dogenous protease inhibitors (e.g., alpha-1-protease inhibitor) our data suggest that the potential for direct proteolysis of HLE by an endogenous trypsin-like enzyme may offer an additional control mechanism for leukocyte elastase. Studies are presently underway exploring further biochemical aspects of the inactivation of HLE as well as accessing its physiological importance.

Abbreviations. HLE, human leukocyte elastase; PPE, porcine pancreatic elastase; BPT, bovine pancreatic trypsin β-trypsin; CT, bovine pancreatic α-chymotrypsin; MeOSuc-, methoxysuccinyl; pNA, p-nitroaniline.

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Bromocriptine-induced removal of endoplasmic membranes from prolactinoma cells[1]

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Summary. Adenomatous prolactin cells lose 39% of their cytoplasm volume within 7 days after the beginning of bromocriptine treatment. A simultaneous reduction of the rough-surfaced endoplasmic reticulum and the Golgi apparatus occurs. Their membranes are removed by rapid transport along the secretory pathway to the cell surface and to lysosomal destruction.
Key words. Pituitary neoplasms; prolactin; bromo-α-ergocriptine; endoplasmic reticulum; biological transport.

Prolactin (PRL) secreting pituitary adenomas (prolactinomas) exposed to bromocriptine for 4–6 weeks (daily dose 7.5 mg) demonstrate a cell shrinkage of 33–39% due to a reduction of the cytoplasm area with regression of the rough surfaced endoplasmic reticulum (RER) and the Golgi apparatus[2–4]. However, the mechanism of the reduction of the RER and of the Golgi apparatus has not yet been elucidated. Measurements of the RER and Golgi surface in the average tumor cell after a 1-week exposure to bromocriptine, i.e. during the phase of ongoing cell shrinkage, were made to obtain an insight into the processes that are active during the phase of endoplasmic membrane removal.

A new, injectable, long-acting form of bromocriptine ensures a therapeutic and sustained plasma drug level within hours after the injection[5]. Long-acting bromocriptine (50 mg) was injected 7 days before surgery in six patients bearing prolactinomas in order to achieve a shrinking and softening of the tumors and thereby facilitate their removal (short-term treatment) (Landolt, unpublished results). 10 biopsy specimens obtained from patients with prolactinomas were selected randomly from a large group of untreated patients; specimens from eight patients treated for 4–6 weeks with peroral bromocriptine (daily dose 7.5 mg) (chronic treatment) were used for comparison. The tissue was fixed in 2% S-collidine buffered osmium tetroxide and embedded in Epon. This fixation technique was preferred to the more commonly used glutaraldehyde-osmium fixation because the different types of cell membranes are better visible due to a 'washing-out' of cytoplasmic ground substance. The thin sections were contrasted with uranyl acetate and lead citrate[6]. Measurement of the average section area of the cell, nucleus, cytoplasm and the nucleolus was done on random electron micrographs of 100–140 cells (magnification $\times 3000$)[3]. The surface densities of the RER and Golgi membranes were measured on 30–40 random electron micrographs (magnification ×108,000) with the superimposed multipurpose test system (21 test lines) designed by Weibel et al.[7]. The total surface per average section of adenoma cell was calculated from the surface density and the cytoplasm area. The number of membrane-fixed ribosomes was determined by measuring the length of random profiles of RER membranes and counting the number of fixed ribosomes[8,9]. No attempt was made to correct for the section thickness[10] since this, as determined by interference color, and the final magnification of the electron micrographs were the same in all cases.

The results of the measurement of the size of the cells, cytoplasm, nuclei, nucleoli, surface of the RER and Golgi apparatus, the number of membrane-fixed ribosomes and ribosome density are shown in the table together with the results of the statistical comparison of the data with the H-test[11]. Median values are used to reduce the influence of outliers. The table shows that the major reduction of most parameters occurs within 1 week, with the exception of the ribosome density. The nuclear size does not decrease significantly.

We have calculated the half life of the cell volume, nucleolus volume, RER surface, Golgi surface and number and density of the membrane fixed ribosomes assuming that the shrinkage of these structures follows an exponential curve to the baseline of adenomas treated for 4–6 weeks (fig. 1). This baseline represents the new steady state since no further cell shrinkage was observed even if the bromocriptine treatment lasted one year[12]. The half life falls into the same range (2.2–3.7 days) for all parameters with the exception of the ribosome density (8.2 days) (table). No half life can be calculated for the cytoplasm volume and the nuclear volume.

