter of CNPA (chapter 8). Future studies are needed to determine the usefulness of this technique for the prediction of clinical responses to dopamine agonists.

Samenvatting

Chromogranine A (CgA) als tumormerker van neuroendocriene tumoren.

CgA is een eiwit dat deel uitmaakt van de secretiegranulae van neuroendocriene cellen. De fysiologische functies van dit eiwit worden geleidelijk aan opgehelderd, hoewel nog veel vragen onopgelost blijven. Tijdens het secretieproces wordt CgA samen met de hormonale inhoud van de granulae vrijgesteld. De concentratie in het serum vormt dus een weerspiegeling van de secretieactiviteit van het neuroendocrien stelsel. Vermits neuroendocriene tumoren in staat blijven om CgA vrij te stellen, kan het gebruikt worden als tumormerker. Endocriene cellen, die niet van neuroendocriene oorsprong zijn, zoals de folliculaire cellen van de schildklier en de steroidhormoon producerende cellen van de bijnierschors en van de gonaden, zijn niet in staat om CgA te produceren.

CgA is een specifiekere merker van neuroendocriene tumoren dan andere algemene neuroendocriene merkers, zoals neuron-specifiek enolase en de α-subunit van glycoproteïne hormonen (hoofdstuk 3).

Het nut van CgA als merker van tumoren, die specifieke peptide hormonen poduceren is natuurlijk beperkt, omdat het qua sensitiviteit en specificiteit niet met deze merkers kan concurreren. Het kan nochtans interessante klinische toepassingen hebben bij zogenaamde "niet-functionerende" neuroendocriene tumoren, waarvoor geen merker voorhanden is, of bij tumoren, zoals carcinoïden of pheochromocytomen, waarvan de beschikbare merkers ofwel onstabiel zijn ofwel onhandig voor klinisch gebruik (hoofdstuk 3).

"Niet-functionerende" neuroendocriene tumoren, die ofwel niet in staat zijn om hormonale stoffen te produceren, ofwel stoffen vrijstellen, die met de huidige technieken niet opgespoord kunnen worden, behouden vaak het vermogen om significante hoeveelheden CgA vrij te stellen. Dit is erg nuttig bij "silentieuze" gastro-entero-pancreatische neuroendocriene tumoren, omdat dit toelaat hen te onderscheiden van tumoren van de exocriene pancreas, met een hoger maligne potentiaal en een slechtere prognose (hoofdstuk 3).

Zeer hoge serum concentraties van CgA worden vaak gevonden bij patiënten met carcinoïden of pheochromocytomen. Vermits CgA een erg stabiele molecule is, zijn er geen speciale maatregelen nodig om de serummonsters te transporteren of te bewaren, in tegenstelling tot de monsters die nodig zijn voor bepaling van serotonine bij carcinoïden of van catecholamines bij pheochromocytomen. Bovendien zijn serumbepalingen gemakkelijker en beter reproduceerbaar dan 24 uur urine verzamelingen voor 5-hydroxy-indoolazijnzuur bij patiënten met carcinoïd tumoren en voor catecholamines en hun afbraakproducten bij patiënten met pheochromocytomen (hoofdstuk 3).

CgA kan niet beschouwd worden als een vroege merker van neuroendocriene neoplasie. Enkel uitgebreide tumoren zijn in staat om de serumconcentraties boven de fysiologische achtergrondspiegel, gevormd door de secretie door de vele neuroendocriene cellen in het lichaam, te doen uitstijgen. Er is dus een duidelijk verband tussen de hoogte van de serumspiegel en het tumorvolume, waardoor kleine tumoren aan detectie ontsnappen (hoofdstuk 3).

Daarom is CgA niet bruikbaar voor vroegtijdige opsporing van neoplastische groei bij syndromen van multipele endocriene neoplasie, waar het nochtans zou kunnen gebruikt worden als universele merker van de verschillende betrokken tumoren. De spiegels van CgA stijgen immers eerder laat en zijn dus minder sensitief dan de specifieke peptide hormoonmerkers van deze neuroendocriene tumoren (hoofdstuk 3).

