Prolactinomas

Prolactinomas

An Interdisciplinary Approach

Edited by

L. M. Auer, G. Leb, G. Tscherne
W. Urdl, G. F. Walter

Walter de Gruyter
Berlin · New York 1985

Prof. Dr. med. Ludwig M. Auer
Universitätsklinik für Neurochirurgie
Landeskrankenhaus
A-8036 Graz

This book contains 157 illustrations and 118 tables.

CIP-Kurztitelaufnahme der Deutschen Bibliothek

Prolactinomas : an interdisciplinary approach ; [proceedings of the Internat. Symposium on Prolactinomas, Graz (Austria), April 29–May 2, 1984] / ed. by L. M. Auer ... – Berlin ; New York : de Gruyter, 1985.
ISBN 3-11-010153-X
NE: Auer, Ludwig M. [Hrsg.]; International Symposium on Prolactinomas ⟨1984, Graz⟩

Library of Congress Cataloging in Publication Data

International Symposium on Prolactinomas (1984 : Graz, Austria)
Prolactinomas.
Includes bibliographies and indexes.
1. Prolactinoma–Congresses. I. Auer, Ludwig M., 1948–
II. Title. [DNLM: 1. Pituitary Neoplasms–congresses. 2. Prolactin–secretion–congresses.
WK 585 I615p 1984]
RC280.P5I55 1984 616.99'247 84-22998
ISBN 0 89925 039 4

ISBN 3 11 010153 X Walter de Gruyter Berlin New York
ISBN 0 89925 039 4 Walter de Gruyter, Inc., New York

Preface

In this volume an attempt is made to provide an up to date survey of the physiology and the pathophysiology of prolactin. Furthermore, new pharmacological developments which may have a favourable effect on diseases resulting from a pathologically high production of prolactin are discussed. Interesting new morphological aspects on the differentiation of pathological entities in the sellar region, such as DNA and S–100 protein, are also presented. We believe that this will mean a promising start towards our efforts to differentiate between hyperplasia and adenoma. A substantial amount of information from follow-up studies has been collected so as to observe the effect of both medical treatment and surgical or combined treatment.

It is demonstrated in patients with microadenomas that the risks of each type of treatment are equally low. As long as the sellar structure is normal, it seems to be commonly accepted to treat very small microadenomas medically, although some favour surgical treatment. It is absolutely essential for hyperprolactinaemia to be treated, because of the danger of osteoporosis as an additional complication of elevated serum prolactin.

For larger microadenomas both medical and surgical treatment are advisable. In this respect, it might be useful to distinguish between women who would like to become pregnant and those who do not; some of us favour surgery in the first category.

For large, and especially for invasive adenomas, it is becoming increasingly difficult to establish a clear concept: medical treatment, especially during pregnancy, is known to cause complications. However, complications do tend to accompany surgery as well. Probably the most important thing we have learned about macroadenomas is the fact that a combination of surgical and medical treatment and radiation therapy is likely to give better results, both from the endocrinological and the clinical point of view.

The present state of knowledge thus highly suggests cooperation of the disciplines involved in this field. Any type of treatment may be ideal for an individual patient, provided the decision is taken jointly by surgeons, endocrinologists and gynecologists at an early stage. Only in such rare cases of apoplectic adenomas should an immediate decision in favour of surgery be taken, assuming joint agreement.

We would like to express our thanks to D. Linnette Iliff, Ph. D. for taking care of the language editing and to Ms. Elfriede Meier for editorial assistance.

The Editors

Contents

I Morphology

Structure and ultrastructure of prolactinomas

A. M. Landolt

Introduction

Before to serum prolactin (PRL) determinations were included routinely in evaluation of all patients with pituitary adenomas, PRL-secreting pituitary adenomas (prolactinomas) were considered rare, and there were few reports describing the histology and ultrastructure of these tumours [6, 11, 12, 17, 31, 32, 38, 41, 45, 48, 56, 57]. Since that time, however, the picture has changed considerably. Hyperprolactinaemia is now the most common clinical syndrome seen in our patients with pituitary adenomas. Among 311 patients operated by the author in the past 5 years, 46% had prolactinomas [24]. The results obtained from examination of this large number of prolactinomas has made it possible to arrive at a characteristic histological description of this tumour type [14, 18, 29, 42, 47].

The histological classification of tumours is a process of defining individual adenomas by morphological methods (histology, immunocytochemistry, electron microscopy) and forming categories of tumours that bear common characteristics. When making a diagnosis, we must bear in mind that a practical and useful classification embraces only *distinguishing* features. Minor differences must not be weighed too heavily because the light and electron microscopic pictures of tumours, prolactinomas in this context, have a certain variability that can be attributed both to naturally occurring differences among tumours and to the results of external influences on the tumour. The implications of such differences must not be overestimated, and the temptation to create new and confusing subdivisions in the classification system must be resisted. To refine the differentiation of prolactinomas it is necessary to focus on changes caused by extratumoral factors. These changes can best be defined with quantitative, morphometric studies and statistical methods.

Light microscopy

Most prolactinomas consist of uniform, oval or polygonal cells with large, oval nuclei and prominent nucleoli (fig. 2a) [42, 47]; a structure that is typical of cells engaged in protein synthesis. Polymorph nuclei, multinuclear cells, and mitoses are rare. Staining with haematoxylin-eosin or PAS-orange G usually shows no secretory granules. Prolactinomas were classified as "chromophobic" in the now obsolete

nomenclature that distinguished eosinophilic, basophilic, and chromophobic adenomas. Herlant's erythrosine and Brookes' carmosine, however, stain fine, bright red, secretory granules in a large number of prolactinoma specimens [14, 49]. The immunoperoxidase reaction with anti-PRL shows a positive result in 93–100% of biopsy specimens studied [10, 23, 47]. About half of these specimens show a concomitant positive reaction with anti-adrenocoticotrophic hormone (ACTH), anti-growth hormone (GH), and anti-luteinizing hormone (LH) in a few prolactinoma cells [23], although sample testing in patients from whom these specimens were obtained showed normal blood levels of these hormones.

Frequently, characteristic variations involving signs of degeneration are found on light microscopic examination: Intraadenomatous microcalcispherites are found in 19–30% of biopsy specimens. Because these components are rare in other types of pituitary adenomas, they are useful in establishing a diagnosis [22, 28, 42, 47]. The presence and extent of calcification in the specimen is not related to tumour size; calcification occurs in microadenomas as well as in large tumours; calcium deposition starts in isolated necrotic cells that are surrounded by apparently healthy adenoma cells rather than in large areas of necrosis.

Amyloid bodies are rarely found in prolactinomas [1, 15, 22, 47]. It appean in these rare cases that the fibrillary substance, showing the typical congo red reaction and birefringence is secreted by the prolactinoma cells.

Table 1 Intraadenomatous cysts observed during surgery or on CT scans of 115 prolactinomas

	Number of patients examined	Number of patients with cysts
Women		
Microadenomas	53	9 (17%)
Macroadenomas	40	9 (22%)
Men		
Macroadenomas	22	3 (13%)
Total	115	21 (18%)

Signs of old or recent microscopic, intratumoral haemorrhage are seen in about 18% of the cases; however, the percentage is much higher (up to 40–60%) in women who have had an oestrogen treatment, and during and after pregnancy [42, 43]. Intratumoral cysts, possibly originating from previous, more extensive, intratumoral haemorrhage, are seen in 18% of the cases (tab. 1); they are somewhat more frequent in women than in men, and in microadenomas than in macroadenomas, but these differences are not statistically significant.

Diffuse, sinusoidal, and papillary adenomas have been differentiated on the basis of the amount and arrangement of their connective tissue [3, 18, 39, 49, 51]. The majority of the biopsy specimens is in the group of diffuse or medullary adenomas that contain only little collagen tissue around the blood vessels [27]. Several architectural types may occur, in a single adenoma, however. A semiquantitative study performed by Robert [47] showed a higher content of intraadenomatous fibrous tissue in prolactinomas obtained from women than from men, and in invasive than in noninvasive adenomas.

Microprolactinomas are only rarely invasive. Focal invasion was seen in only 2% of intrasellar adenomas in one study [47]. However, routine examination of biopsy specimens removed from the basal sellar dura during transsphenoidal surgery demonstrated microscopic invasion in about one-third of a small group microadenomas [19]. Invasive growth is observed frequently in larger adenomas. The invasion, particularly if it is microscopic, may be undetectable on radiographs or CT scans obtained with techniques that are currently available. However, such invasive behaviour may explain the low cure rate observed in patients with macroprolactinomas, particularly in the group producing serum prolactin levels above 200 ng/ml [20]. Invasion into the cavernous sinus is seen in patients with preoperative PRL levels above 1000–2000 ng/ml. But of course, lower levels do not exclude invasion into the cavernous sinus [21, 52].

Electron miscroscopy

An examination of the ultrastructure of prolactinomas with electron microscopy confirms the light microscopy findings with cytological features typical of cells engaged in active protein synthesis (fig. 1). The cytoplasm of biopsy specimens from prolactinomas contains differentiated complexes of the rough surfaced endoplasmic reticulum, the cell organelle engaged in protein synthesis, and a prominent Golgi apparatus engaged in the formation of the secretory granules. Annulate lamellae, a form of specially differentiated rough-surfaced endoplasmic reticulum that is considered an indicator of very active protein synthesis, are found in some specimens [17, 50]. The secretory granules are usually 100–300 nm in diameter. Only few secretory granules remain stored in the cytoplasm; most are released rapidly after their formation, and ongoing granule release can be observed along the entire cell circumference in about 60% of the biopsy specimens [47]. However, a dense accumulation of larger secretory granules (diameter 600 nm or greater) can be observed in a few cases [14, 17, 18, 41, 61]. Lysosomes engaged in crinophagy are found in most specimens. Cytoplasmic microtubules may be seen, primarily in the region of the Golgi apparatus. In rare cases, a few cells contain small bundles of fine (type I) microfilaments [14]. Desmosomes and the formation of abortive follicles are rare, but they are more frequent in prolactinomas than in the GH-secreting

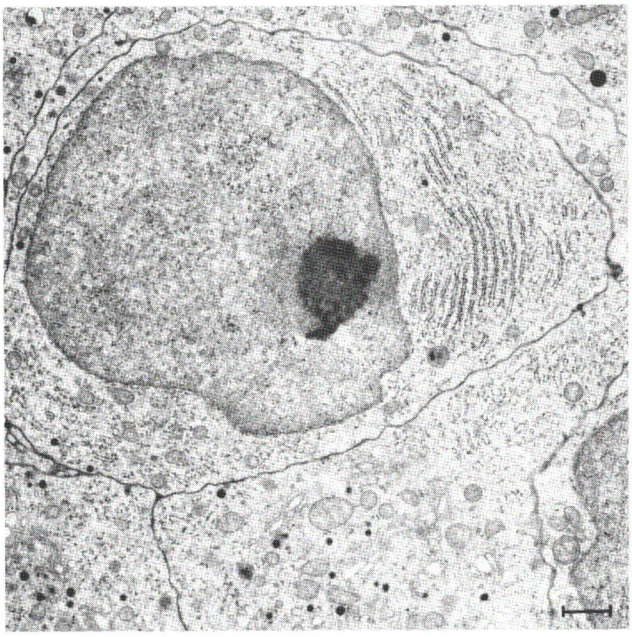


Fig. 1 Electron micrograph. Prolactinoma demonstrating oval nuclei with large nucleoli and large cytoplasma areas with a well differentiated rough-surfaced endoplasmic reticulum and few secretory granules. Osmium fixation, scale 1 μ.

adenomas [18]. Follicle formation is exaggerated in the very rare variant that is called cystic prolactinoma [16]. This type of adenoma, which must not be confused with adenomas containing an intratumoral cyst, consists of two cell types: (1) Cuboidal cells lining multiple microscopic, mucin-containing cysts, and (2) the usual prolactin cells.

