The differential distribution of NSE consistently shown at all levels of the gut primarily parallels the distribution of the innervation. In fact, the major amounts were found in the muscle layer, containing the large ganglia of Auerbach's plexus, while the lower concentrations measured in the submucosa parallel the looser network of Meissner's plexus. In the lamina propria of the mucosa no neurons are present, but nerve fibers form a very rich network[12]. Our findings are in keeping with previous investigation, which showed much higher concentrations of NSE to be present in the 'muscle' (muscle layer and submucosa) compared to the mucosa in the rat intestine[13].

The possibility that the low amounts of NSE found in the separated epithelium are due to contamination from the other layers cannot be ruled out in our study. However, in a previous investigation[10], we detected less than 0.5% of the total content of vasoactive intestinal polypeptide and substance P (exclusively neural peptides in the human gut) in the epithelium, while the adjacent lamina propria contained the highest concentration of both, thus indicating the negligible extent of contamination of the separated epithelium by nerve structures when this separation procedure is used.

By immunocytochemistry, endocrine cells were found to be the only source of NSE in the gut epithelium[8]. In the distal intestine they showed a lower immunostaining intensity[8], thus suggesting that their content of NSE may decrease distally. The finding of a low, but clearly detectable proportion of the NSE content in the separated ileal and colonic epithelium fits well with this finding, while indicating that the NSE concentration in the individual endocrine cells is likely to be significantly lower than that in enteric neurons.

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3 Marangos, P.J., and Schmechel, D., in: Essays in neurochemistry and neuropharmachology, vol.4, p.211. Eds M.B.H. Youdim, W. Lovenberg, D.F. Sherman and J.R. Lagnado. John Wiley & Sons, New York 1980.
4 Moore, B.W., in: International review of neurobiology, vol.15, p.215. Eds C.C. Pfeiffer and J.R. Smythies. Academic Press, New York 1972.
5 Marangos, P.J., Zomzely-Neurath, C.E., Luk, D.C.M., and York, C., J. biol. Chem. *250* (1975) 1884.
6 Pickel, V.M., Reis, D.J., Marangos, P.J., and Zomzely-Neurath, C., Brain. Res. *105* (1975) 184.
7 Schmechel, D., Marangos, P.J., and Brightman, M., Nature *276* (1978) 834.
8 Bishop, A.E., Polak, J.M., Facer, P., Ferri, G.-L., Marangos, P.J., and Pearse, A.G.E., Gastroenterology *83* (1982) 902.
9 Ferri, G.L., Probert, L., Cocchia, D., Michetti, F., Marangos, P.J., and Polak, J.M., Nature *297* (1982) 409.
10 Ferri, G.-L., Adrian, T.E., Ghatei, M.A., O'Shaughnessy, D.J., Probert, L., Lee, Y.C., Buchan, A.M.J., Polak, J.M., and Bloom, S.R., Gastroenterology *84* (1983) in press.
11 Parma, A.M., Marangos, P.J., and Goodwin, F.K., J. Neurochem. *36* (1981) 1093.
12 Gabella, G., Int. Rev. Cytol. *59* (1979) 129.
13 Kato, K., Ishiguro, Y., Suzuki, F., Ito, A., and Semba, R., Brain Res. *237* (1982) 441.

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Immobilization of rat brain synaptic vesicles on positively-charged glass microspheres

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Summary. Synaptic vesicles from rat brain were immobilized on glass microspheres covalently coated with poly-L-lysine. Using a potassium tartrate perfusion medium, the vesicular accumulation and methamphetamine-induced release of (L)-^{3}H-norephinephrine could be conveniently monitored in a flow experiment.

Synaptic vesicles from mammalian brain display a large, negative surface charge at pH 7.4[2]. It was felt that if this property could be utilized to electrostatically immobilize vesicles, then drug-induced release of neurotransmitters from vesicles could easily be monitored in a perfusion experiment. Such a method would eliminate such isolation procedures as membrane filtration, where separation of vesicles from the incubation medium precludes further use of the tissue. It had been reported[3] that glass microspheres of 25 µm diameter could be covalently functionalized with poly-L-lysine to provide a bead with a strong positively-charged surface at pH 7.4. This elegant technique was used to immobilize and expose the cytoplasmic surface of erythrocyte membranes[4]. The current report details the immobilization of synaptic vesicles from rat brain on glass microspheres functionalized with poly-L-lysine, and the methamphetamine-induced release of accumulated (L)-^{3}H-norephinephrine (NE) from perfused vesicles.

Methods. Tartrate buffer was prepared from neutral potassium tartrate (110 mM; pH 7.4)[5]. Ascorbic acid (10^{-5} M) and iproniazid (10^{-5} M) were included.