CgA kan gebruikt worden als merker van Cushing syndroom veroorzaakt door ectopische secretie van adrenocorticotroof hormoon (ACTH) of corticotropin releasing hormoon (CRH) door niet-hypofysaire neuroendocriene tumoren, zoals carcinoïden, medullaire schildkliercarcinomen en kleincellige longtumoren (hoofdstuk 4). CgA spiegels zijn doorgaans niet verhoogd bij hypofysaire Cushing omdat de corticotrofe microadenomen meestal zeer klein zijn. Bovendien zijn cortisol producerende tumoren van de bijnieren niet in staat om CgA te maken omdat ze niet tot het neuroendocriene stelsel behoren. Spijtig genoeg biedt CgA geen hulp in de moeilijke gevallen van zogenaamde occulte ACTH-secreterende neuroendocriene tumoren, meestal bronchuscarcinoïden, vermits deze tumoren erg klein zijn en daarom niet genoeg CgA aanmaken.

Licht gestegen CgA spiegels worden eigenaardig genoeg ook gevonden bij sommige patiënten met niet-endocriene tumoren (hoofdstuk 3). Veel niet-endocriene weefsels bevatten cellen, die tot het diffuse neuroendocriene systeem behoren. Vershillende recente immunohistochemische studies hebben aangetoond dat deze cellen ook aanwezig zijn in de meeste niet-endocriene tumoren. Ze liggen ofwel diffuus verpreid in de tumor of multifocaal in kleine celnesten. Verder onderzoek is nodig om uit te maken of de aanwezigheid van verhoogde serum spiegels van CgA bij patiënten met deze tumoren prognostisch belang heeft.

Klinisch niet-functionerende hypofyseadenomen (NFA) als een paradigma van neuroendocriene tumoren waarvoor geen klassieke merkers beschikbaar zijn.

NFA vertegenwoordigen ongeveer één vierde van alle hypofyseadenomen. Vermits zij geen symptomen van hormonale overproductie induceren, worden zij doorgaans laattijdig ontdekt. Daarom kunnen zij meestal niet volledig chirurgisch verwijderd worden. Momenteel bestaat er nog geen afdoende farmacologische behandeling voor deze tumoren (hoofdstuk 9).

De laatste jaren werd aangetoond dat de meeste van deze zogenaamde "niet-secreterende" tumoren toch een zwakke hormoonsecretie vertonen. In celculturen stellen zij vaak de gonadotrofe hormonen follikelstimulerend hormoon (FSH) en/of luteïnizerend hormoon (LH) en/of vrije α- of β-subunits vrij (hoofdstuk 5).

Spijtig genoeg secreteren de meeste NFA slechts kleine hoeveelheden glycoproteïne hormonen en/of subunits, waardoor deze secretieproducten vaak moeilijk in het serum kunnen aangetoond worden. Daarom gebruikt men vaak een stimulatietest met thyrotropin releasing hormoon (TRH). NFA reageren immers vaak op TRH met een paradoxale vrijstelling van hun secretieproducten, terwijl normale gonadotrofe cellen geen reactie vertonen. Bij patiënten met niet-endocriene, en dus niet hormonaal actieve tumoren van de hypofysestreek kan men echter ook een vrijstelling van LH of α-subunits zien na inspuiting van TRH. Hierdoor wordt de mogelijkheid van de test om een ondersheid te

maken tussen endocriene en niet-endocriene tumoren in twijfel getrokken (hoofdstuk 5).

Met immuunhistochemie kan men *in vitro* in bijna alle NFA CgA aantonen, zelfs in zogenaamde nulcel-adenomen, die alle neuropeptide synthesecapaciteit verloren hebben. Door het vrij kleine tumorvolume zijn de serumconcentraties van CgA echter slechts zeldzaam verhoogd. Vrijstelling van CgA kan ook geïnduceerd worden door inspuiting van TRH. Dit lijkt specifieker te zijn voor NFA dan de vrijstelling van gonadotrofines en/of hun subunits in antwoord op TRH. De sensitiviteit van deze test is echter laag (hoofdstuk 5).