The presence of oncocytes is generally interpreted as a sign of cell degeneration. Oncocytes dispersed in an actively secreting adenoma can only be detected with the electron microscope. They occur only in macroadenomas and not in microadenomas [19].

Changes in prolactinoma morphology caused by bromocriptine

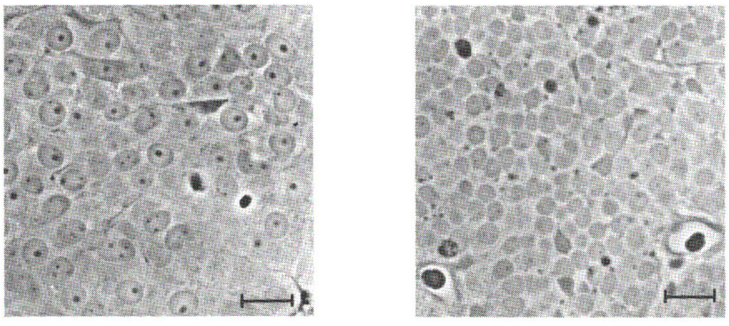
Bromocriptine has been introduced as an alternative to the surgical extirpation of prolactinomas [53]. A number of reports published since 1979 have documented not only that this drug is able to normalize hyperprolactinaemia within a few days in about 66% of the patients, but also that is shrinks the tumour effectively in about 62% of the cases [2, 7, 9, 13, 30, 33, 36, 37, 58, 60]. Clinical experience has shown, however, that an ostensible cure achieved with bromocriptine seldom persists after treatment with the drug is discontinued; recurrence of hyperprolactine-

mia and reenlargement of the tumour usually occur within 1 or 2 weeks [54]. This observation casts some doubt on a previous assumption, based on experimental work in vitro [4, 40, 44], that bromocriptine exerts a cytostatic or even cytocidal effect on prolactinomas.

Light and electron microscopic studies of bromocriptine treated prolactinomas confirm that the drug causes pronounced shrinkage of adenoma cells (figs. 1–3). The nuclei are surrounded by a narrow rim of cytoplasm only, causing a dense accumulation of the cell nuclei that produces a lymphocyte-like picture [26, 46, 55]. A quantitative comparison of untreated and bromocriptine treated adenomas shows that the size of the nucleolus is most severely affected by the drug (tab. 2), and that the cell body, the cytoplasm, and the nucleus shrink less. The complexes of the rough-surfaced endoplasmic reticulum are reduced to single, dispersed tubules and vesicles. The Golgi cisterns become inconspicuous. Secretory granules may become rare. The cells present the morphological pattern of elements with reduced protein synthesis. The intensity of immunostaining and the number of immunostainable cells are markedly reduced [55].

These changes are caused by the bromocriptine-induced inhibition of prolactin gene transcription from DNA to messenger RNA [34, 35]. As the nucleolus is the site of the DNA to messenger RNA transcription, the pronounced reduction in the size of the nucleolus is a direct consequence of this inhibition by the drug. The reduction in the size of the rough-surfaced endoplasmic reticulum, the site of messenger RNA action during protein synthesis, is a secondary effect that leads ultimately to the reduction in prolactin synthesis and lowering or normalization of hyperprolactinaemia.

In a study by Gen et al. [8], microscopic examination of biopsy specimens from only one bromocriptine treated prolactinoma showed signs of tumour cell necrosis.



a b

Fig. 2a, b Phase contrast micrographs of osmium fixed, Epon embedded biopsy specimens of an untreated (a) and a bromocriptine-treated (6 weeks, 7.5 mg per day) prolactinoma (b). Scale 20 μ.

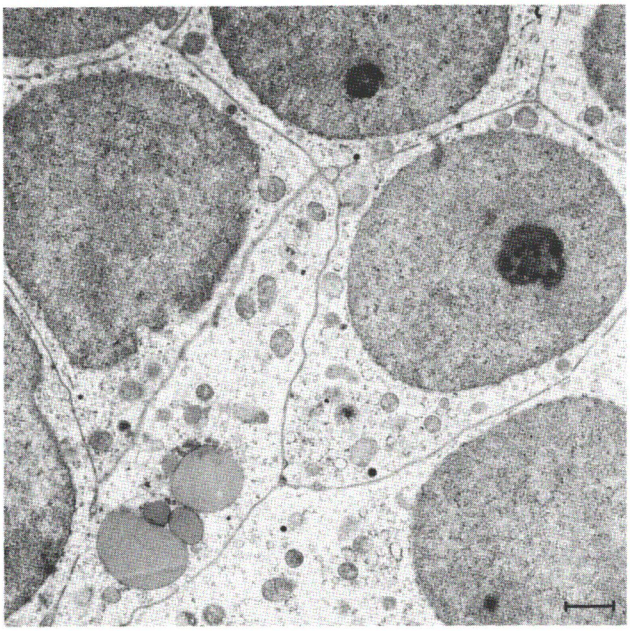


Fig. 3 Electron micrograph. Bromocriptine-treated prolactinoma with small adenoma cells demonstrating a narrow rim of cytoplasm with a reduced rough-surfaced endoplasmic reticulum. The nucleoli are reduced. Some lipid bodies are present. Note that the magnification is the same as in figure 1. Osmium fixation, scale 1 μ.

Table 2 Bromocriptine-induced changes in prolactinoma cell size*

Morphological structure	Percent decrease in size, as compared with untreated controls, caused by bromocriptine treatment of a least 4 weeks' duration
Cell size	−23
Cytoplasm area	−29
Cell nucleus	−18
Nucleolus	−49

* Data from Landolt et al. [26].

Our study has shown neither necrotic cells nor macrophages in the biopsy specimens from our cases, nor have we seen an increase in intraadenomatous calcification.

The rapid shrinkage of the prolactin cells after bromocriptine treatment is initiated causes the intercellular spaces to increase and the tissue structure of the adenoma to break up. Because the softened tissue facilitates surgical removal of the tumour, bromocriptine can be instrumental in improving surgical results in patients with

prolactinomas [25, 59]. Prolonged bromocriptine treatment, however, causes deposition of fibrous tissue in the intercellular spaces. This can be measured in the perivascular area: a significantly increased perivascular fibrosis is observed in biopsy specimens obtained from prolactinomas that are treated with bromocriptine for periods of 3 months or longer [27]. The intraadenomatous fibrosis causes the adenoma to develop a rubber-like consistency, rending surgical removal more difficult at this stage because the adenoma has become more adherent to the surrounding normal tissue. In our series reported in 1982, postoperative normalization of prolactin levels was not achieved in patients who had been treated with bromocriptine for 1 year or more [25].

Prolactin levels maintained after the completion of bromocriptine therapy may be lower after withdrawal than were the pretreatment values. Although this effect has been interpreted as the result of an antiproliferative effect of the drug [5], we suspect that it may rather be a consequence of the perivascular fibrosis, which acts as a diffusion barrier between the secreting adenoma cells and the blood stream.

Discontinuation of bromocriptine treatment is followed by a rapid reenlargement of the adenoma. The cell increases to its pretreatment size within I week [26], and the ultrastructural features typical of untreated adenomas reappear [54].

Summary and conclusions

Most prolactinomas have the light and electron microscopic features typical of cells engaged in active protein synthesis. They often show secondary changes, however, such as intratumoral haemorrhage. calcification, amyloid deposition, and the appearance of dispersed oncocytes. Intraadenomatous fibrosis is present in some biopsy specimens, particularly those from invasive adenomas. Bromocriptine treatment changes the structure and ultrastructure of prolactinomas by blocking the transcription of the prolactin gene from DNA to messenger RNA. This causes a pronounced shrinkage of the nucleolus, the rough-surfaced endoplasmic reticulum, and the Golgi cisterns. The cells shrink by an average of 25% in comparison with cells in untreated samples, and the simultaneous enlargement of the intercellular spaces results in softening of the tumour tissue. Prolonged treatment, however, causes intraadenomatous, particularly perivascular, fibrosis. The fibrosis renders radical and selective removal of the adenoma difficult, if not impossible. We conclude that bromocriptine should not be used in patients suffering from prolactinoma – except briefly, to obtain preoperative tumour shrinkage – unless both the patient and physician accept that such treatment may render later surgery less effective and make continuation of the drug treatment necessary, perhaps for a lifetime.

Acknowledgements

The author thanks Prof. Ph. U. Heitz, Dept. of Pathology, University of Basle, for the immunohistological examination of the adenomas, Miss V. Osterwalder, Dept. of Neurosurgery, University of Zürich, for technical assistance, Mrs. Susan Eastwood-Berry, B. A., Berkeley, California, USA, for editorial assistance, the Sandoz Foundation, Basle, and the EMDO Foundation Zürich, for financial support.

References

[1] Bilbao, J. M., F. Horvath, A. R. Hudson, et al.: Pituitary adenoma producing amyloid-like substance. Arch. Pathol. (Chicago) **100** (1975) 411–415.

[2] Chiodini, P. G., A. Liuzzi, R. Cozzi, et al.: Size reduction of macroprolactinomas by bromocriptine or lisuride treatment. J. Clin. Endocrinol. Metab. **53** (1981) 737–743.

[3] Crompton, M. R.: The pathology of pituitary tumours. In: Pituitary tumours (J. S. Jenkins, ed.), pp. 22–37. Butterworth, London 1973.

