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Effect of dopamine agonist medication on prolactin producing pituitary adenomas

A morphological study including immunocytochemistry, electron microscopy and in situ hybridization

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Summary. Conventional light microscopy, immunocytochemistry, electron microscopy and in situ hybridization were used to evaluate the effect of dopamine agonists (bromocriptine-LAR and bromocriptine) on the morphology of surgically removed prolactin (PRL)-producing pituitary adenomas. Dopamine agonist therapy resulted in decrease of serum PRL, clinical improvement and tumour shrinkage. Using light and electron microscopy cellular atrophy, interstitial and perivascular fibrosis were noted; in several tumours connective tissue accumulation was pronounced. The cellular response was not uniform. In some adenomas populations of large cells and small cells were distinguished. The large cells contained immunoreactive PRL and expressed the PRL gene indicating resistance to dopamine agonists. It appears that these cells retained the potential to secrete PRL and proliferate despite exposure to dopamine agonists. In the small cells, PRL immunoreactivity and PRL gene expression decreased providing evidence that both PRL release and synthesis were blocked. Small cells can persist in tumours after discontinuation of dopamine agonist medication suggesting these small cells are irreversibly suppressed and are not capable of regaining their endocrine function and proliferative capability. The formation of irreversibly suppressed PRL cells may explain why some PRL-producing adenomas do not recur after withdrawal of dopamine agonists.

Key words: Bromocriptine – Pituitary neoplasm – Prolactin – Ultrastructure

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Introduction

A large number of studies have provided conclusive evidence that dopamine agonist drugs decrease serum prolactin (PRL) levels, stop amenorrhoea and galactorrhoea, restore fertility and libido and cure impotence and hypogonadism in patients with PRL-producing pituitary adenoma (Barrow et al. 1984; Corenblum 1985; Corenblum and Taylor 1983; Johnston et al. 1983; Jordan and Kohler 1987; Molitch 1989; Molitch et al. 1985; Nabarro 1982; Vance and Thorner 1987). Beside the marked clinical and biochemical improvement, substantial tumour shrinkage has also been documented (Bassetti et al. 1984; Chiodini et al. 1981; Corenblum and Hanley 1981; Fahlbusch et al. 1987; Hassoun et al. 1985; Landolt et al. 1987; McGregor 1979; Nillius et al. 1978; Rengachary et al. 1982; Saitoh et al. 1986; Schottke et al. 1986; Thorner et al. 1980). In most patients tumour involution is reversible, since after discontinuation of dopamine agonist medication the tumour regrows, serum PRL levels rise and the clinical signs and symptoms recur (Molitch et al. 1985; Thorner et al. 1981).

Despite the experience obtained in dopamine agonist therapy and the progress in understanding the mechanism of dopamine agonist action, several questions remain unresolved. It is not clear whether only PRL release or both release and synthesis are inhibited in human PRL-producing adenomas, nor is it known what are the mechanisms and sequence of events in tumour involution and regrowth. Further studies are required to clarify why some adenomas are resistant to dopamine agonist drugs (Bannister and Sheridan 1987; Breidahl et al. 1983; Cheyne et al. 1988; Kupersmith et al. 1989; Liuzzi et al. 1985; Molitch et al. 1985) whereas other

tumours show inreversible shrinkage, serum PRL levels fail to increase and the clinical signs and symptoms do not return after withdrawal of dopamine agonist medication (Johnston et al. 1984; Moriondo et al. 1985; Wang et al. 1987; Zarate et al. 1983). Some of these questions were addressed by previous morphological investigations (Esiri et al. 1986; Hassoun et al. 1985; Horvath and Kovacs 1986; Horvath et al. 1988; Rengachary et al. 1982; Saitoh et al. 1986; Tindall et al. 1982); however, no definite conclusions were drawn.

The aim of the present study was to obtain a deeper insight into these problems by studying the tumours in detail using light microscopy, immunocytochemistry and electron microscopy. We correlated the morphology of dopamine agonist exposed PRL-producing adenomas with PRL gene expression, applying in situ hybridization.

