Endocytotic pathways in the melanotroph of the rat pituitary

NILS BÄCK, SEppo SOINILA and ISMO VIRTANEN

Department of Anatomy, University of Helsinki, Siltavuorenpenger 20A, SF-00170 Helsinki, Finland

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Summary

The internalization of the extracellular markers horseradish peroxidase (HRP) and cationized ferritin (CF) by the melanotrophs of the intermediate lobe of the rat pituitary was studied during short-time incubation of mechanically dissociated cells or in cell culture after 5 days. After a 30 min exposure, the tracers were found in electron-lucent granules or vacuoles of approximately the same size as the secretory granules, situated 200–500 nm from the cell membrane. In the cultured cells, which showed a higher rate of tracer uptake, internalization was followed for 1, 2 and 5 min after labelling and during 2 h of exposure. Initially, the label was seen only in coated pits and coated vesicles at the cell membrane. Larger vacuoles were first seen after 2–5 min of incubation. After 2 h of exposure the labelling pattern was distinctly different for the two tracers. CF was found in larger vacuoles of varying morphology, in dilatations at the base of cilia, within Golgi saccules and at the edge of the electron-dense core of forming secretory granules. HRP was found in an extensive array of tubulovesicular structures extending throughout the cytoplasm. The Golgi complex and forming granules were, however, not labelled with HRP. The study identifies part of the electron-lucent granules or vacuoles in the melanotroph as endosomes, and shows that the melanotrophs sort CF and HRP via diverting pathways after internalization, suggesting that granule membrane, and possibly its functional components, can be recycled in these cells.

Introduction

During exocytotic secretion the membrane of the secretory granule is incorporated into the plasma membrane. A reverse process, membrane internalization, has been demonstrated in a number of cell types by using extracellular markers, such as dextran, horseradish peroxidase and ferritins (see Herzog & Farquhar, 1977; Farquhar, 1982, 1985; Oliver et al., 1989). Of these tracers dextran, horseradish peroxidase and anionic ferritin serve as markers for extracellular fluid, while cationized ferritin binds to the cell membrane. Two major pathways have been described for the internalized membrane material; degradation in lysosomes or eventual re-utilization for granule formation in the Golgi complex (see Oliver & Hand, 1978; Farquhar, 1978, 1982, 1985; Herzog & Miller, 1979; Thyberg, 1980; Rosenzweig & Kanwar, 1984). Several lines of evidence suggest that the membrane of the exocytosed secretory granule is specifically re-internalized, not merely random areas of plasma membrane (de Camilli et al., 1976; Theodosis, 1983; Lotshaw et al., 1986; Patzak & Winkler, 1986).

New interest in the process of membrane re-internalization has been raised by the demonstration that several post-translational steps in the processing of secretory peptides occur within the secretory granule, where the corresponding enzymes are also contained, some of them membrane-bound (Gainer et al., 1985; Loh, 1987; Darby & Smyth, 1990). This raises the question of whether functional components of the granule membrane can also be re-utilized.

One of the best documented examples of peptide processing by granular enzymes is the post-translational processing of pro-opiomelanocortin (POMC) in the secretory granules of the melanotrophs in the intermediate lobe of the rat pituitary (Gainer et al., 1985; Loh, 1987; Mains et al., 1987; Schnabel et al., 1989). The endocytotic pathways of these cells have not been studied. Electron-lucent granules or vacuoles have been observed in these cells, and have been interpreted as a subtype of secretory granules, or as endosomes, or as a mixture of both (Bäck, 1989, 1990; Carr et al., 1991). The aim of the present study was to define the endocytotic pathways in the melanotroph, and characterize the role of electron-lucent granules or vacuoles in this process by following the internalization of two extracellular markers, horseradish peroxidase and cationized ferritin.

Material and methods

Adult (2–4 month old) Sprague-Dawley and Wistar rats of both sexes were used. The rats were decapitated under
pentobarbital anaesthesia (Mebunat, Orion, Helsinki, Finland),
the pituitary was dissected, and the neurointermediate lobe
separated from the anterior lobe under a dissection microscope.
For incubation *in vitro*, fragments of intermediate lobe tissue
were dissected from the neurointermediate lobe and transferred
to test tubes containing the incubation medium. The medium
was constantly oxygenated HEPES-Tricine-buffered Krebs–
Ringer-glucose, pH 7.35–7.40, at 37°C (Bäck *et al.*, 1988). After
a 30–45 min pre-incubation the medium was changed, 0.5 mg
ml⁻¹ cationized ferritin (Sigma, St Louis, MO, USA) or 5 mg
ml⁻¹ horseradish peroxidase (Sigma, type VI) was added, and
the incubation was continued for 30 min.

Fig. 1–4. Uptake of cationized ferritin (CF) and horseradish peroxidase (HRP) into melanotrophs during a 30 min incubation *in vitro.*

Fig. 1. Uptake of CF into two electron-lucent vacuoles (arrows) at some distance from the cell membrane. The lower vacuole shows a labelled connection towards the cell membrane. ×34 000.

Fig. 2. Uptake of CF into an electron-lucent vacuole (arrow). Two small labelled structures (thin arrows) are seen closer to the cell membrane. ×34 000.

Fig. 3. CF label in a small vesicle (arrow) at some distance from the cell membrane. Two unlabelled electron-lucent granules or vacuoles are shown by asterisks. ×49 600.

Fig. 4. Uptake of HRP (arrows) from the labelled surface of a melanotroph. The vacuole on the left shows direct communication with the cell membrane, the lower vacuole a labelled extension. ×34 000.

Fig. 5–7. Uptake of cationized ferritin (CF) into cultured melanotrophs exposed to CF for 30 min on ice, then incubated at 37°C for 1 or 2 min.

Fig. 5. Uptake of CF into a coated pit. One-minute incubation. ×70 000.

Fig. 6. CF in a coated vesicle near the labelled cell membrane. One-minute incubation. ×70 000.

Fig. 7. CF in a large, irregular vacuole with a tubular extension (arrows). Two-minute incubation. ×35 000.