[4] Davies, C., J. Jacobi, H. M. Lloyd, et al.: DNA synthesis and the secretion of prolactin and growth hormone by the pituitary gland of the male rat: Effects of diaethylstilboestrol and 2-bromo-alpha-ergocryptine methanesulphonate. J. Endocrinol. **61** (1974) 411–417.

[5] Flückiger, E., E. del Pozo, K. v. Werder: Prolactin: Physiology, pharmacology and clinical findings. Springer-Verlag, Berlin 1982.

[6] Foncin, J. F.: Morphologie ultra-structurale de l'hypophyse humaine. Neurochirurgie **17**, Suppl. 1 (1971) 10–24.

[7] Fossati, P., G. Strauch, J. Tournaire: Etude de l'activitée de la bromocriptine dans les états d'hyperprolactinémie. Nouv. Presse Méd. **5** (1976) 1687–1691.

[8] Gen, M., T. Uozumi, S. Shinohara et al.: Does bromocriptine have a cytocidal effect on prolactinoma cells? Report of a case. Neurol. Med.-Chir. **23** (1983) 61–65.

[9] George, S. R., G. N. Burrow, B. Zinman, et al.: Regression of pituitary tumors, a possible effect of bromoergocryptine. Am. J. Med. **66** (1979) 697–702.

[10] Girod, C., M. Mazzuca, J. Trouillas, et al.: Light microscopy, fine structure and immunohistochemistry studies of 271 pituitary adenomas. In: Pituitary adenomas – biology, physiopathology and treatment (P. Derome, C. P. Jedynak, F. Peillon, eds.), pp. 3–18. Asclepios-Publishers, France 1980.

[11] Hachmeister, U.: Ultrastructural aspects of pituitary tumors. In: Modern aspects of neurosurgery, vol. 4 – Proceedings of the German Society of Neurosurgery (H. Kuhlendahl, M. Brock, D. Le Vay, T. J. Weston, eds.), pp. 108–115. Excerpta Medica, Amsterdam 1973.

[12] Hachmeister, U., R. Fahlbusch, K. v. Werder: Ultrastructural identity of pituitary adenoma cells in Forbes-Albright syndrome and of adenohypophyseal pregancy cells. Acta Endocrinol. (Kbh) Suppl. **152** (1972) 42.

[13] Hall, K., A. M. McGregor, M. F. Scanlon, et al.: Metrizamide cisternography in assessment of pituitary tumour size, with special reference to the demonstration of tumour (prolactinoma) shrinkage on bromocriptine therapy. Progr. Reprod. Biol. **6** (1979) 232–243.

[14] Horvath, E., K. Kovacs: Pathology of the pituitary gland. In: Pituitary diseases (C. Ezrin, E. Horvath, K. Kaufman, K. Kovacs, M. H. Weiss, eds.), pp. 1–83. CRC Press, Boca Raton, Florida 1980.

[15] Kuratsu, J.-I., Y. Matsukado, M. Miura: Prolactinoma of pituitary with amyloid-like substances. Case report. J. Neurosurg. **59** (1983) 1067–1070.

[16] Kurisaka, M., S. C. Tindall, Y. Takei: Cystic prolactinoma of the pituitary following surgery for polycystic ovaries. Neurol. Med.-Chir. (Tokyo) **23** (1983) 55–60.
[17] Landolt, A. M.: Ultrastructure of human sella tumors. Acta Neurochir. (Wien) Suppl. **22** (1975) 1–167.
[18] Landolt, A. M.: Progress in pituitary adenoma biology. In: H. Krayenbühl, (ed.): Advances and technical standards in neurosurgery **5** (1978) 3–49.
[19] Landolt, A. M.: Biology of pituitary microadenomas. In: Pituitary microadenoma (C. Faglia, M. A. Giovanelli, R. M. McLeod, eds.), pp. 107–122. Academic Press, London 1980.
[20] Landolt, A. M.: Surgical treatment of pituitary prolactinomas: postoperative prolactin and fertility in seventy patients. Fertil. Steril. **35** (1981) 620–625.
[21] Landolt, A. M.: Cerebrospinal fluid rhinorrhea: a complication of therapy for invasive prolactinomas. Neurosurgery **11** (1982) 395–400.
[22] Landolt, A. M.: Secreting pituitary adenomas: morphological and biological aspects. In: Pituitary hyperfunction: physiopathology and clinical aspects (F. Camanni, E. F. Müller, eds.), pp. 135–155. Raven Press, New York 1984.
[23] Landolt, A. M.: Morphology and natural course of prolactinomas. In: Proceedings of the 3rd european symposium on pituitary adenomas (S. W. J. Lamberts, J. Assies, F. J. H. Tilders, F. A. van der Veen, eds.). Blackwell Publishers, London 1984 (in press).
[24] Landolt, A. M.: Prolactinomas: Structure, ultrastructure and bromocriptine-induced changes. In: Functioning pituitary adenoma; proceedings of the fourth japanese workshop on pituitary tumors (T. Uozumi, ed.). Osaka 1984 (in press).
[25] Landolt, A. M., P. J. Keller, E. R. Froesch, et al.: Bromocriptine: does it jeopardise the result of later surgery for prolactinomas? Lancet **2** (1982) 657–658.
[26] Landolt, A. M., H. Minder, V. Osterwalder, et al.: Bromocriptine reduces the seize of cells in prolactin-secreting pituitary adenomas. Experientia **39** (1983) 625–626.
[27] Landolt, A. M., V. Osterwalder: Perivascular fibrosis in prolactinomas: is it enhanced by bromocriptine? Clin. Endocrinol. Metab. **58** (1984) 1179–1183.
[28] Landolt, A. M., V. Rothenbühler: Pituitary adenoma calcification. Arch. Pathol. Lab. Med. **101** (1977) 22–27.
[29] Landolt, A. M., V. Rothenbühler, G. S. Kistler: Morphology of the chromophobe adenoma. In: Treatment of pituitary adenomas (R. Fahlbusch, K. v. Werder, eds.), pp. 154–171. Georg Thieme Verlag, Stuttgart 1978.
[30] Landolt, A. M., R. Wüthrich, H. Fellmann: Regression of pituitary prolactinoma after treatment with bromocriptine. Lancet **1** (1979) 1082–1083.
[31] Le Beau, J., J. F. Foncin: Contribution à l'étude ultrastructurale des adénomes à prolactine. Ann. Endocr. (Paris) **33** (1972) 353–356.
[32] Lewis, P. D., S. van Noorden: "Nonfunctioning" pituitary tumors. Arch. Pathol. **97** (1974) 178–182.
[33] Matsumura, S., S. Mori, T. Uozumi: Size reduction of a large prolactinoma by bromoergocriptine (CB-154) treatment. In: Pituitary adenomas: biology, physiopathology and treatment (P. J. Derome, C. P. Jedynak, F. Peillon, eds.), pp. 336–339. Asclepios Publishers, France 1980.
[34] Maurer, R. A.: Dopaminergic inhibition of prolactin synthesis and prolactin messenger RNA accumulation in cultured pituitary cells. J. Biol. Chem. **255** (1980) 8092–8097.
[35] Maurer, R. A.: Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP. Nature **294** (1981) 94–97.
[36] McGregor, A. M., M. F. Scanlon, K. Hall, et al.: Reduction in size of a pituitary tumor by bromocriptine therapy. N. Engl. J. Med. **300** (1979a) 291–293.

[37] McGregor, A. M., M. F. Scanlon, R. Hall, et al.: Effects of bromocriptine on pituitary tumour size. Br. Med. J. **2** (1979b) 700–703.
[38] Mirouze, J., C. Jaffiol, P. Mary, et al.: Deux syndromes originaux "Amenorrhée-Galactorrhée" par tumeur hypophysaire. Discussion anatomo-clinique. Etude ultrastructurale de l'un d'eux. Ann. Endocrinol. (Paris) **30** (1969) 810–821.
[39] Nurnberger, J. I., S. R. Korey: Pituitary chromophobe adenomas. Springer Publishing Comp., New York 1953.
[40] Pawlikowski, M., J. Kunert-Radek, H. Stepien: Direct antiproliferative effect of dopamine agonists on the anterior pituitary gland in organ culture. J. Endocrinol. **79** (1978) 245–246.
[41] Peake, G. T., D. W. McKeel, H. L. Jarett, et al.: Ultrastructural, histologic and hormonal characterization of a prolactin-rich human pituitary tumor. J. Clin. Endocrinol. Metab. **29** (1969) 1385–1393.
[42] Peillon, F., J. Racadot, D. Moussy, et al.: Prolactin-secreting adenomas. A correlative study of morphological and clinical data. In: Treatment of pituitary adenomas (R. Fahlbusch, K. v. Werder, eds.), pp. 114–122. Georg Thieme Verlag, Stuttgart 1978.
[43] Peillon, F., E. Vila-Porcile, L. Olivier, et al.: L'action des œstrogènes sur les adénomes hypophysaires chez l'homme. Documents histopathologiques en microscopie optique et électronique et àpport à l'expérimentation. Ann. Endocr. (Paris) **31** (1970) 259–270.
[44] Quadri, S. K., K. H. Lu, J. Meites: Ergot-induced inhibition of pituitary tumor growth in rats. Science **176** (1972) 417–418.
[45] Racadot, J., F. Vila-Porcile, F. Peillon, et al.: Adénomes hypophysaires à cellules à prolactine: étude structurale et ultrastructurale, corrélations anatomo-cliniques. Ann. Endocr. (Paris) **32** (1971) 298–305.
[46] Rengachari, S. S., T. Tomita, B. F. Jeffries, et al.: Structural changes in human pituitary tumor after bromocriptine therapy. Neurosurgery **10** (1982) 242–251.
[47] Robert, F.: Le prolactinome – aspects pathologiques. Neurochirurgie **27**, Suppl. 1 (1981) 61–73.
[48] Robert, F., J. Hardy: Prolactin-secreting adenomas: A light and electron microscopical study. Arch. Pathol. **99** (1975) 625–633.
[49] Saeger, W.: Hypophyse, In: Spezielle, pathologische Anatomie (W. Doerr, G. Seifert, eds.), vol. 14/I, pp. 2–226. Springer-Verlag, Berlin 1981.
[50] Scanarini, M., R. Giordano, S. Mingrino, et al.: Fine structure of prolactin pituitary adenoma with special reference to annulate lamellae. Acta Neuropath. (Berl.) **46** (1979) 163–166.
[51] Schnitker, M. T., E. C. Cutler, O. T. Bailey, et al.: The chromphobe adenomas of the pituitary: pathologic features and response to irridiation based on a study of 81 verified cases. Am. J. Roentgenol. **40** (1938) 645–659.
[52] Shucart, W. A.: Implications of very high serum prolactin levels associated with pituitary tumors. J. Neurosurg. **52** (1980) 226–228.
[53] Thorner, M. C., A. S. McNeilly, C. Hagan, et al.: Long-term treatment of galactorrhea and hypogonadism with bromocriptine. Br. Med. J. **2** (1974) 419–422.
[54] Thorner, M. O., R. C. Perryman, A. D. Rogul, et al.: Rapid changes of prolactinoma volume after withdrawal and reinstitution of bromocriptine. J. Clin. Endocrinol. Metab. **53** (1981) 480–483.
[55] Tindall, G. T., K. Kovacs, E. Horvath, et al.: Human prolactin-producing adenomas and bromocriptine: a histological, immunocytochemical, ultrastructural, and morphometric study. J. Clin. Endocrinol. Metab. **55** (1982) 1178–1183.