Glass beads (Ferro Corp., 25 µm diameter) were functionalized with poly-L-lysine (Sigma Chem. Co. mol.wt > 80,000) as described by Jacobson et al.[3]. Briefly, this procedure involved sequential treatment of 20 g portions of beads with nitric acid (to expose free surface silol groups), triethoxyaminopropylsilane (Pierce Chem. Co.) to covalently link aminopropyl residues via siloxy bridges, succinic anhydride (to amidate the aminopropyl residues), and poly-L-lysine (amidation via dicyclohexylcarbodiimide). Beads prepared in this manner were stable for several months at −90 °C.

Crude vesicles were isolated from rat brain by the method of Seidler et al.[6]. Sprague-Dawley rats of either sex (150–200 g) were decapitated, and the brain tissue (less cerebellum) quickly removed and weighed. The tissue was homogenized in 4 vols of cold 0.32 M sucrose by 6 up-down strokes of a hand-held Duall glass/glass homogenizer. The resulting suspension was sequentially centrifuged (3000 × g for 20 min; 20,000 × g for 30 min; 100,000 × g for 30 min) to yield a crude vesicle pellet (2.73 mg protein/g wet wt of brain). The pellet was resuspended in 500 µl of 0.32 M sucrose at 4 °C using slow up-down strokes of a hand-held teflon-glass homogenizer.

The resuspended pellet was transferred to a microcolumn (5 × 30 mm) containing 250 mg of beads supported on a small plug of glass wool, and the slurry allowed to stand for 5 min at 4 °C. The jacketed column was then warmed to

37 °C and tartrate buffer perfusion initiated at 1 ml/min. After an equilibration period of 10 min, the perfusion rate was diminished to 0.25 ml/min and L-^{3}H-NE (10^{-5} M, 1 μCi/ml; MgATP 10^{-3}M) perfused for 10 min. After loading, perfusion with buffer alone was resumed at a rate of 1 ml/min. Washing was conducted for periods of 15–30 min.

When drug-induced release was examined, the drug in question (plus Mg-ATP 10^{-3} M) was included in the buffer for 2 min. Fractions were collected each minute and the tritium content determined by liquid scintillation counting (minimum efficiency 30%). All samples were corrected for quenching.

MgATP, (±) methamphetamine sulfate, and (L)-NE bitartrate were obtained from Sigma Chemical Co. (L)-^{3}H-NE (44 Ci/mmole) was obtained from New England Nuclear Corp. Sprague-Dawley-derived rats were obtained from Biological Research Labs, Arvada, Colorado.

When employed, reserpine pretreatment consisted of a single dose of reserpine (Ciba, Serpasil, 2.5 mg/kg, i.p.) administered 18 h prior to sacrifice.

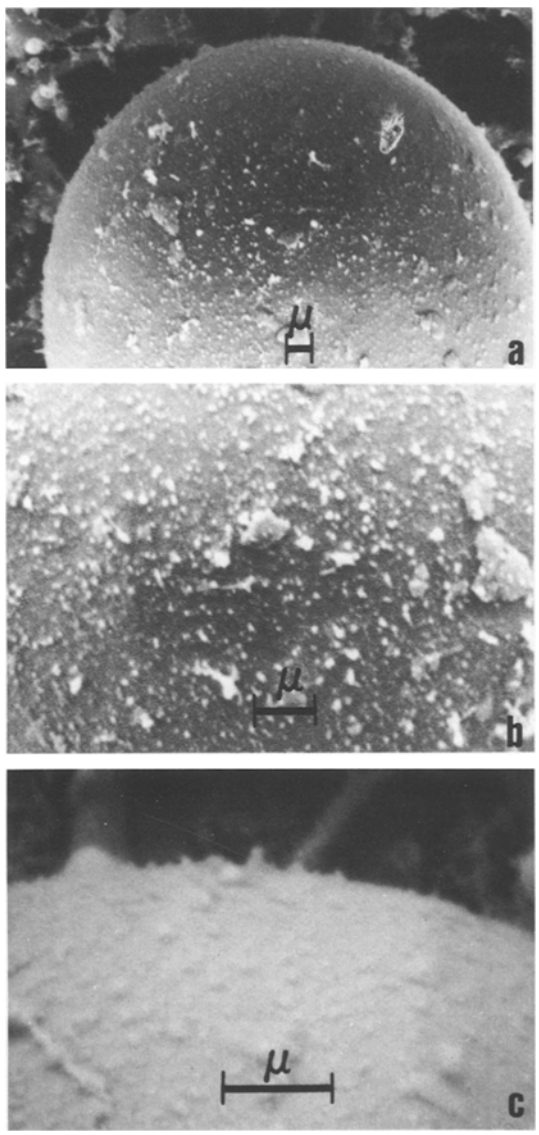

Figure 1. Scanning electron micrographs of rat brain storage vesicles immobilized on poly-L-lysine functionalized glass microspheres. *a* × 5400, *b* × 11,000, *c* × 21,000.