Patients and methods

Twenty-two patients underwent trans-sphenoidal surgery for PRL-secreting pituitary adenomas. All patients had marked hyperprolactinaemia. Six patients received no dopamine agonist therapy. Seven patients were treated with one intramuscular injection of 100 mg bromocriptine (2-bromo-α-ergocryptine, BEC) LAR, a new long-acting repeatable injectable form of bromocriptine (Parlodel LAR, Sandoz, Basel, Switzerland). This drug was found to be effective in the management of patients with PRL-producing adenoma (Ciccarelli et al. 1987; Grossman et al. 1986; Montini et al. 1986; Schettini et al. 1988; van't Verlant et al. 1988). Nine patients were given bromocriptine (2.5–7.5 mg) daily. In 6 of these patients, bromocriptine treatment was discontinued for at least 2 months before surgery. The duration of bromocriptine therapy varied from 3 weeks to 11 months. Serum PRL levels were measured before, during and after cessation of bromocriptine administration by radioimmunoassay. The clinical signs and symptoms were monitored and tumor sizes estimated by various imaging techniques.

Tumour tissues were fixed in buffered formalin and embedded in paraffin. Sections of 4–6 μm were stained with haematoxylin and eosin (H&E) and the periodic acid-Schiff (PAS) method. The avidin-biotin-peroxidase complex (ABC) technique was used for demonstration of hormone content. The following antisera were applied: anti-hGH (1:1500 dilution, DAKO, Santa Barbara, Calif.), anti-hPRL (1:4000 dilution, provided by Dr. H. Friesen, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada), anti-hACTH (1:2000 dilution), anti-β hTSH (1:4000 dilution), anti-β hFSH (1:2000 dilution); anti-β hLH (1:2000 dilution), all donated by NIDDKD (Bethesda, Md.); monoclonal anti α-hSU (1:800 dilution, Biogenex, Dublin, Calif.). The immunostaining and the control stains were performed as described elsewhere (Asa et al. 1986).

For electron microscopy small pieces of the tumours were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded series of ethanol, processed through propylene oxide and embedded in Epon-Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with a Philips 410-LS electron microscope.

Using in situ hybridization (ISH) for PRL mRNA and GH mRNA demonstration, oligonucleotide probes complementary to the region of hPRL 66–72 and hGH 11–17 were exploited. The probes were synthesized on an automated DNA synthesizer (Gene Assembler) by Pharmacia (Milwaukee, Wis.) using the solid-phase beta cyanoethyl phosphor amidite method. The 3-end labelling method with 35-S was applied. The sequence of the probes and details of the procedures have been described previously (Kovacs et al. 1989).

ISH was performed on 5-μm-thick paraffin sections applying 5×10^5 cpm of probe as described elsewhere. For combined ISH and immunocytochemistry for PRL the ABC method was carried out after 2× standard saline citrate (SSC: 0.15 MNaCl/0.015 M trisodium citrate) washings (Kovacs et al. 1989). The following controls for ISH were performed: (1) predigestion of tissue sections with 100 ng/ml RNase A (Sigma, St. Louis, Mo); (2) competition studies with 100-fold excess of unlabelled probe to assure specificity; (3) human liver and parathyroid adenomas were used as negative controls.

Results

Biochemical, clinical and imaging findings will not be reported here and the effects of the two different dopamine agonists will not be compared; these results will be the subject of a clinical paper (Fahlbusch et al., in preparation). It suffice to say that dopamine agonist medication resulted in reduction of serum PRL levels in most cases, clinical improvement and varying degrees of pituitary adenoma shrinkage.

By light microscopy, the pituitary tumours represented chromophobic, slightly acidophilic adenomas. The adenomas removed from untreated patients had a diffuse growth pattern and were PAS negative. By immunocytochemistry, the presence of PRL with the characteristic disposition in the Golgi area was demonstrated in most adenoma cells.