[56] Tournaire, J. P. Guinet, I. Bachelot, et al.: Les adénome à prolactine. Oto-Neuro-Ophth. **47** (1974) 303–320.
[57] Trouillas, J., M. Curé, M. Lheritier, et al.: Les adénomenes hypophysaires avec aménorrhée-galactorrhée ou galactorrhée isolée. Lyon Méd. **236** (1976) 359–375.
[58] Wass, J. A. H., P. J. H. Moult, M. O. Thorner, et al.: Reduction of pituitary tumour size in patients with prolactinomas and acromegaly treated with bromocriptine with or without radiotherapy. Lancet **2** (1979) 66–69.
[59] Weiss, M. H., R. R. Wycoff, R. Yadley, et al.: Bromocriptine treatment of prolactin-secreting tumors: surgical implications. Neurosurgery **12** (1983) 640–642.
[60] Woodhouse, N. J. Y., F. Khouqueer, J. O. Sieck: Prolactinomas and optic nerve compression: Disappearance of a suprasellar extension and visual recovery after two weeks bromocriptine treatment. Hormone Res. **14** (1981) 141–147.
[61] Yaghmai, F.: Clinical pathological correlation of pituitary adenomas. In: The pituitary – a current review (M. B. Allen, V. B. Mahesh, eds.), pp. 373–386. Academic Press, New York 1977.

Prolactinomas and mixed adenomas with prolactin cells: an immunohistochemical study of the subcellular localization of hormones

G. Jautzke, J. Iglesias

Introduction

Since the introduction of immunocytochemical investigations it has been known that, in most cases, pituitary adenomas are prolactinomas [3]. Increased prolactin values in serum in the case of a pituitary adenoma may either be due to a prolactinoma or to a disinhibition mechanism [5]. Therefore, only the immunohistochemical detection of prolactin production in the adenoma cells can provide evidence for the presence of a prolactinoma.

Materials and methods

In a retrospective study, we examined the surgical biopsies of 98 patients, who had been operated in Berlin between 1969 and 1983 mostly because of large pituitary adenomas. The material which was routinely fixed in formalin and embedded in paraffin, was examined by light microscopy using the PAP method [7], and more recently predominantly using the avidin-biotin-complex method [2]. From the paraffin blocks, suitable tissue specimens were re-embedded in resin (Epon). Several methods can be used for material embedded in resin:

1. After removal of the resin [4], the immune reaction can be performed in the semithin section, and, in addition, semithin serial sections can be examined for different hormones.

2. In the semithin section, the immunocytochemical reaction can be performed, and in the contrasted thin section, which follows immediately thereafter, the ultrastructure can be analyzed.

3. Immunohistochemistry can be performed in thin sections using either the PAP method or the protein A gold method [6].

The following antisera were used:
anti-human prolactin (Radioassay Systems Cab. Cal.), anti-porcine ACTH (1–39) (Immuno Nuclear Corporation), anti-human-GH (Serono), anti-TSH (Serono), monoclonal anti-alpha subunit (Medix). The bridge antiserum and the PAP-complex were obtained from Dako. The ABC-method was performed using a kit from Vector.

Results

In 36 of 98 pituitary adenomas, we found prolactin cells. In 17 cases, only prolactin was found, in 14 cases, prolactin and growth hormone (GH), and in 5 cases, prolactin and hormones other than GH.

Prolactinomas

In the 17 cases of "pure" prolactinomas, the ratio of men to women was 8:9, the age distribution in men was 15 to 69 years with an average of 40 years, in women it was 22 to 65 years with an average of 36 years. Clinically, and most adenomas were large and involved visual disorders, and in the women they also involved concomitant secondary amenorrhoea. Light-microscopically, all the adenomas were chromophobic. In immunohistochemistry, most tumour cells were labelled. In the semithin section, the prolactin immune reactivity manifested itself in the form of partly arched, partly ring-shaped bands near the nuclei. Only in one case, there were also individual cells (less than 5% of the adenoma cells) in which the cytoplasm showed a positive reaction along the cell membrane. The semithin-thin section technique, in which the immune reaction was performed in the semithin section and the subsequent thin section was contrasted with uranyl acetate, yielded the following results (fig. 1a, b, c):

The prolactin-positive areas corresponded to the cytoplasmic zones in the thin section which

1. were free from rough endoplasmic reticulum,
2. contained immature secretory granules, and
3. yielded indications of Golgi's membranes that were barely discernible due to the absence of contrast with osmium oxide.

The protein A gold method yielded a picture in the thin section which corresponded to the results already mentioned (fig. 2). Gold particles, which label the prolactin, were never found in the rough endoplasmic reticulum but only in the Golgi field, with the immature granules being densely labelled and the granula-free

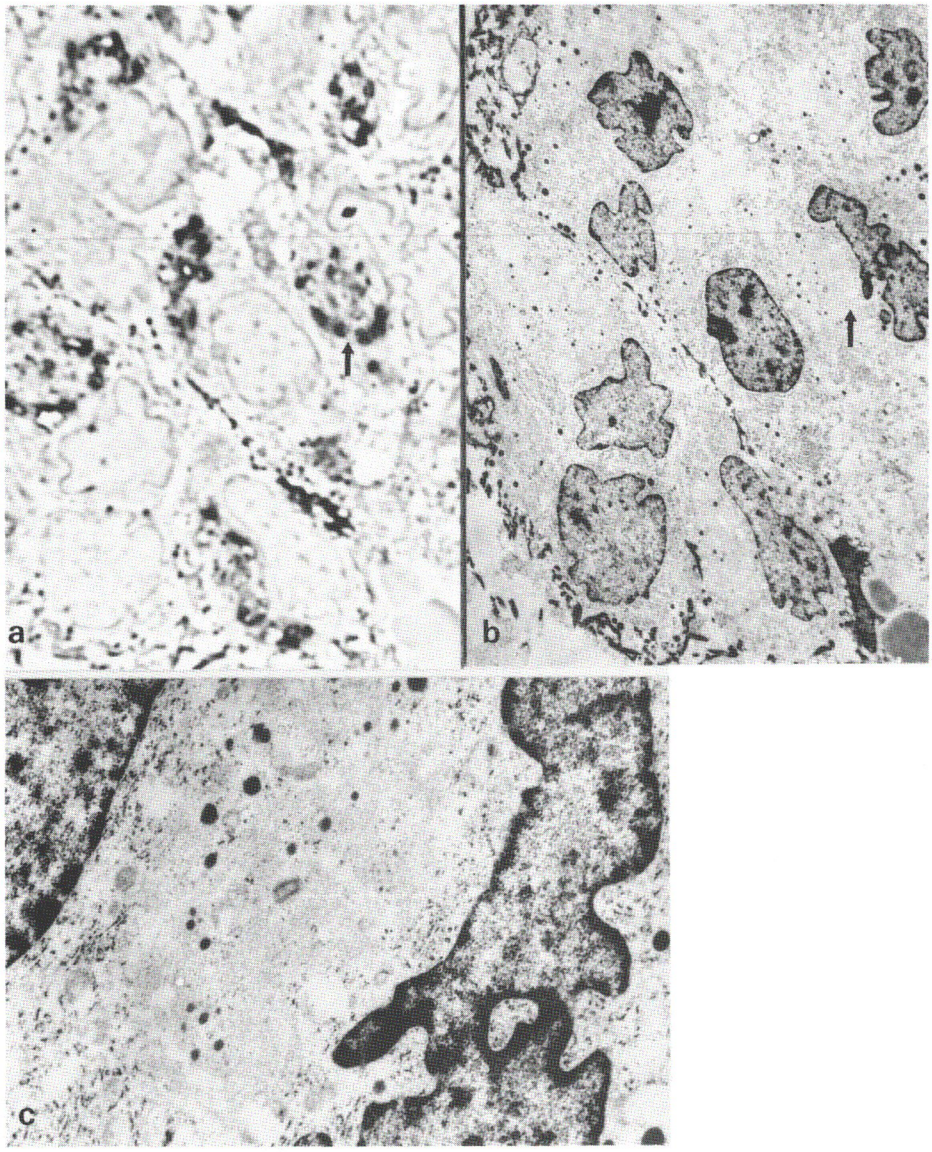


Fig. 1 a) Semithin section of a sparsely granulated prolactinoma immunostained for prolactin, which is found in circumscribed areas near the nucleus as dark deposits (arrow), (3600×). b) Thin section adjacent to the one shown in Figure 1a, contrasted with uranyl acetate. The immunostaining in Figure 1a corresponds to areas that are free of RER and contain immature granules (arrow), (3600×). c) Same section as Figure 1b at higher magnification shows barely discernible Golgi's membranes surrounding the immature secretory granules and a centriole (15000×).

areas of the Golgi fields being more weakly labelled. The labelled secretory granules were also found to be fused with the cell membrane (fig. 3). Some single granules had been released into the extracellular space, a process that has been designated as "misplaced exocytosis" [1].

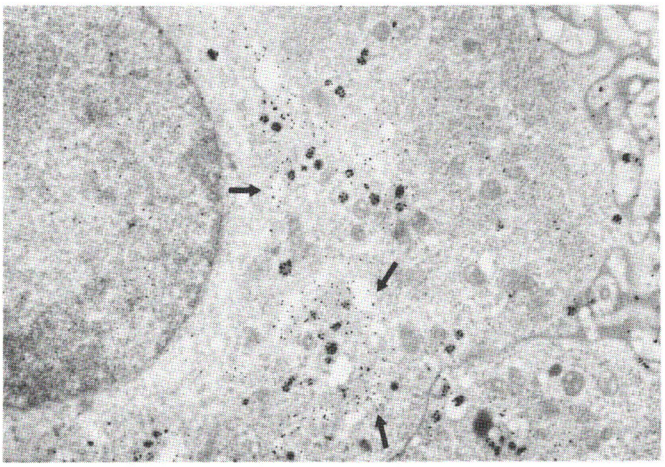


Fig. 2 Sparsely granulated prolactinoma immunostained for prolactin with the protein A gold method. Immature granules are densely labelled. Weak labelling of granule-free areas of the Golgi field (arrows), (12000×).