Results. We have previously demonstrated[5,7], that, depending upon the non-permanent medium employed, the vesicle fraction described accumulates 13.9–15.7 pmoles of ^{3}H-NE/mg protein (K_m 7.6×10^{-7} - 1×10^{-6} M), and that NE accumulation is reduced approximately 72% after in vivo reserpine pretreatment, 60–70% in the absence of MgATP, and by 90% at 4 °C. These biochemical parameters are characteristic of NE accumulation by vesicles from a variety of central and peripheral neural tissues[8].

Scanning electron-microscopical examination of the beads treated with the crude rat brain vesicle suspension reveal numerous spherical structures (500–1500 Å) attached to the bead surfaces (fig. 1).

The release of previously-accumulated L-^{3}H-NE by 10^{-5} M methamphetamine is shown in figure 2. Release did not occur in the absence of tissue. Tissue from animals pretreated with reserpine would not accumulate NE (data not shown).

Discussion. In view of the extended periods of biochemical viability of brain vesicles observed in media comprised of membrane-impermeant anions[5,7], it was of great interest to develop a perfusion technique to study drug-induced neurotransmitter release from synaptic vesicles. It was felt that a large negative charge existing on the vesicle surface would provide a means of electrostatically immobilizing vesicles. Using the recently reported method of functionalizing glass microspheres with poly-L-lysine[3], it was found that synaptic vesicles could be immobilized on the beads in much the same manner as has been reported for immobilization of erythrocytes.

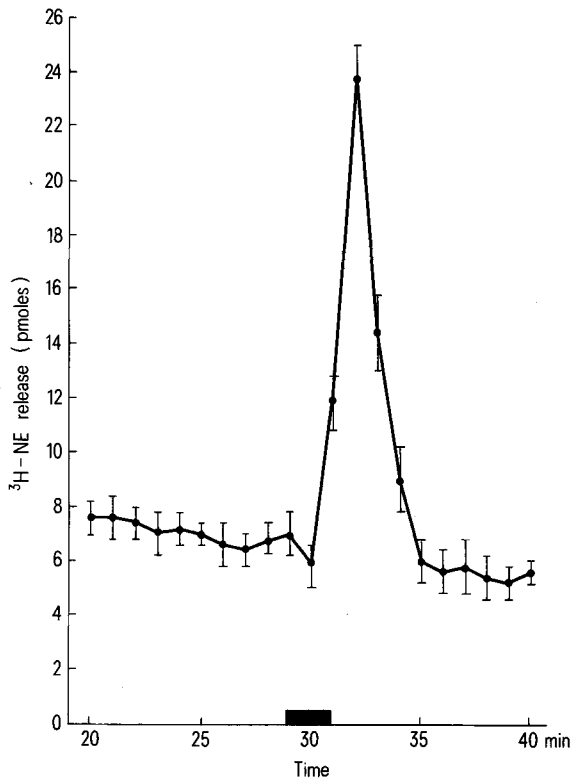

Figure 2. The release of (L)-^{3}H-norephinephrine from immobilized rat brain storage vesicles by 10^{-5} M methamphetamine. Vesicles were perfused with (L)-^{3}H-NE (10^{-5} M) and MgATP (10^{-3} M) for 10 min, followed by buffer for 18 min. Methamphetamine (10^{-5} M) and MgATP (10^{-3} M) were then included in the perfusion medium for 2 min (indicated by heavy bar). Samples were collected every minute and assayed for radioactivity. Mean ± SEM for 3 determinations.

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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2 Matthews, E.K., and Nordmann, J.J., Molec. Pharmac. *12* (1976) 778.
3 Jacobson, B.S., Cronin, J., and Branton, D., Biochim. biophys. Acta *506* (1978) 81.
4 Jacobson, B.S., and Branton, D., Science *195* (1977) 302.
5 Disbrow, J.K., Gershten, M.J., and Ruth, J.A., Experientia *38* (1982) 1323.
6 Seidler, F.J., Kirksey, D.F., Lau, C., Whitmore, W.L., and Slotkin, T.A., Life Sci. *21* (1977) 1075.
7 Disbrow, J.K., and Ruth, J.A., Life Sci. *29* (1981) 1989.
8 Philippu, A., in: The mechanism of neuronal and extraneuronal transport of catecholamines, p.215. Ed. D.M. Paton. Raven Press, New York 1976.
9 Johnson, R.G., Carty, S.E., Hayflick, S., and Scarpa, A., Biochem. Pharmac. *31* (1982) 815.

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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-