In adenomas exposed to bromocriptine-LAR or bromocriptine up to surgery the most conspicuous changes were the decrease in size of tumour cells and the presence of interstitial and perivascular fibrosis. In several tumours accumulation of connective tissue was pronounced. The degree of cellular response to dopamine agonist varied not only from case to case but also, sometimes, within different areas of the same tumour (Table 1). The most marked morphological alterations were noted in two adenomas (cases 1 and 2) exposed to bromocriptine-LAR. The small PAS-negative adenoma cells possessed scanty cytoplasm around dark, heterochromatic nuclei and were arranged in small groups surrounded by abundant connective tissue; deposits of endocrine amyloid were seen in one of these adenomas; intense perivascular fibrosis was evident as well. PRL immunoreactivity was absent or weak and restricted to few adenoma cells. Three adenomas (cases 5, 8 and 9) displayed small cells, but fibrosis was limited to the perivascular areas and PRL immunopositivity was present in many cells. In four adenomas (cases 3, 4, 7 and 10) areas of small cells with dark nuclei alternated with groups of cells with euchromatic nuclei and PRL immunopositivity was restricted to the latter population of cells. Two adenomas (cases 6 and 7) were composed of cells with relatively large, lightly stained nuclei, and narrow cytoplasm containing evenly distributed PRL immunoreactivity.

Three adenomas removed from patients who had been off BEC for 2 or more months before surgery had the same morphology as untreated ones; PRL immunoreactivity was intense and present in most adenoma cells. In two adenomas (cases 3 and 4) marked cellular heterogeneity was evident: one adenoma was composed of

Table 1. Summary of morphological results in prolactin (PRL)-producing adenomas from ten patients treated with dopamine agonists up to surgery

Case	Sex, age	BEC dose	Duration	ICC for PRL	ISH for PRL	Ultrastructure
1.	F, 20	100 mg (LAR)	5 weeks	Rare cells	Most cells 0; rare cells +, ++	Marked suppression; strong fibrosis
2.	M, 42	100 mg (LAR)	5 weeks	Rare cells	Most cells 0; rare cells +, ++	Marked suppression; strong fibrosis; amyloid deposits
3.	F, 48	100 mg (LAR)	15 weeks	Few groups of cells	Most cells +; few cells ++, +++	Marked or less often moderate suppression; moderate fibrosis
4.	M, 49	100 mg (LAR)	2 weeks	Many cells	NA	Moderate to marked suppression; slight fibrosis
5.	F, 40	2 × 50 mg (LAR)	3 weeks	Most cells	Diffuse +, ++	Moderate to marked suppression; perivascular fibrosis; few mitoses
6.	M, 59	100 mg (LAR)	8 weeks	Many cells	Diffuse +, ++	Moderate suppression and crinophagy; few cells with marked suppression; fibrosis
7.	M, 56	100 mg (LAR)	6 weeks	Many cells	NA	Moderate suppression; some dead cells; strong fibrosis
8.	F, 23	2.5 mg/day 5.0 mg/day 7.5 mg/day	1 week 1 week 6 weeks	Most cells	Diffuse +, ++	Marked suppression; some dead cells; perivascular fibrosis
9.	F, 27	2.5 mg/day 5.0 mg/day 7.5 mg/day	1 week 2 weeks 3 weeks *1 week off	Most cells	Most cells +; few ++, +++	Marked suppression; perivascular fibrosis
10.	F, 50	10.0 mg/day	4 weeks	Most cells, uneven intensity	Many cells 0; some ++	Moderate to marked suppression; perivascular fibrosis

0, negative labelling; +, weak labelling; ++, moderate labelling; +++, intense labelling; ++++, very intense labelling; NA not available; BEC, 2-bromo-α-ergocryptine; ICC, immunocytochemistry; ISH, in situ hybridization