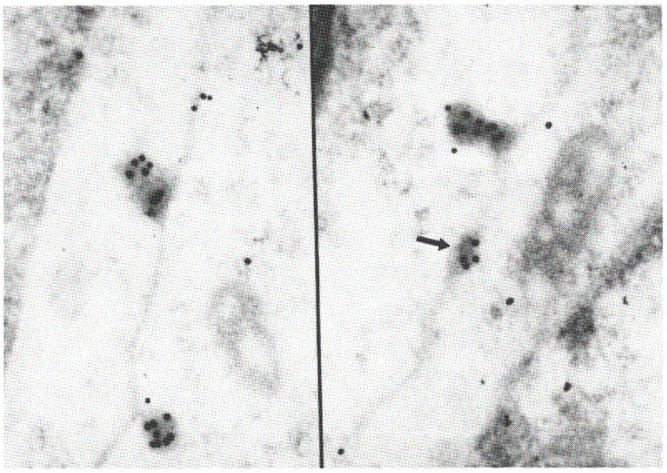


Fig. 3 Same section as in figure 2. Labelled secretory granules fuse with the cell membrane, some of which are released into the extracellular space (arrow), (25000×).

Adenomas with immunohistochemically detectable prolactin and GH-cells

In 14 cases, we found pituitary tumours which contained both prolactin and GH-immunoreactive cells (tab. 1). The majority of these cases (10 cases) showed a predominance of growth hormone cells. 4 of them were densely granulated, 6 were sparsely granulated. Only those cases with a predominance of GH-cells in pituitary adenoma clinically showed an acromegaly. In the densely granulated adenoma, the number of labelled growth hormone cells was between 60% and 100%; that of the

Table 1 Adenomas with prolactin cells and GH-cells

1. Predominance of prolactin cells	((?) densely granulated)	0 cases
	sparsely granulated	4 cases
2. Predominance of GH-cells	densely granulated	4 cases
	sparsely granulated	6 cases

prolactin-immunoreactive cells ranged from about 10% to 30% of adenoma cells. In semithin serial sections (fig. 4c and d), it became evident that the granula-containing positive prolactin cells were also positively labelled with growth hormone. However, there were also a few poorly granulated cells which were exclusively prolactin-labelled. In thin sections, in which double labelling was performed with prior visualization of prolactin by the protein A gold method and subsequent localization of growth hormone by the PAP method, it became evident that, apart from the gold labelling within the granules, the prolactin-positive cells showed concomitant labelling with the PAP complexes (figs. 5a and 6). The exclusively prolactin-labelled cells showed immune reactivity in the Golgi field (fig. 5c). Methodologically, this double-labelling does, of course, present a problem, since protein A, which labels prolactin, could also bind the antisera of the second incubation, although between these two incubations, incubation with a strong excess of normal serum was performed. Therefore, we only assess those places as double-stained, in which the PAP complexes and the gold labelling are recognizable as being spatially separated above the granules. Though the method of double-labelling in the present form must be treated with caution, our other results also indicate that the granules of these cells contain both hormones:

1. In the semithin serial sections, all granula-containing prolactin cells showed concomitant labelling with growth hormone.

2. The cells that were exclusively growth-hormone-positive showed the same intensity of immune reactions as the cells containing both growth hormone and prolactin: the majority of the granules could thus be assumed to be growth-hormone-labelled.

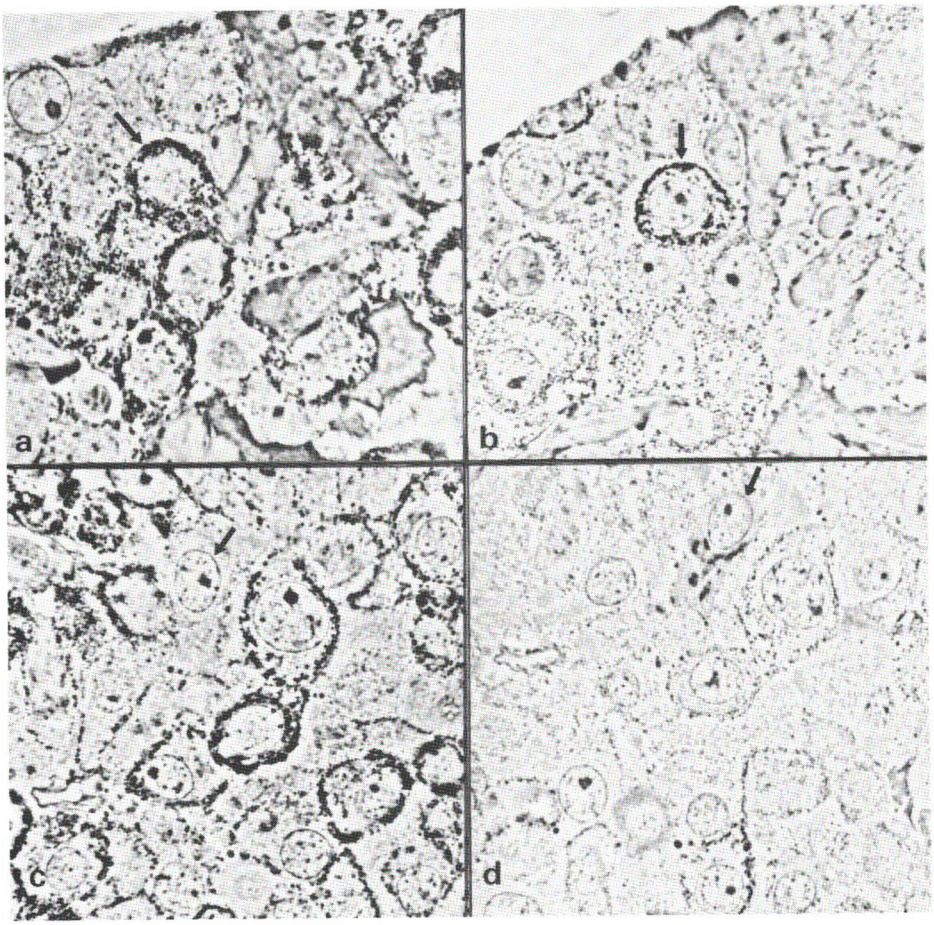
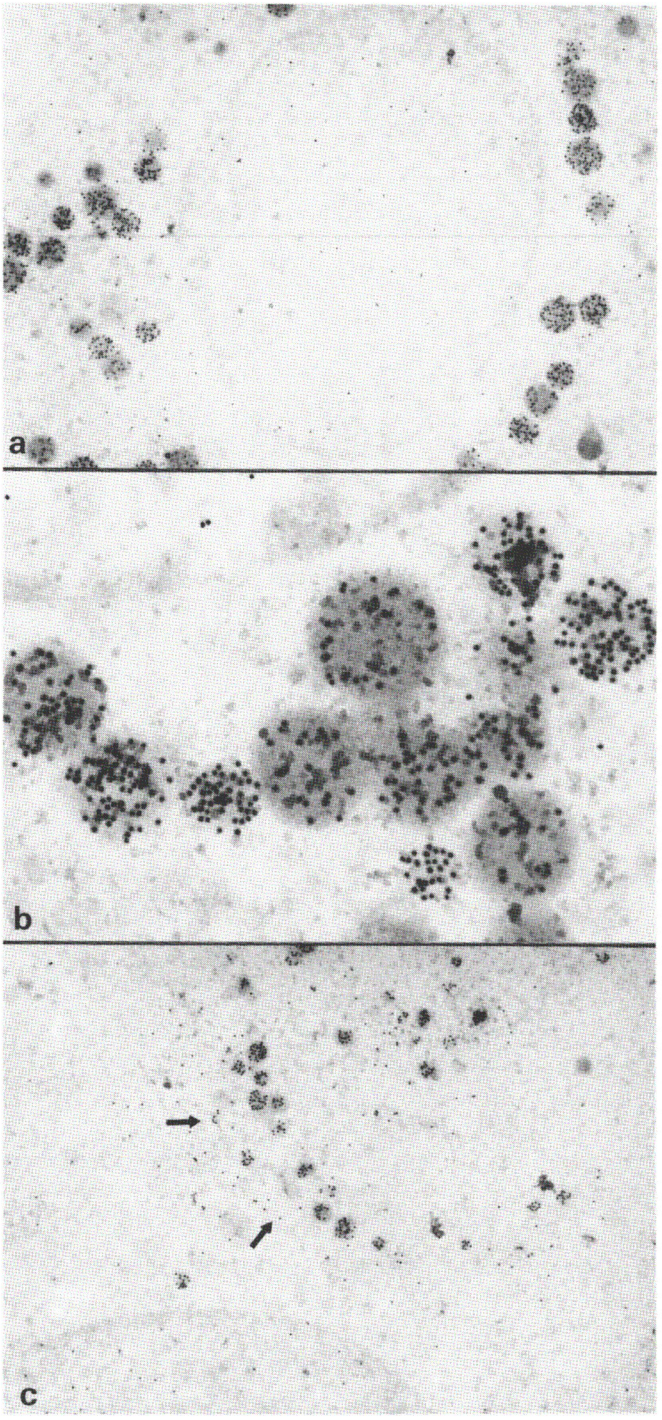


Fig. 4 Densely granulated mixed growth hormone cell and prolactin cell adenoma. a) Semithin section immunostained for growth hormone. Most cells are stained (1600×). b) Semithin section adjacent to the one shown in figure 4a immunostained for prolactin. The only cell stained is a double-stained cell, since the same cell is stained in Figure 4a for growth hormone (arrows) (1600×). c) Semithin section immunostained for growth hormone (1600×). d) Semithin section adjacent to the one shown in figure 4c immunostained for prolactin. One sparsely granulated cell is stained in the Golgi region. This cell is not stained with growth hormone in figure 4c (arrows) (1600×).

