Scanning electron microscopy confirmed the presence of spherical structures, 500–1500 Å in diameter, densely covering the bead surfaces following incubation of funtionalized beads with a rat brain crude vesicle pellet resuspended in sucrose. The vesicles accumulated (L)-^{3}H-NE from the perfusate in a MgATP-dependent fashion. The accumulation was temperature dependent, and was inhibited in tissue prepared from animals pretreated with reserpine. A control column of beads alone accumulated less than 5% of the radioactivity found in beads incubated with synaptic vesicles.

Release of previously accumulated (L)-^{3}H-NE was accomplished after washing the vesicles to a continuous low level of spontaneous NE efflux. This low level of efflux was achieved after 15–20 min of perfusion. It was observed that the methamphetamine-induced release of NE required the presence of MgATP, possibly needed to drive the incorporation of methamphetamine into the vesicle[9]. MgATP alone was without effect on NE release. Although the methamphetamine-induced release of ^{3}H-NE was concentration-dependent, a cumulative concentration-effect curve could not be obtained on a single tissue sample, since the releaseable pool of (L)-^{3}H-NE was rapidly exhausted with increasing concentration of methamphetamine, or with continued exposure to low concentrations.

The results suggest that immobilization of brain synaptic vesicles on poly-L-lysine coated glass microspheres and perfusion with media comprised of membrane-impermeable anions may provide a useful technique for investigation of the interaction of drugs with vesicular transmitter stores.

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Bromocriptine reduces the size of cells in prolactin-secreting pituitary adenomas[1]

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Summary. The morphometric analysis of the size of adenomatous prolactin cells shows that bromocriptine-induced cell shrinkage halts if treatment with the drug is discontinued for more than 2 days. Different cell components (nucleus, cytoplasm, nucleolus) do not react to treatment to the same extent.

Reduction in the volume of prolactin-secreting pituitary adenomas produced by bromocriptine has been documented by clinical improvement of the patients and by radiological evidence[2]. It is not yet known however, if bromocriptine exerts this effect by reducing the number of cells or by causing simple diminution of the cell size. On one hand, experimental work with cell cultures suggests that the drug may interfere with tumor cell proliferation[3,4]. On the other, histological examination of 2 biopsy specimens obtained from 1 patient before and after bromocriptine treatment demonstrated a pronounced reduction of the tumor cell size after treatment[5]. Clinical observations indicate that the changes in the volume of the adenomas occur rapidly and are reversible[6]. This finding would be better explained by assuming that the cell size is reduced and that no significant cell loss occurs. Our morphometric analysis demonstrates a reversible, bromocriptine dependent prolactinoma cell shrinkage.

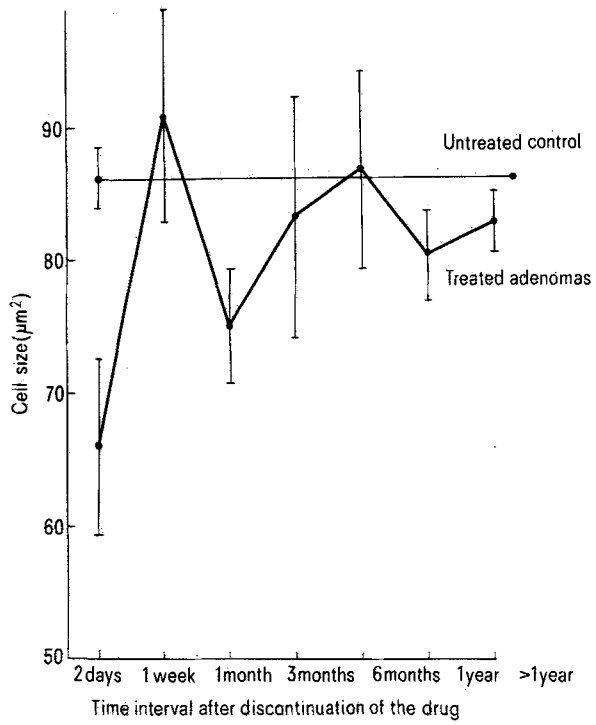
Changes in the size of prolactinoma cells were investigated in 100 prolactinomas. The tumor biopsies were obtained from 33 patients who had been treated with bromocriptine for various intervals before surgery, and from 67 untreated control patients. All patients had suffered from hyperprolactinemia. The tumors had been extirpated using a transsphenoidal approach. The diagnosis of prolactinoma was proven by positive immunostaining with anti-prolactin. The biopsy tissue used for this investigation was fixed in 2% s-collidine buffered osmium tetroxide immediately after extirpation and was embedded in Epon. The ultrathin sections were contrasted with uranyl acetate and lead citrate[7]. 10 random, low-power electron micrographs of each biopsy were photographed together with a calibration grid, and were enlarged to a final magnification of about ×3000. In 100–140 cells from each biopsy specimen depicted completely in the individual random pictures, the cross sectional area of the entire cell, and of the cytoplasm, nucleus, and nucleolus were measured with a Kontron MOP 02 (Kontron Messgeraete GmbH, Munich, FRG). The average areas were computed with an on-line connected programmable desk calculator (HP 8915A, Hewelett-Packard, Palo Alto, California, USA). Arithmetical means and SE for the groups of treated vs untreated were calculated with the same calculator from the data obtained from the individual cases. The nucleolus-nucleus and the nucleus-cytoplasm relations were calculated also. All results were compared using Student's t-test.