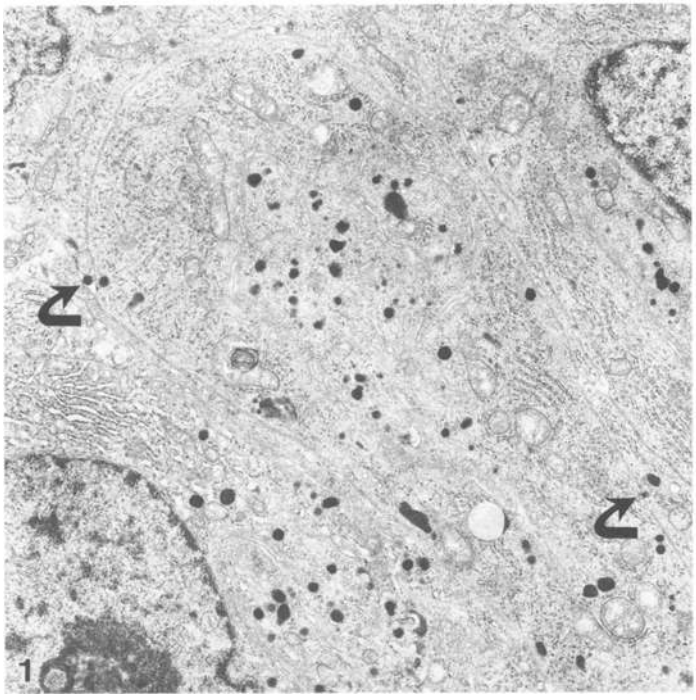
Table 2. Summary of morphological results in six PRL-producing adenomas from patients who discontinued dopamine agonist therapy before surgery

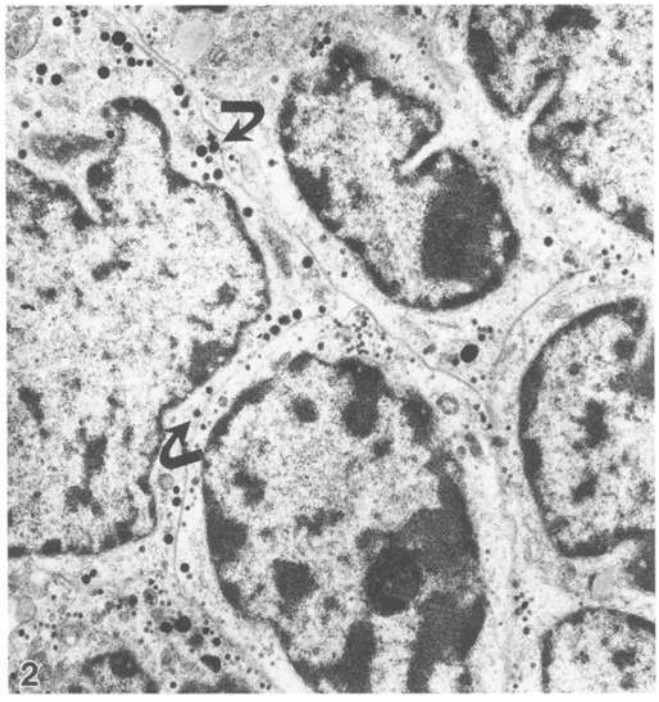
Case	Sex, age	BEC dose	Duration	ICC for PRL	ISH for PRL	Ultrastructure
1.	F, 17	7.5 mg/day	3 months 2 months off	Most cells	Diffuse ++, +++	Typical
2.	F, 37	5.0 mg/day	6 months 4 months off	Most cells	Diffuse ++	Typical; some giant mitochondria; mitoses
3.	F, 31	3.7 mg/day	18 months 2 months off	NA	NA	Few typical; most cells with marked suppression
4.	F, 30	NA	NA 6 months off	Most cells	NA	Typical and suppressed cells
5.	F, 40	7.5 mg/day	4 months 2 months off	Most cells	Diffuse +++	Typical; 15% of cells densely granulated
6.	M, 24	5.0 mg/day	11 months 2 months of	Most cells	Diffuse +++, ++++	Densely granulated cell adenoma

For legend, see Table 1

small cells with dark nuclei intermingled with large cells containing euchromatic nuclei; however, the PRL immunostaining was evenly distributed. The other adenoma exhibited large areas of small cells with dark nuclei and a minor population of large cells with euchromatic nuclei (Table 2). PRL immunopositivity was uneven and perivascular fibrosis was marked in this tumour.