De meeste NFA hebben dopamine receptoren op hun celmembranen, maar hun aantal en hun affiniteit voor dopamine is kleiner dan bij prolactinomen. *In vitro* gegevens suggereren dat langdurige behandeling met hoge dosis dopamine agonisten in staat zou kunnen zijn om de tumorgroei *in vivo* te onderdrukken. De klinische resultaten zijn echter teleurstellend omdat bromocriptine meestal niet goed verdragen wordt wanneer gebruikt in hoge dosissen. De nieuwe generatie van krachtigere en selectievere dopamine agonisten kan een nuttig alternatief bieden. Langdurige behandeling van patiënten met NFA met de dopamine agonist quinagolide (CV 205-502) induceert echter doorgaans geen tumorverkleining. Ondanks het feit dat de hormoonproductie van de NFA permanent onderdrukt wordt door quinagolide, ziet men bij de meeste patiënten een progressieve stijging van het tumorvolume (hoofdstuk 7).

Waarom sommige NFA reageren en andere niet zou kunnen te wijten zijn aan verschillen in expressie van dopamine receptoren op hun membraan. De densiteit van dopaminereceptoren in de tumor kan nu *in vivo* aangetoond worden door middel van scintigrafie met ^{123}I-IBZM of ^{123}I-epidepride. Positieve scans, wijzend op een hoge densiteit van dopamine receptoren, worden in ongeveer 1 op 4 NFA gevonden (hoofdstuk 8). Of deze techniek zinvol is om uit te maken welke tumoren gunstig zullen reageren op behandeling met dopamine agonisten zal in toekomstige studies uitgemaakt moeten worden.

Dankbetuiging

Dit proefschrift kwam tot stand door samenwerking met verschillende personen. Het doet mij veel plezier hen hiervoor te bedanken.

Prof. Steven Lamberts, beste Steven, bedankt dat je een rondrijzende Belg in je team hebt willen opnemen. Na mijn uiterst leerzame klinische stage op je dienst heb jij mij er toe aangezet om een doctoraatsthesis te maken. Je hebt mij steeds met raad en daad bijgestaan en mij alle faciliteiten gegeven voor dit werk.

Prof. Roger Bouillon, beste Roger, dank voor het ter beschikking stellen van de chromogranine A assay die in jouw laboratorium werd oppuntgesteld, en voor de opbouwende kritiek die je leverde bij het nalezen van mijn manuscripten.

Dr. Dik Kwekkeboom, beste Dik, jouw scherpe geest heeft het interpreteren van de vele data een stuk eenvoudiger gemaakt. Bedankt voor de hulp bij de statistische analyses!

Dr. Wouter de Herder, beste Wouter, wij hebben samen vele aangename uren gespendeerd. Bedankt voor je steun, zowel tijdens mijn klinische stage in Dijkzigt, als nadien bij het werk aan dit proefschrift.

Dr. Paul Van Crombrugge, beste Paul, jij hebt mij steeds gestimuleerd om ondanks het vele klinische werk toch tijd vrij te maken om aan deze thesis te werken. Bedankt voor je collegialiteit, je vriendschap en je vertrouwen.

Marleen en mijn kinderen Anne, Bart en Stijn dank ik om mij nog maar eens te steunen bij weer één van mijn zoveelste professionele "avonturen". Gelukkig vinden we nog tijd om tussendoor samen van het leven te genieten!