The comparison of the morphometric parameters of cells obtained from the 67 adenomas that had never been treated with bromocriptine before surgery with the data obtained from the specimens from the small groups of patients whose treatment had been stopped for various time intervals before their operation, showed that the size of the average tumor cell decreased significantly during treatment with bromocriptine. This effect ceased and the tumor cells enlarged when bromocriptine therapy was withdrawn for an interval of 1 week or more (fig.). Data from adenomas that had been without bromocriptine therapy for intervals longer than 1 week did not differ significantly from those for the untreated group. In the further analysis, therefore, we compared 3 groups of adenomas: a) untreated, b) treat-

Morphometric evaluation of bromocriptine-induced changes of cells and nuclei in prolactinomas

Morphologic feature	Untreated adenomas (N = 67)	Bromocriptine-treated adenomas BrCr stopped 0–2 days before surgery (N = 5)	Bromocriptine-treated adenomas BrCr stopped more than 2 days before surgery (N = 28)
Cell (μm^2)	86.2 ± 2.2	66.0 ± 6.6*	83.2 ± 2.5**
Cytoplasm (μm^2)	46.8 ± 1.7	33.2 ± 6.2	43.5 ± 1.6**
Nucleus (μm^2)	41.7 ± 1.0	34.0 ± 0.9*	41.2 ± 1.2**
Nucleolus (μm^2)	2.51 ± 0.14	1.29 ± 0.09*	2.31 ± 0.17**
Nucleus-cytoplasm relation	0.92 ± 0.04	1.11 ± 0.15	0.94 ± 0.05
Nucleus-nucleolus relation	0.062 ± 0.003	0.038 ± 0.004*	0.059 ± 0.005**

Value: average ± SE. *Difference from untreated adenomas significant, $p < 0.05$. **Difference from treated adenomas with interval shorter than 2 days significant, $p < 0.05$.



Reenlargement of prolactinoma cells after bromocriptine-induced shrinkage.

ed, but with a preoperative bromocriptine-free interval of 2 days or less, c) treated, but with a preoperative bromocriptine-free interval of 1 week or more.

In the table, the cross-sectional areas of randomized cells are proportional to the volumes of the cell compartments measured[8]. As the table shows, the cells and all the cell structures measured were smaller in the group of adenomas operated on 2 days or less after bromocriptine treatment ceased than were those in the other 2 groups; however, only the differences obtained for the values of total cell size, nucleus, and nucleolus were significant. The nucleolus shrank more than did the nucleus (decreasing nucleolus-nucleus relation); this difference was also significant. An increase in the nucleus-cytoplasm relation indicated that the nucleus shrank more than did the cytoplasm during the treatment with bromocriptine, but this difference was not significant.

The cell size and other morphometric features of prolactinomas that had not been treated with bromocriptine differed significantly from those of adenomas that were operated on during or shortly after bromocriptine treatment. However, no significant differences were noted between the values obtained from untreated adenomas and those from adenomas operated on 1 week or later after bromocriptine treatment stopped. The values reflecting the extent of reduction in tumor-cell size in this study are not as pronounced as those for the single case reported earlier, in which there was a 60% reduction in cell size and a 40% reduction in nuclear size[5]. This difference can probably be attributed to our figures being the average values for cells in a larger number of cases. No doubt the change of cell size varies considerably in individual cases.

It is known that bromocriptine causes reduction of the prolactin messenger RNA (mRNA) in normal prolactin cells by inhibition of the prolactin gene transcription[9,10]. We suspect that the drug has a similar effect on neoplastic prolactin cells. The nucleolus is the site of mRNA transcription[11]; its pronounced shrinkage during bromocriptine treatment may be the morphological expression of the decreased prolactin mRNA production. We have further seen, in our electron micrographs, that the area of cytoplasm occupied by the cisternae of the rough-surfaced endoplasmic reticulum (RER) was reduced in cases of bromocriptine treatment, although it was not possible to obtain a measurement with our present technique. This reduction of the RER, which may be the basis of the cytoplasm shrinkage with bromocriptine treatment, may be caused in turn by the reduced mRNA supply. The inhibition of prolactin mRNA synthesis in normal prolactin cells is fully reversible within 4 days if bromocriptine treatment is stopped[9]. This time interval correlates well with the interval after which reexpansion of adenoma cells was observed in our patients.

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