On electron microscopy the six tumours removed from untreated patients showed the characteristic ultrastructural features of sparsely granulated PRL cell adenomas (Horvath and Kovacs 1986; Kovacs and Hor-





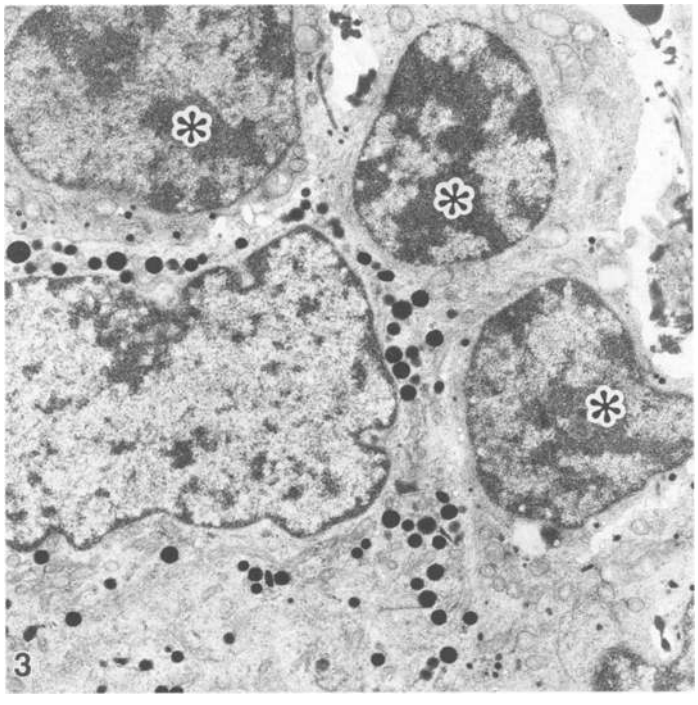


Fig. 1. Sparsely granulated prolactin (PRL) cell adenoma not exposed to 2-bromo-α-ergocryptine (BEC) therapy. Note euchromatic nucleus with prominent nucleolus. The abundant cytoplasm contains well-developed rough endoplasmic reticulum (RER) and Golgi complex with pleomorphic forming secretory granules; extruded secretory granules are seen (*arrows*). ×9,600

Fig. 2. Sparsely granulated PRL cell adenoma exposed to BEC-LAR up to surgery. The small cells possess indented heterochromatic nuclei, small nucleoli and narrow cytoplasm. Few RER profiles and scattered small secretory granules, some extruded (*arrows*) are noted. ×9,600

Fig. 3. Sparsely granulated PRL cell adenoma exposed to BEC up to surgery. Large, active cell with well developed cytoplasm and euchromatic nucleus is surrounded by small cells with heterochromatic nuclei and scarce cytoplasmic organelles (*asterisks*). ×9,600

vath 1986) (Fig. 1). The adenoma cells possessed euchromatic nuclei with prominent nucleoli and abundant cytoplasm. The rough endoplasmic reticulum (RER) was well developed, consisting of parallel cisternae or concentric whorls. Golgi complexes were prominent and contained pleomorphic developing granules. The cytoplasmic secretory granules were usually sparse, electron-dense and measured 125–300 nm. The extrusion of secretory granules into the intercellular space was a common event.

Five adenomas (cases 1, 2, 7–9) exposed to dopamine agonists up to surgery showed uniform, marked ultrastructural signs of functional suppression (Fig. 2). The very small, ovoid, slightly polyhedral adenoma cells contained smaller, often indented nuclei with coarsely clumped heterochromatin and small nucleoli. The narrow rim of cytoplasm contained scanty RER and few free ribosomes. In most adenoma cells, the Golgi complex was not recognizable. When it was noticeable, it was formed by few collapsed sacculi and numerous vesicles with occasional small forming granules. The majority of secretory granules were very small, measuring 50–

200 nm, but few of them reached 300 nm. Misplaced exocytoses of one or multiple secretory granules were frequently encountered.

Three adenomas (cases 3–5) consisted of sparsely granulated cells with ultrastructural signs of marked, or often moderate suppression. The small polyhedral cells contained irregular nuclei with discrete or moderate heterochromatinization and nucleoli with variation in size. Beside the adenoma cells with small cytoplasm, occasional adenoma cells with large, well-differentiated cytoplasm and secretory granules measuring up to 450 nm were seen (Fig. 3).