▶

Fig. 5 Densely granulated mixed growth hormone cell and prolactin cell adenoma. Thin section with double immunolabelling. Prolactin is visualized using the protein A gold technique in a first step. In a second step, growth hormone is stained with the PAP method. a) Double-stained cell. All granules contain prolactin-labelling gold particles and growth-hormone-labelling PAP-complexes (16500×). b) Double-stained granules at higher magnification (47500×). c) A few cells are solely prolactin-stained with gold particles over the Golgi region (arrows) (16500×).


a
b
c

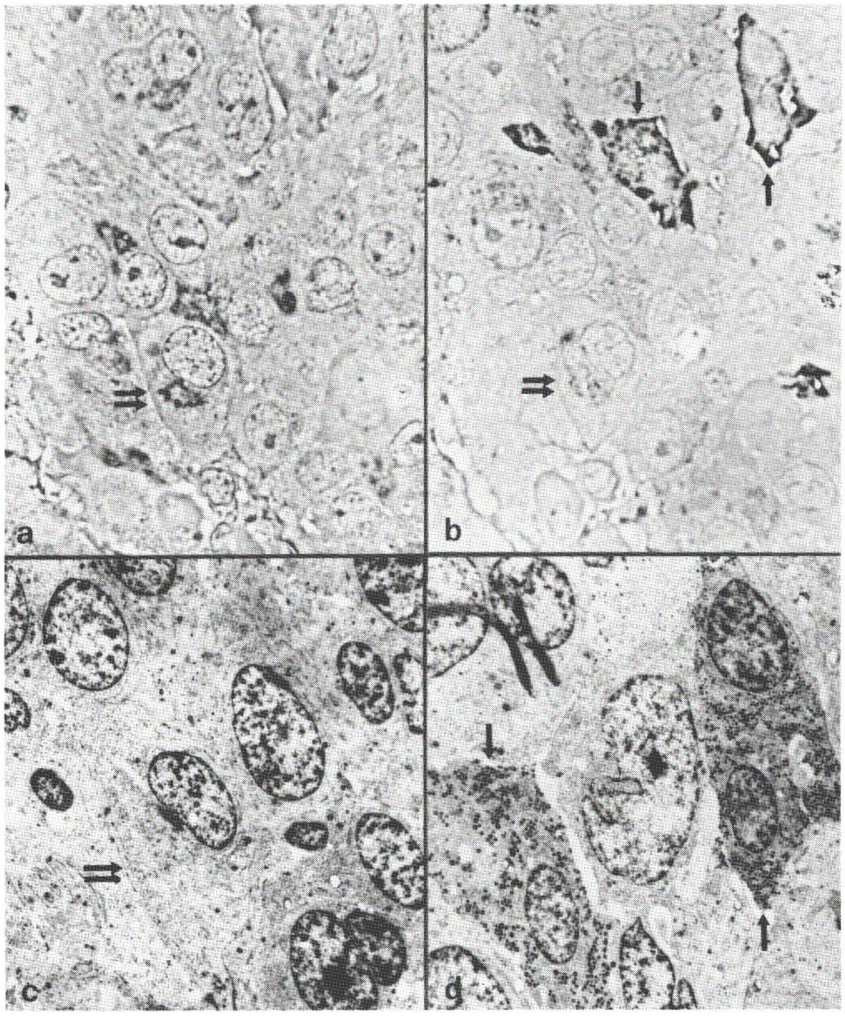


Fig. 6 Sparsely granulated mixed growth-hormone cell and prolactin cell adenoma. a) Semithin section immunostained for prolactin, which is found only in sparsely granulated cells over the Golgi region (1600×). b) Semithin section adjacent to the one shown in Figure 6a immunostained for growth hormone. Two densely granulated cells (arrows) and one sparsely granulated cell (double arrows) are labelled. The latter is a double-labelled cell, since this cell is also labelled for prolactin in figure 6a (1600×). c) Adjacent thin section contrasted with uranyl acetate. The double-labelled cell (double arrows) does not differ from the cells that are only prolactin-labelled (3600×). d) Same section as in Figure 6c. Densely granulated cells containing only growth hormone (arrows), (3600×).

3. In the thin section, the protein A gold method showed that nearly all granules were also labelled for prolactin in the double-stained cells.

In the sparsely granulated adenomas, the fraction of growth-hormone-positive cells amounted to 10% in one case and nearly 100% in 2 cases with values varying between these two in the other cases. The number of prolactin-immunoreactive cells ranged between 5% and 40% of the adenoma cells. In semithin serial sections (fig. 6a, b, c, d), it became evident that the few granula-containing cells were exclusively GH-labelled; in the ultrathin section, these cells contained granules ranging in size between 130 and 220 nm. The Golgi-field-labelled adenoma cells were largely prolactin-positive cells, though there were some cells that showed both prolactin and growth hormone in the Golgi region. These cells did not differ electronmicroscopically from the cells that were only prolactin-labelled.

Similar results were observed in the 4 adenomas which showed a clear predominance of the prolactin cells. In these cases as well, there were some cells that were granulated and contained only growth hormone and a few cells that contained both growth hormone and prolactin in the Golgi region.

Mixed adenomas with immunostainable prolactin cells and hormones other than GH

We found two cases in which, in addition to prolactin, the alpha-chain of the glycoprotein hormones was also detectable. These adenomas did not differ clinically from the "pure" prolactinomas. They showed cells with concomitant prolactin and alpha-chain-labelling and identical distribution in the cytoplasm.

Furthermore, we found 3 adenomas in which prolactin appeared together with other hormones (tab. 2). One case contained ACTH-immunoreactive cells concomitantly labelled with prolactin. This adenoma was large and endocrinologically silent. A further adenoma contained prolactin, growth-hormone and the alpha-chain of the glycoprotein hormones. Some cells were concomitantly labelled with all 3 hormones (fig. 7a, b, c). Another adenoma contained prolactin in 30% of cells,

Table 2 Other mixed adenomas with prolactin cells

Hormones	Number of cases	Double-stained cells found	not found
Prolactin + α-chain	2	+	
Prolactin + ACTH (clinically silent)	1	+	
Prolactin + GH + α-chain	1	+ (3-fold)	
Prolactin + GH + TSH + α-chain (clinical hyperthyroidism)	1		+

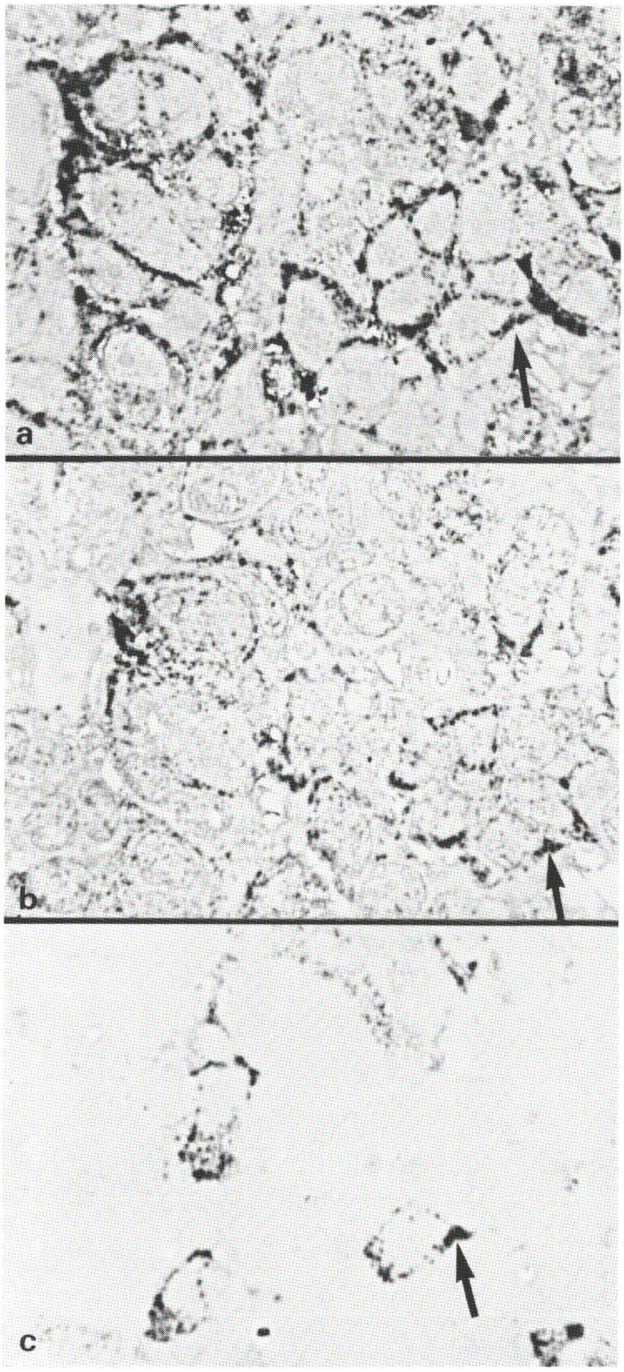


Fig. 7 Serial semithin sections of a mixed adenoma immunostained for alpha-chain (7a), prolactin (7b) and growth hormone (7c). One cell produces all three hormones (arrows).

growth-hormone in 10%, alpha chain in 30%, and TSH in 10% of cells. Nevertheless, the adenoma corresponded clinically to a TSH-producing adenoma with increased TSH-values in the blood.

Conclusions

1. The "pure" prolactinomas were all sparsely granulated in our material. Densely granulated prolactin cells were only found in mixed adenomas.

2. Prolactin- and GH-producing mixed adenomas are frequent. In addition to cells that only form one hormone, double-labelled cells also occur. If these are densely granulated, both hormones are found in one granule in the Golgi field in sparsely granulated cells. Only the cases in which the GH-cells predominate were clinically associated with acromegaly.

3. Mixed adenomas that form prolactin and ACTH or prolactin and alpha-chain are rarely found. Both hormones can also be formed in one cell in these adenomas.

4. In one adenoma, we found prolactin, GH and alpha-chains, in which these 3 hormones occasionally occurred in one cell. This means that, in cases of mixed pituitary adenomas, the thesis "one hormone, one cell" must be revised.

5. In one adenoma, we found prolactin, GH, alpha chain and TSH. Since this patient presented endocrinologically with hyperthyroidism, only the TSH-cell fraction was clinically active. This suggests that plurihormonal adenomas must be classified not only by immunohistochemical methods but by clinical parameters as well.

References

[1] Horvath, E., K. Kovacs: Misplaced exocytosis: Distinct ultrastructural feature in some pituitary adenomas. Arch. Pathol. **97** (1974) 221.
[2] Hsu, S. M., L. Raine, H. Fanger: Use of Avidin-Biotin-Peroxidase Complex (ABC). In: Immunoperoxidase Techniques. J. Histochem. Cytochem. **29** (1981) 577–580.
[3] Kovacs, K., E. Horvath, N. Ryan: Immunocytology of the human pituitary. In: Diagnostic Immunohistochemistry Masson Monographs in Diagnostic Pathology (A. Delellis, ed.), pp. 17–35. Masson, New York–Paris 1981.
[4] Mayor, H. D., J. C. Hampton, B. Rosario: A simple method for removing the resin from epoxy-embedded tissue. J. Biophys. Biochem. Cytol. **9** (1961) 909–910.
[5] Reichlin, S.: The prolactinoma problem. N. Engl. J. Med. **300** (1979) 313–315.
[6] Roth, J., M. Bendagan, L. Orci: Ultrastructural localization of intracellular Antigens by the use of Protein A Gold Complex. J. Histochem. Cytochem. **26** (1978) 1074–1081.
[7] Sternberger, L. A., P. H. Hardy, J. J. Cuculis et al.: The unlabelled antibody enzyme method of immunohistochemistry: Preparation and properties of soluble antigen-antibody complex (horseradish peroxydase – antihorseradish peroxydase) and its use in identification of spirochetes. J. Histochem. Cytochem. **18** (1970) 315–333.