Curriculum vitae

1 september 1961	Geboren in Hamme (België)
1974-1979	Humaniora (latijn-wiskunde) St-Lievenscollege Antwerpen
1979-1986	Studie geneeskunde aan Rijks Universitair Centrum Antwerpen (RUCA) en Universitaire Instelling Antwerpen (UIA)
1986-1991	Opleiding in inwendige geneeskunde in Universitair Ziekenhuis Antwerpen (UZA) en geaffilieerde ziekenhuizen
1990-1992	Opleiding in endocrinologie en metabole aandoeningen in Universitair Ziekenhuis Antwerpen (Prof. Dr. I. De Leeuw), Centre Hospitalier Régional et Universitaire de Lille, Frankrijk (Prof. Dr. P. Fossati, Prof. Dr. D. Dewailly), Dijkzigt Ziekenhuis Rotterdam (Prof. Dr. S. Lamberts, Prof. Dr. J. Birkenhäger, Prof. Dr. G. Hennemann)
1992-	Internist-endocrinoloog in de afdeling endocriene en metabole ziekten van het O.L.Vrouwziekenhuis te Aalst
1994-1998	Hoofdredacteur van de Vlaamse Diabetes Vereniging (VDV)
1998-	Coördinator van het Diabetes Project Vlaanderen
1999-	Voorzitter van de Vlaamse Diabetes Vereniging (VDV)

Publications

1. Nobels F, Rillaerts E, D'Hollander M, Van Gaal L, De Leeuw I. Plasma zinc levels in diabetes mellitus: relation to plasma albumin and amino-acids. Biomed 1986;40:57-60.
2. De Leeuw I, Van Gaal L, Nobels F, Bekaert J. Long-term efficacy of semi-synthetic human insulins in newly-diagnosed insulin-dependent diabetic patients. A two year clinical study. Acta Clin Belg 1986;41:381-87.
3. Van Gaal L, Nobels F, Rillaerts E, Creten W, De Leeuw I. Hypertension in obese and non-obese non-insulin dependent diabetics. A matter of regional adiposity ? Diab Metab 1988;14:289-93.
4. Nobels F, Vandenbroeck F, De Clercq R, Rutsaert R. Beleid bij intracraniële bloedingen. Belg Tijdschr Gen 1988;44:1237-42.
5. Nobels F, Van Gaal L, Rillaerts E, De Leeuw I. Effect of oral antidiabetic drug withdrawal in type II diabetes. Diab Med 1989;6:149-51.
6. De Bock R, Nobels F, Schoemaker I, Van Der Planken M, Peetermans M. Allogeneic bone marrow transplantation for plasma cell leukemia following melphalan, cyclophosphamide and total body irradiation. Bone marrow transplantation 1989;4:331 (letter).
7. Nobels F, Colemont L, Van Moer E. A case of diversion rectitis. Acta Clin Belg 1989;44:202-4.
8. Nobels F, Van Gaal L, De Leeuw I. Weight reduction with a high protein, low carbohydrate, calorie-restricted diet: effects on blood pressure, glucose and insulin levels. Neth J Med 1989;35:295-302.
9. Nobels F, Colemont L, Van Moer E. Short Bowel Syndroom. Belg Tijdschr Gen 1990;46:1593-1600.
10. Nobels F, Colemont L, Goethals M, Abs R. Nephrogenic diabetes insipidus: an unusual presentation of recurrent rectal cancer. Cancer 1991;68:2056-59.
11. Van Gaal L, Nobels F, De Leeuw I. Effects of acarbose on carbohydrate metabolism, electrolytes and vitamins in non-insulin dependent diabetes. Z Gastroenterol 1991;29:642-44.
12. Nobels FRE, Kwekkeboom DJ, De Herder WW, Lamberts SWJ. Klinisch niet-functionerende hypofyseadenomen: nieuwe diagnostische mogelijkheden en therapeutische opties. Ned Tijdschr Gen 1992;136:1148-52.
13. Dewailly D, Cortet-Rudelli C, Nobels F, Henric B, Longelin C, Fossati P. Insulinorésistance et syndrome des ovaires polymicrocystiques. Ann Endocrinol 1992;53:1-7.

14. Nobels F, Dewailly D. Puberty and polycystic ovarian syndrome: the insulin/insulin-like growth factor I hypothesis. Fertil Steril 1992;58:655-66.

15. Abs R, Nobels F, Verhelst J, Chanson P, Mahler C, Corthouts B, Blockx P, Beckers A. Hyperfunctioning unilateral adrenal macronodule in three patients with Cushing's disease: hormonal and imaging characterization. Acta Endocrinol (Copenh) 1993;129:284-290.