One sparsely granulated PRL cell adenoma (case 6) was composed of small cells with long cytoplasmic processes. The relatively large nuclei contained stippled heterochromatin and moderately developed nucleoli. The narrow cytoplasm harbored poorly to moderately developed RER and Golgi apparatus. The sparse secretory granules were small and many were engaged in exocytosis. In case 7, the adenoma contained cells with heterochromatic nuclei but fairly prominent nucleoli. In the narrow cytoplasm a surprisingly abundant RER, active Golgi region and quite numerous small secretory granules were present.

The ultrastructure of adenomas of patients in whom bromocriptine was discontinued showed some variations (Table 2). Two adenomas (cases 1 and 2) had the same ultrastructure as those not exposed to the drug. Case 5 was composed of a predominant population of typical sparsely granulated PRL cells and a minor component (approximately 15%) of densely granulated PRL cells with secretory granules measuring up to 900 nm. One tumour (case 6) represented densely granulated PRL cell adenoma. The abundant spherical secretory granules measured 150–600 nm. Many adenoma cells showed extrusion of secretory granules. The cytoplasmic organelles involved in hormone synthesis were well developed as in the sparsely granuled variant. Lysosomes and crinophagy were frequently encountered. Two tumours (cases 3 and 5) had heterogeneous ultrastructural features with adenoma cells showing considerable variation in shape and size. In one adenoma some nuclei were