In normal rat PRL cells examined in vitro, ergocryptine and bromocriptine treatment cause a rapid fall of PRL messenger RNA (mRNA) content and PRL release[13,14]. Both drugs block the DNA to mRNA translation of the PRL gene. Bromocrip-

Change of cytological parameters during bromocriptine treatment of prolactinomas

	Untreated adenomas n = 10	Short-term treatment n = 6	Chronic treatment n = 8	H-test chi^2	p <	$T_{½}$ days
Cell volume (μm^2)	92.4 (100%)	71.0 (77%)	68.3 (74%)	8.992	0.05	2.2
Cytoplasm volume (μm^2)	51.3 (100%)	31.1 (61%)	34.3 (67%)	8.773	0.05	–
Nucleus volume (μm^2)	38.7 (100%)	41.0 (106%)	35.0 (90%)	4.329	–	–
Nucleolus volume (μm^2)	2.4 (100%)	1.6 (67%)	1.3 (54%)	5.620	0.1	3.7
RER surface/cell (μm)	189 (100%)	113 (69%)	97 (51%)	12.910	0.01	2.7
RER ribosomes/cell	2267 (100%)	824 (36%)	420 (18%)	18.095	0.001	3.2
Ribosomes/mm membrane	10406 (100%)	8003 (77%)	5034 (48%)	17.568	0.001	8.2
Golgi surface/cell (μm)	60 (100%)	32 (53%)	25 (42%)	5.562	0.1	3.0

All results are shown as measured in the thin section of the average adenoma cell and *not* as partial volumes or volume densities. $T_{½}$: half life.

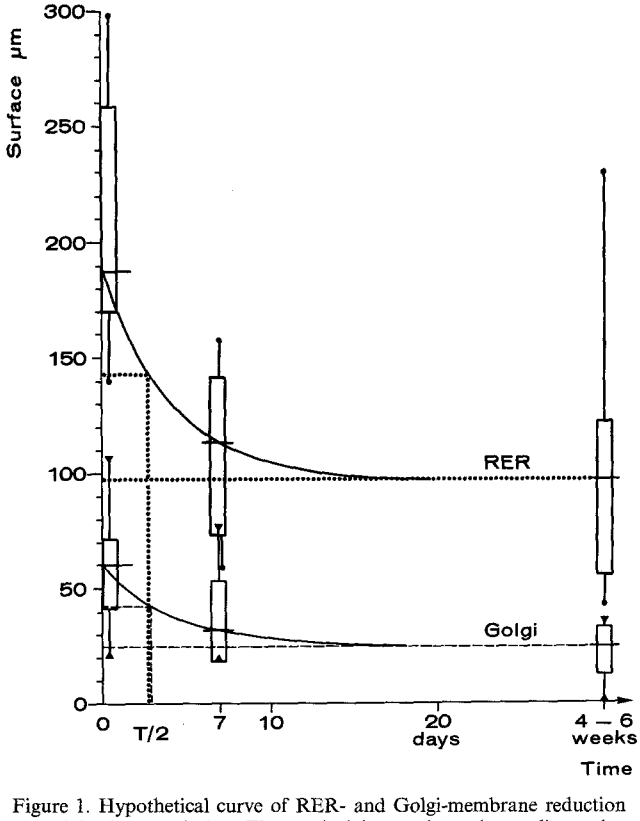

Figure 1. Hypothetical curve of RER- and Golgi-membrane reduction caused by bromocriptine. The vertical boxes show the median value (horizontal bar) and the 25 and 75 percentiles. The vertical lines connect the maximum and minimum values.

tine seems to work similarly on human adenomatous RPL cells. This leads to a rapid reduction of PRL synthesis due to a reduction of the membrane fixed ribosomes, as demonstrated by our results. However, there is not only a reduction of ribosomes engaged in PRL synthesis but probably also of a second subclass of ribosomes engaged in production and insertion of RER-membrane proteins[15,16], since the RER membranes shrink with a speed that is only slightly below the reduction of the ribosome number. The small difference in the two parameters causes the slower reduction of the ribosome density (number of ribosomes per mm membrane). Similar values to those from our material for distance apart, and change of distance of membrane-fixed ribosomes have been observed in rat hepatocytes during the period of cell differentiation 3 days before birth (average distance apart of ribosomes 50 nm) and 3 days after birth (average distance 150 nm)[15]. The average distance of ribosomes from each other in our material is 95 nm before treatment, 125 nm after 7 days and 200 nm after 4–6 weeks of bromocriptine treatment. The half life of the RER membrane (2.7 days) corresponds to the values published by Franke et al.[17] that were determined by in vivo pulse labeling (2.5–3.3 days).

Two possible mechanisms exist for reducing the membranes of the RER and Golgi apparatus. The membranes may be removed by autophagic vacuoles, as are the phenobarbital-induced membranes of the smooth surfaced reticulum in rat liver cells. These are removed somewhat faster than the PER membranes in our material, since the end point in the rat liver cell reticulum is reached within 5 days[18]. The other possibility is a rapid transport of membrane material along the secretory pathway to the Golgi apparatus. Qualitative analysis of the pictures of short-term treated prolactinomas demonstrates that there is an increased number or autophagic vacuoles. They contain, however, only remnants of electron dense secretory granules and no membrane material as in the pictures of phenobarbital-treated rat liver cells. Pictures of closely stacked membranes of the PER without ribosomes on their facing surfaces suggest that PER membranes may be transformed directly into Golgi membranes (fig. 2).