16. Vanuytsel JL, Nobels FR, Van Gaal LF, De Leeuw IH. A case of malnutrition after biliopancreatic diversion for morbid obesity. Int J Obesity 1993;17(7):425-6.

17. Nobels FRE, Kwekkeboom DJ, Coopmans W, Hoekstra R, Bouillon R, Lamberts SWJ. A comparison between the diagnostic value of the measurement of gonadotropins, α-subunits and chromogranin A and their response to TRH in clinically nonfunctioning, α-subunit secreting and gonadotroph pituitary adenomas. J Clin Endocrinol Metab 1993;77:784-789.

18. Lamberts SW, De Herder WW, Kwekkeboom DJ, Van der Lely AJ, Nobels FR, Krenning EP. Current tools in the diagnosis of pituitary tumours. Acta Endocrinol (Copenh) 1993;129(suppl. 1):6-12.

19. Baert D, Nobels F, Van Crombrugge P. Combined Conn's and Cushing's syndrome: an unusual presentation of adrenal adenoma. Acta Clinical Belgica 1995;50:310

20. De Herder WW, Krenning EP, Hofland LJ, Reubi JC, Kwekkeboom D, Oei HY, Bruining G, Nobels F, Lamberts SWJ. Somatostatin receptor scintigraphy: its value in tumor localisation in patients with the Cushing syndrome caused by ectopic corticotropin and/or CRH secretion. Am J Med, 1994;96:305-312.

21. Nobels F, De Herder W, Kwekkeboom D, Coopmans W, Mulder A, Bouillon R, Lamberts S. Serum chromogranin A in the differential diagnosis of Cushing' s syndrome. Eur J Endocrinol 1994;131:589-93.

22. De Herder WW, Reijs AEM, Kwekkeboom DJ, Hofland LJ, Nobels F, Oei HY, Krenning EP, Lamberts SWJ. In vivo imaging of pituitary tumours using a radiolabelled dopamine D2 receptor radioligand. Clin Endocrinol 96;45:755-767.

23. Nobels FR, Kwekkeboom DJ, Coopmans W, Schoenmakers CH, Lindemans J, de Herder WW, Krenning EP, Bouillon R, Lamberts SW. Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. J Clin Endocrinol Metab 1997;82:2622-8.

24. Nobels FR, Lamberts SW. Neuroendocrine tumor markers. Clinical Endocrine Oncology, Blackwell Science Ltd., 1997;60:377-382.

25. Nobels FR, Kwekkeboom DJ, Bouillon R, Lamberts SW. Chromogranin A: its clinical value as marker of neuroendocrine tumors. Eur J Clin Invest 1998;28;431-440.

26. Abs R, Verhelst J, Maiter D, Van Acker K, Nobels F, Coolens J-L, Mahler C, Beckers A. Cabergoline in the treatment of acromegaly: a study in 64 patients. J Clin Endocrinol Metab 98;83:374-378.

27. Nobels F, Pauwels I. Diabetes, verhalen uit het leven (boek). Lannoo/Terra 1998.

28. Nobels FR, de Herder WW, Kwekkeboom DJ, Lamberts SW. Long-term treatment of clinically non- functioning and gonadotroph pituitary adenomas with the dopamine agonist quinagolide (CV205-502). (Submitted for publication).

29. Nobels FR, Kwekkeboom DJ, de Herder WW, Lamberts SWJ. Clinically non-functioning pituitary adenomas: new diagnostic and therapeutic options. (Submitted for publication).

30. Hermans MP, Nobels FR, Schmitt H, De leeuw I. Insulin lispro (Humalog®), a novel fast-acting insulin analogue for the treatment of diabetes mellitus: overview of the pharmacological and clinical data. (Submitted for publication).

31. Bex M, Bouillon R, Buysschaert M, *et al.* Insulin Lispro (Humalog®) in the treatment of diabetes mellitus: overview of Belgian clinical data from global studies. (Submitted for publication).

32. Nobels FR, Hermans MP, De Leeuw I. Insulin lispro (Humalog®), a novel fast-acting insulin analogue: guidelines for its practical use. (Submitted for publication).