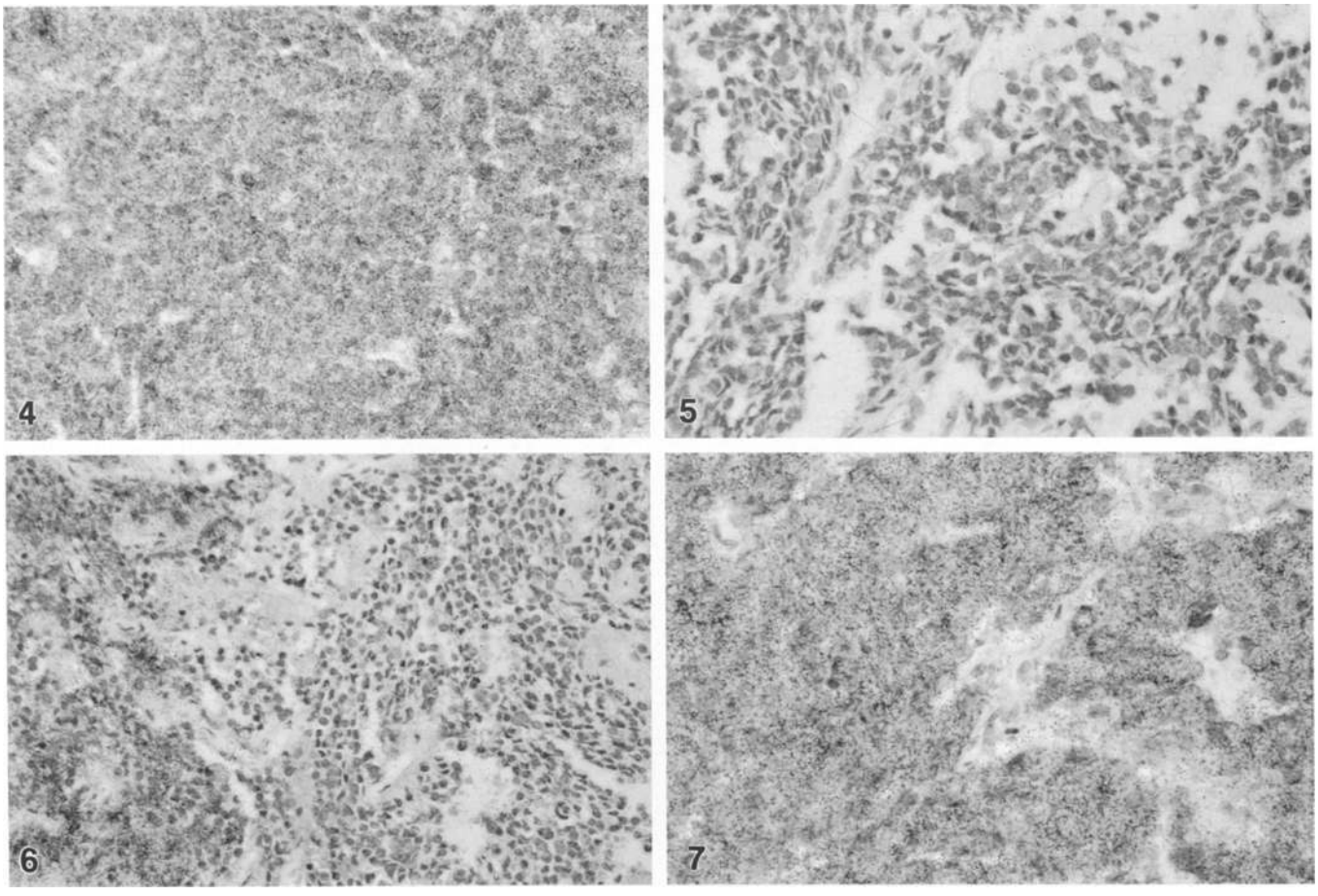


Fig. 4. PRL cell adenoma unexposed to BEC. Most cells are immunoreactive for PRL and have an even, intense distribution of PRL mRNA. ×400

Fig. 5. PRL cell adenoma exposed to BEC-LAR up to surgery shows marked functional suppression as indicated by the lack of both PRL gene expression and gene product. ×400

Fig. 6. PRL cell adenoma with an uneven reponse to BEC: the PRL immunoreactive cells contain abundant PRL mRNA, while cells devoid of PRL lack its messenger, as well. ×400

Fig. 7. PRL cell adenoma in which BEC therapy was discontinued 2 months before surgery shows the same pattern of PRL immunoreactivity and intensity of PRL mRNA as seen in adenoma unexposed to BEC. ×400

euchromatic, while others had coarsely clumped heterochromatin; there were marked variations in size of the cytoplasm and prominence of RER and Golgi complexes; in many adenoma cells secretory granules were sparse and measured up to 300 nm; in some adenoma cells the secretory granules were larger and more numerous than usual. In the other adenoma, most of the cells had hyperchromatic nuclei and small rim of cytoplasm with few organelles.

Using ISH, none of the PRL-producing adenomas examined expressed the GH gene. All six untreated PRL cell adenomas contained an intense or very intense hybridization signal for PRL mRNA (Fig. 4). In adenomas exposed to dopamine agonists a marked decrease in PRL mRNA content was found when compared with the untreated (Table 1). Among PRL cell adenomas with signs of marked suppression, the lowest signal level was found in two adenomas treated with bromocriptine-LAR. In these tumours most adenoma cells were devoid of PRL mRNA (Fig. 5). In the other adenomas, including those in which moderate morphological response was predominant, a weak or mild hybridization signal level prevailed. ISH combined with immunocytochemistry revealed that in tumours with uneven PRL immunoreactivity, PRL mRNA was usually abundant in PRL immunopositive cells and scanty or absent in the adenoma cells which failed to stain for PRL (Fig. 6).

In adenomas in which bromocriptine therapy was discontinued a few months before surgery, the intensity of labelling for PRL mRNA was comparable with that of untreated adenomas (Table 2) (Fig. 7). A high level of mRNA was found in both sparsely and densely granulated adenoma cells. In the two tumours with different degrees of suppression, ISH revealed adenoma cells with variable intensity of labelling for PRL mRNA and adenoma cells lacking the messenger.

Discussion

The present investigation confirms and extends previous findings and provides conclusive evidence that treatment with dopamine agonists causes profound morphological alterations in PRL-producing pituitary adenomas (Hassoun et al. 1985; Horvath and Kovacs 1986; Horvath et al. 1988; Saitoh et al. 1986; Schottke et al. 1986; Tindall et al. 1982). In earlier studies (Tindall et al. 1982) dopamine agonist medication was found to induce marked cellular involution manifested by reduction of cellular, cytoplasmic, nuclear and nucleolar sizes, increase of nuclear-cytoplasmic ratio and decrease of cytoplasmic volume densities of endoplasmic reticulum and Golgi complexes. Compared to PRL producing adenomas removed from patients not exposed to dopamine agonists, the cytoplasmic volume densities of mitochondria and in many cases, those of the lysosomes exhibited no major change and the cytoplasmic volume densities and diameters of secretory granules showed slight increase in some adenomas and no change or slight decrease in others. In a few tumours accumulation of lysosomes was noted especially in those in which cellular shrinkage was not marked. Although no morphometry was applied in the present study, the cellular involution was obvious. The clinical and biochemical results were concordant with the morphological alterations and were manifested by decrease of serum PRL levels as well as restoration of menstruation, cure of galactorrhoea, hypogonadism and impotence and improvement of libido. Tumour shrinkage was documented before surgery by various imaging techniques.