The surface of the cisterns of the Golgi apparatus is reduced at a similar speed to that observed for the RER (half life: 3.0 days). This seems to be achieved by an increased formation of secretory granules that are either destroyed by autophagic vacuoles or released at a higher than normal speed. This activated granule release is illustrated by the frequent occurrence of a simultaneous release of several secretory granules into one secretory pit of the cell membrane (fig. 3) similar to those seen in stimulated rat PRL cells during lactation [19]. Such formations can be seen only in adenomas removed from patients who unterwent surgery after 7 days treatment with bromocriptine-retard and not in the biopsy specimens of controls and/or of patients treated chronically with bromocriptine. We have observed the same active granule release in the biopsy specimen from one patient treated with bromocriptine-retard for 22 h only.

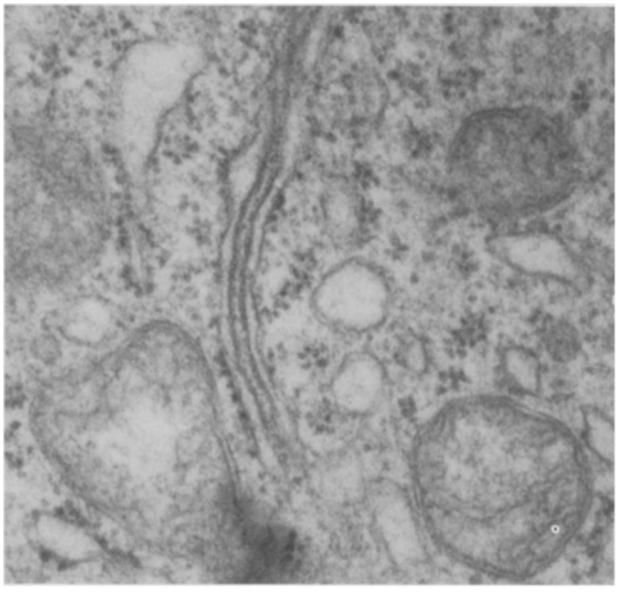

Figure 2. Electron micrograph of closely packed membranes of the RER without ribosomes on their facing membranes, suggesting transformation into Golgi cisterns in a biopsy specimen obtained from a patient after short-term bromocriptine treatment. Osmium fixation, × 40,000.

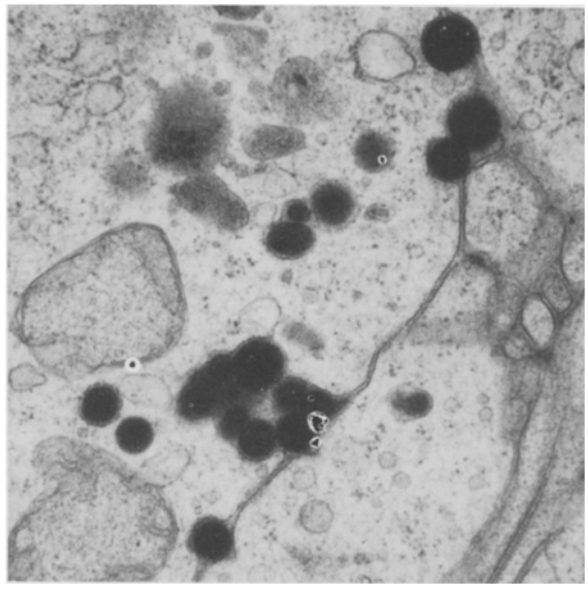

Figure 3. Electron micrograph of a secretory pit with simultaneous release of seven secretory granules in a biopsy specimen obtained from a patient after short-term bromocriptine treatment. Osmium fixation, × 40,000.

Clinical studies demonstrate a rapid decrease of PRL plasma levels during the first days of bromocriptine treatment in patients suffering from prolactinomas [20–22]. The release of an increased number of secretory granules observed in the adenoma cells suggests that these granules have a reduced PRL content. We consider that bromocriptine has several effects on prolactinomas: It inhibits the DNA to mRNA translation of the PRL gene, which leads to a reduction of membrane-fixed ribosomes engaged in PRL synthesis. This blocks the renewal of RER membranes directly or indirectly. Finally, bromocriptine increases the rate of transport of RER membrane material of the Golgi apparatus, secretory granules and cell surface at least during the phase of cell shrinkage. A similar transport of membrane material from the RER to secretory granules has been suggested to occur in the normal turnover of endoplasmic membranes[17,23,24].

The transport of membrane material is slower than the transport of the secreted PRL. The half life of the membranes for reaching the new steady state is three to four times longer than the fall of the PRL synthesis after inhibition with bromocriptine (half life: 0.8 days as calculated from the data of Maurer[13]) which suggests also that the electron dense material that ultimately reaches the cell surface probably has a lower PRL content in adenomas removed 7 days after the injection of long-acting bromocriptine. The membrane constituents may be recycled, since a remarkable number of coated vesicles can be found[25].

A 2nd possible pathway to membrane destruction leads from the RER, Golgi apparatus and secretory granules to autophagic vacuoles[18,19]. Part of their contents may be spilled into the intercellular space. However, the final evolution of the coated vesicles in bromocriptine-treated prolactinomas requires further investigation.

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