Immunocytochemical, chemical and nuclear-DNA studies of pituitary tumours*

P. O. Lundberg, C. Muhr, L. Grimelius, Y. Olsson, R. Hugosson, J. Stahle, L. Wide, K. Bergström

Introduction

The introduction in recent years of immunocytochemical and chemical techniques in the diagnosis of pituitary adenomas have made it clearly evident that the previously used classification into chromophobe and chromophile types of these tumours is inadequate. Newer classification systems have been proposed in which adenomas are grouped on the basis of a combination of clinical symptoms, blood hormone activity, immunocytochemistry and electron microscopy (e.g. [4]). In other studies, however, considerable variability has been found in the characteristics of adenomas as revealed by the above methods [7, 10, 13, 14, 16].

DNA studies of tumour cells have contributed greatly towards solving problems of differentiation between different tumour types in many endocrine organs, for example the parathyroid gland [3] and the pituitary [1, 2].

The aim of the present investigation was to gain a more thorough knowledge of the characteristics of pituitary adenomas, in the hope of making possibly more accurate prognostic evaluations and of providing better grounds for the choice between different treatment alternatives.

Patients

Patients who had undergone surgery for a pituitary adenoma in the Department of Neurosurgery (29 cases) or the Department of Oto-Rhino-Laryngology (17 cases) at the University Hospital of Uppsala during the last 6-year period were studied. There were 31 females and 15 males. Their age at the time of operation ranged from 17–72 years (mean 46.8 years).

The patients were divided into three groups on the basis of clinical symptoms and determinations of serum growth hormone and serum prolactin. Group A consisted

* This work was supported by a grant from the Swedish Medical Research Council (project no. 102).

of 16 patients with acromegaly (basal serum growth hormone 6.8–156 μg/l). Twelve of them were females and four males. Eight of the females and one male had slight hyperprolactinaemia (serum prolactin 32–75 μg/l).

Group B consisted of 20 further patients with hyperprolactinaemia – 15 females and 5 males. All the males and 12 of the females had slight or moderate hyperprolactinaemia, with serum prolactin values between 23 and 124 μg/l. In three females the serum prolactin concentrations were 410, 419 and 1100 μg/l, respectively. In none of these patients was the serum level of growth hormone elevated.

Group C comprised 10 patients, 4 females and 6 males, with presumably functionless pituitary adenomas. None of them had any clinical symptoms or signs, or blood hormone values, indicating endocrine hyperfunction.

The indication for surgery in the group A was acromegaly and in groups B and C progressive tumour growth with compression of the optic chiasm and visual defects.

All patients had an asymmetrical or enlarged sella turcica on plain sellar films. Computed tomography showed a pituitary adenoma with higher attenuation after injection of contrast medium in all cases. The case material thus did not include any patients with so called microadenomas (a pituitary adenoma with a diameter of less than 10 mm). Nor were any cases of invasive pituitary adenoma included.

Methods

Surgery

Transfrontal surgery was performed with an osteoplastic bone flap in the right frontal region of the skull. The adenoma was reached by traction of the frontal lobes to the rear and by splitting the arachnoidea in the chiasmatic cistern. In all cases the adenoma was protruding from the midline in between the optic nerves. The adenoma tissue was removed in pieces. Necrotic or cystically transformed tumour tissue was not saved. The biopsy material thus examined represented the more solid parts of the adenoma.

In the transsphenoidal approach an orbital incision was made and the sphenoidal sinus was reached via the right ethmoid. The posterior part of the nasal septum was removed. The frontal inferior sella wall was then excised and a cruciate incision was made through the dura to expose the pituitary gland. The tumour tissue was removed with pituitary forceps.

After both procedures all tumour tissue was immediately taking care of in accordance with a predetermined scheme for further specialized preparation.

Tumour tissue examination

1. The major tissue portion was fixed in 10% buffered formalin or Bouin's fluid at room temperature for 24 hours. After dehydration, the specimens were embedded in paraffin. Sections 5 μ thick were then stained with haematoxylin-eosin, van Gieson's stain and immunostained with the peroxidase-antiperoxidase (PAP) method of Steinberger [17] for GH, prolactin, ACTH, TSH, β-endorphin and leu- and methenkephalin. The controls were those recommended by Goldman [6] including exposure to respective antisera in excess (100—200 μg/ml diluted antisera). The following antisera and dilutions were used: ACTH (1:400) and TSH (1:1600) from Dakopatts; GH (1:1600) and prolactin (1:400) from L Wide, Uppsala; β-endorphin (1:3200) and leu- and methenkephalin (1:400) a gift from L Terenius, Uppsala.

2. Tissue imprints were prepared before fixation by pressing glass slides against the tissue. The imprints were immediately fixed in acetone/absolute alcohol (1:1) at room temperature. The imprints were then incubated at 37 °C in 0.5 mg/ml ribonuclease solution and stained with buffered ethidium bromide solution. The DNA content of the cells was measured by a fluoroscence cytophotometric method [3]. Lymphocytes were used as reference cells.

3. Very small tissue specimens were fixed by immersion in 3% buffered glutaraldehyde, pH 7.4, at +4 °C. After post fixation for one hour in 1% osmium tetroxide the specimens were dehydrated and embedded in Epon. Semithin sections were cut and stained with toluidine blue for orientation. From selected areas ultrathin sections were cut and counterstained with uranyl acatate and lead citrate and examined in a JEOL −100 C transmission electron microscope.

4. In 18 of the 46 cases some tumour tissue specimens could be stored frozen in liquid nitrogen at −65 °C. GH, prolactin, TSH, FSH and LH in the tumour tissue were extracted in phosphate buffer, pH 5.7 followed by extraction with glycine buffer, pH 9.8. A radioimmunoassay technique [19] was used for determination of hormones in the tumour tissue extracts as well as in serum.

Results

Routine histopathology and electron microscopy

The histopathological characteristics and the ultrastructural features were in accordance with the morphology as described earlier in the literature. The details will be published elsewhere.

DNA measurements

DNA measurements showed a normal DNA content in 26 of the 46 patients (tab. 1). In 13 of these 26 patients tetraploidy was observed in 1–4% of the cells, but this finding is apparently not of pathological significance and can be made in normal pituitary tissue [5].

Twenty patients had a pathological DNA content. In 12 of these patients tetraploidy was found in 5 to 12% of the cells, and occasionally octaploid cells were also found. Eight further patients had grave DNA abnormalities with an aneuploid DNA content.

Table 1 Nuclear DNA-content in pituitary tumours

	DNA	
	normal	pathological
Acromegaly	5 (31%)	11 (69%)
Hyperprolactinaemia	14 (70%)	6 (30%)
Functionless	7 (70%)	3 (30%)

DNA abnormalities were much more common among the 16 acromegalic patients compared with the other two groups. In five of them more than 5% of the cells were tetraploid. Aneuploidy was found in six further cases. The DNA content was normal in only five (31%) of the acromegalic patients. Among the 20 patients with hyperprolactinaemia the DNA content was normal in 14 (70%); aneuploidy was found in two patients and increased tetraploidy in a further four patients. The DNA content was pathological in two of the three patients with high serum prolactin values. Among the 10 patients with functionless pituitary tumours, DNA abnormality in the form of increased tetraploidy was found in only three (70%) and in the other seven patients the DNA content was entirely normal.

Two of the 46 patients were operated on because of tumour recurrency. In both of these cases the DNA content was normal. During the rather short period of observation after surgery (less than 4 years), five patients have displayed clinical and roentgenological signs of tumour recurrency. In three of these patients the DNA content was definitely abnormal at the first operation.

Immunocytochemistry

Cells containing immunoreactive growth hormone were found in tumours from 11 of the 16 acromegalic patients. In three adenomas no GH immunoreactive cells could be found and in two adenomas the staining reaction was unsuccessful due to

less well preserved tissue. Of the 11 tumours in which GH immunoreactive cells were found, eight also contained immunoreactive prolactin. In eight of the patients the prolactin reaction was negative. There was no correlation between the intensity of the immunoreactive staining for growth hormone or prolactin and the serum levels of these hormones.

Of the adenomas from the 20 patients with hyperprolactinaemia nine contained prolactin immunoreactive cells and five contained GH immunoreactive cells. In one patient the staining reaction was unsuccessful for technical reasons. In some further patients the specimens also contained normal pituitary tissue with GH and prolactin immunoreactive cells. There was no apparent correlation between the intensity of the staining and the serum prolactin values. Two of the three patients with high serum prolactin values had tumours containing both prolactin immunoreactive cells and GH immunoreactive cells. In the third case the reaction was negative to both prolactin and GH.

Among the 10 patients with functionless pituitary adenomas only two had tumours containing prolactin immunoreactive cells. In none of the adenomas from these patients were any GH immunoreactive cells found.

In 44 of the 46 cases minor cell populations were immunostained with antisera against ACTH, TSH, endorphin and enkephalin (tab. 2). Eight of the tumours con-

Table 2 Number of patients with positive immunoreactivity in adenoma cells

	n	ACT	TSH	Endorphin	Enkephalin
Acromegaly	15	1 (7%)	6 (40%)	2 (13%)	4 (27%)
Hyperprolactinaemia	19	4 (21%)	6 (32%)	4 (21%)	7 (37%)
Functionless adenomas	10	3 (30%)	2 (20%)	2 (20%)	4 (40%)

tained ACTH immunoreactive cells, 16 TSH immunoreactive cells, eight endorphin immunoreactive cells and 15 enkephalin immunoreactive cells. The frequency of tumours containing ACTH, TSH, endorphin and enkephalin immunoreactive cells did not differ significantly between the three groups. It has not been possible so far to correlate the occurrence of ACTH, TSH, endorphin or enkephalin immunoreactive cells with the clinical picture or blood hormone levels in any of the three groups of patients.