Apart from the morphological changes affecting the adenoma cells varying degrees of interstitial and perivascular fibrosis was noted by previous workers (Esiri et al. 1986; Landolt and Osterwalder 1984) and in the present study. A marked individual difference was evident among various tumours. Accumulation of connective tissue was more pronounced in the tumours of patients who had protracted dopamine agonist medication with relatively large doses and in whom tumour shrinkage was extensive. The factors accounting for the development of fibrosis are not known. No widespread cellular death was evident; thus it is unlikely that extensive necrosis preceded and was responsible for the accumulation of connective tissue. It may be that mediators were released from the shrinking tumour cells which evoke proliferation of connective tissue. This interpretation is, however, speculative and is not supported by any evidence so far.

Similar to the clinical and biochemical results the morphological changes were found to be reversible in many tumours. In some tumours following withdrawal of dopamine agonist medication the structural features were indistinguishable from those removed from patients not receiving dopamine agonist drugs. In these tumours the adenoma cells were large, possessing abundant cytoplasm, conspicuous endoplasmic reticulum network, many free ribosomes and polysomes and prominent Golgi complexes.

Hybridization histochemistry using an oligonucleotide probe demonstrated a marked decrease of PRL mRNA in many tumours indicating that not only PRL release but also PRL synthesis was suppressed by dopamine agonist medication. These results, using ISH methodology, are in agreement with previous findings obtained in animal pituitaries which showed a significant reduction of PRL gene expression secondary to dopamine agonist exposure (Maurer 1980).

A marked individual variation in the cellular response to dopamine agonist medication was a striking finding in the present study. In some tumours removed from patients treated with dopamine agonists two different cell populations were clearly identified; one seemed to have escaped dopamine agonist suppression. These adenoma cells were large, their PRL content was not decreased and PRL gene expression appeared to be within the normal range. The other cell population consisted of small cells exhibiting the effect of dopamine agonist suppression characterized by marked cellular involution, decrease of immunoreactive PRL content and PRL mRNA.

The finding that differences exist in cellular responsiveness to dopamine agonists is difficult to explain. It may be that functional dopamine receptors (Bression

et al. 1980; Bronin 1982; Koga et al. 1987) are missing on the surface of some adenomatous PRL cells or impairment of some intracellular event accounts for the resistance to dopamine agonists. The degree of clinical and biochemical resistance depends on the number of unresponsive large cells which are capable of expressing the PRL gene and its product and presumably have a potential for growth despite exposure to dopamine agonists. If unresponsive large cells predominate in a tumour exposed to dopamine agonist medication, little suppressive effect can be expected from the drug.

It has been noted by several authors that some patients are resistant to dopamine agonist therapy (Bannister and Sheridan 1987; Breidahl et al. 1983; Cheyne et al. 1988; Kupersmith et al. 1989; Liuzzi et al. 1985; Molitch et al. 1985). In other patients tumour involution is permanent, tumour regrowth fails to occur and hyperprolactinaemia as well as the clinical symptoms do not revert after discontinuation of dopamine agonist medication (Johnston et al. 1984; Moriondo et al. 1985; Wang et al. 1987; Zarate et al. 1983). It appears that such tumours are composed of markedly suppressed small PRL cells that are not able to secrete PRL in excess and do not continue to proliferate. It is conceivable that those small cells which contain no immunoreactive PRL and do not express the PRL gene even after discontinuation of dopamine agonist therapy lose the potential to regain endocrine activity and growth, in some cases irreversibly. The development of a clone of irreversibly suppressed PRL cells may provide an explanation of why some adenomas do not cause hyperprolactinaemia and do not regrow after withdrawal of dopamine agonist medications.

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