Hormonal extraction from tumour tissue

Unfortunately it was only possible to obtain specimens large enough for hormonal extraction procedures in 18 of the 46 patients. Evaluation of the results is difficult because of the insufficient knowledge of values for non-tumourous pituitary tissue

[18]. Very high GH extraction values (more than 1000 ng/mg wet weight) were found in specimens from three of the acromegalic patients but in none of the patients in groups B and C. High prolactin extraction values (more than 100 ng/mg wet weight) were noted in five of the acromegalic patients and in two of the patients with hyperprolactinaemia in group B, and in one of the patients with a functionless pituitary adenoma. In five of the patients with hyperprolactinaemia the tissue extraction values for prolactin were low.

Discussion

DNA measurements have gained increasing interest in the last years. In several studies an abnormal DNA content has been demonstrated in tumours. The knowledge concerning the DNA content in normal human pituitary tissue and pituitary tumours is limited. However, a low percentage of tetraploidy seems to be common even in normal pituitary tissue [5]. If a tetraploidy frequency of up to 4 per cent is accepted as normal, then 20 of 46 patients (43.5%) in the present series had tumours with a pathological DNA content. This frequency is similar to that reported by Anniko et al. [2], who found aneuploidy in nine out of 24 patients (35.5%) with pituitary adenomas of different types, using a flow-cytofluorometric method.

It has generally been considered that an abnormal, and especially an aneuploid DNA content implies malignancy [5]. Pituitary adenomas expressing malignancy in the form of metastasis are extremely rare. However, invasive tumour growth, that is tumours growing in an infiltrative and destructive manner, destroying bone structures cranial nerves and the brain, are not uncommon [11, 15]. No such tumour was included in the present series, as we do not consider them suitable for surgery. In the present series routine histopathology did not reveal any indication of malignancy in any of the adenomas.

It is extremely difficult to evaluate the rate of tumour growth in such a series of patients as this, since the development of symptoms is often vague. Two patients operated on because of recurrency did not show an abnormal DNA content in the tumour tissue. On the other hand, three out of five patients who had already developed a recurrency after the DNA determination were included among those who had an abnormal DNA content. This might represent a tendency, but the question whether an abnormal DNA content carries an increased risk of recurrency can only be judged after a long period of follow-up.

The most conspicuous finding was that a pathological DNA content was much more common among patients with acromegaly (69%) than among those with hyperprolactinaemia or a functionless pituitary adenoma (30%).

In a recent study of 55 adenomas from patients with acromegaly, it was found that all of them contained GH immunoreactive cells and 25 (45%) contained prolactin

immunoreactive cells [8]. Our findings of 78% and 50%, respectively, are in good accordance with these figures.

Hyperprolactinaemia in patients with a pituitary adenoma does not necessarily mean that the adenoma is actively producing hormone, i.e. that it is a prolactinoma. Serum prolactin values of up to 100 μg/l, or according to some authors even somewhat higher, may occur in patients with other types of tumours such as meningeomas, metastastic carcinomas and so on ([12] and others). This may be caused by impingement on the pituitary stalk. Suprasellar extension of a purely GH-secreting adenoma or a functionless pituitary adenoma may also give rise to hyperprolactinaemia in this way [8, 9].

Three patients in the present series had a hyperprolactinaemia of 410, 490 and 1100 μg/l, respectively. However, only in the latter two prolactin immunoreactive cells were found. The correlation between tissue extraction values of prolactin, the occurrence of prolactin immunoreactive cells and the level of serum prolactin was also poor both in the nine acromegalic patients with hyperprolactinaemia and in the other 20 patients with hyperprolactinaemia. One should bear in mind, however, that the techniques were employed on different tissue specimens.

The results from this series of patients shed no light on the clinical importance of the presence of ACTH, TSH, enkephalin and endorphin immunoreactive cells.

Conclusion

1. Pathological DNA content is much more common in pituitary adenomas from acromegaly patients than in those from patients with hyperprolactinaemia or functionless pituitary adenomas.

2. There is good correlation between GH immunoreactive cells in pituitary adenomas and increased serum GH levels.

3. Pituitary adenomas with moderately increased serum prolactin levels probably do not actively produce hormone, i.e. they are not prolactinomas.

References

[1] Anniko, M., L.-E. Holm, B. Tribukait, et al.: DNA characteristics of human pituitary tumours. Acta Otolaryngol. Suppl. 379 (1981a) 5.

[2] Anniko, M., L.-E. Holm, C. Silfverswärd, et al.: Cellular DNA content of pituitary tumours: Its relationship to morphology and hormonal type of tumour. Acta Otolaryngol. Suppl. **379** (1981b) 13.

[3] Bengtsson, A., L. Grimelius, H. Johansson, et al.: Nuclear DNA-content of parathyroid cells in adenomas, hyperplastic and normal glands. Acta Pathol. Microbiol. Scand., Sect A **85** (1977) 455.

[4] Berry, R. C., H. J. Caplan: An overview of pituitary tumours. Ann. Clin. Lab. Science **9** (1979) 94.
[5] Böhm, N., W. Sandritter: DNA in human tumours. A cytophotometric study. Curr. Top. Pathol. **60** (1975) 151.
[6] Goldman, M.: Fluorescent antibody methods, p. 159. Academic Press, New York 1968.
[7] Horwath, E., K. Kovacs: Ultrastructural classification of pituitary adenomas. J. Can. Sci. Neurol. Feb. (1976) 9.
[8] Kanie, N., N. Kageyama, A. Kuwayama, et al.: Pituitary adenomas in acromegalic patients: An immunohistochemical and endocrinological study with special reference to prolactin-secreting adenoma. J. Clin. Endocrinol. Metab. **57** (1983) 1093.
[9] Kruseman, A. C. N., G. Th. A. M. Bots, F. Roelfsma, et al.: Immunocytochemical growth hormone and prolactin in pituitary adenomas causing acromegaly and their relationship to basal serum hormone levels and the growth response to thyrotropin releasing hormone. Clin. Endocrinol. **19** (1983) 1.
[10] Landolt, A. M.: Praktische Bedeutung neuer Erkentnisse über Struktur und Funktion von Hypophysenadenomen. Schw. Med. Wschr. **108** (1978) 1521.
[11] Lundberg, P. O., B. Drettner, A. Hemmingsson et al.: The invasive pituitary adenoma. Arch. Neurol. **34** (1977) 742.
[12] Lundberg, P. O., P. O. Osterman, L. Wide: Serum prolactin in patients with hypothalamus and pituitary disorders. J. Neurosurg. **55** (1981) 194.
[13] Martinez, A. J., A. Lee, J. Moossy, et al.: Pituitary adenomas: Clinicopathological and immunohistochemical study. Ann. Neurol. **7** (1980) 24.
[14] McCormick, W. F., N. S. Halmi: Absence of chromophobe adenomas from a large series of pituitary tumours. Arch. Pathol. **92** (1971) 231.
[15] Robert, F.: Morphology of human prolactinomas: A study of invasive prolactin-secreting adenomas. In: Prolactin and Prolactinomas (G. Tolis et al., eds.), pp. 339–349. Raven Press, New York 1983.
[16] Saeger, W.: Modern concepts of pituitary morphology and pathology of pituitary adenomas. Per. Biol. **85**, Suppl. 1 (1983) 9.
[17] Sternberger, L. A.: Immunohistochemistry, pp 143–144. Prentice-Hall, Englewood Cliffs, NJ, 1974.
[18] Trouillas, J., C. Girod, G. Sassolas, et al.: A human β-endorphin pituitary adenoma. J. Clin. Endocrinol. Metab. **58** (1984) 242.
[19] Wide, L.: Radioimmunoassays employing immunosorbents. Acta Endocrinol. **142**, Suppl. (1969) 207.

Hyperplasia of prolactin pituitary cells with or without microadenoma

M. Scanarini, S. Mingrino, I. Mastrogiacomo, E. Serafini, D. Cervesato

Introduction

Among the various types of pituitary adenomas more and more interest has been focussed on prolactinomas, since many questions need to be answered concerning their biology and the therapeutic indications for these tumours [1, 2]. The aim with the present study was to correlate the clinical course and surgical results in a series of 58 prolactin-pituitary adenomas with the morphological findings and to assess the value and reliability of the immunocytochemical methods in the clinicopathological investigations of pituitary tumours.

Materials and methods

During the last three years 1981–1983, fifty-eight patients with prolactin-secreting pituitary adenomas were operated in the Department of Neurosurgery of Padua University. The clinical features of these cases are summarized in table 1. All patients underwent a formal protocol of testing for pituitary function before and one month after surgery. Endocrinological evaluation included, beside basal PRL levels, prolactin serum level variations after TRH. The radiological investigation consisted of anteroposterior and lateral hypocycloidal polytomography and thin-section computerized tomography (CT). Twenty-two patients had microadenomas,

Table 1 Clinical features of immunocytochemically defined PRL-pituitary adenomas (58 patients)

Sex	f = 49; m = 9
Age (average)	17–54 years
Amenorrhoea	30
Amenorrhoea – Galactorrhoea	19
Hypogonadism	7 (m)
Gynecomastia	2 (m)
PRL (average)	70–3500 ng/ml

24 had intrasellar macroadenomas and 12 patients presented with extrasellar macroadenomas.

In the 22 patients with microadenomas, abnormalities of the sella turcica were seen in 18 cases, while in the remaining 4 cases the sella turcica was normal. Only in 9 cases the CT scan showed an intrasellar focal area of decreased attenuation confirmed later as a tumour at surgery. In all these cases the unilateral transseptal, transsphenoidal microsurgical approach was performed; in all 22 patients it was possible to perform a selective removal of the microadenoma while saving the normal pituitary tissue. A soft tumour was found in all cases with a diameter of less than 10 mm (in 5 cases the diameter was less than 5 mm).

Histological verification of a pituitary adenoma was obtained in 19 cases while in 3 cases hyperplastic cell nests without microadenoma were found. Only in 6 cases it was possible to examine the surrounding peri-adenomatous tissue (tab. 2). The immunoperoxidase method was performed on serial sections of the formalin-fixed, paraffin-embedded tissue.

Sternberger's peroxidase-antiperoxidase (PAP)-method was used for detection of human prolactin hormone (PRL), human growth (GH) and human adrenocorticotropic hormone (ACTH) [3].

The tumours in 19 patients were classified as acidophil or chromophobe adenomas by light microscopy.

On immunoperoxidase staining all 19 tumours were immunoreactive for PRL; in 3 cases of this group up to 10% of the tumour cells were immunoreactive for GH. The immunoperoxidase staining on peri-adenomatous tissue showed prolactin po-

Table 2 Histological findings (22 patients)

Adenoma	Periadenomatous tissue	Adenohypophyseal cell hyperplasia
19	6	3

Table 3 Immunoperoxidase staining on adenohypophyseal cell hyperplasia

Hormones detected	No.
PRL	3
PRL-GH	0
PRL-ACTH	0
Negative	0
Total	3