Effect of monensin on intracellular transport and receptor-mediated endocytosis of lysosomal enzymes

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In cultured human fibroblasts we observed that monensin, a Na^+/H^+ -exchanging ionophore, (i) inhibits mannose 6-phosphate-sensitive endocytosis of a lysosomal enzyme, (ii) enhances secretion of the precursor of cathepsin D, while inhibiting secretion of the precursors of β -hexosaminidase, (iii) induces secretion of mature β -hexosaminidase and mature cathepsin D, and (iv) inhibits carbohydrate processing in and proteolytic maturation of the precursors remaining within the cells; this last effect appears to be secondary to an inhibition of the transport of the precursors. If the treated cells are transferred to a monensin-free medium, about half of the accumulated precursors are secreted, and the intracellular enzyme is converted into the mature form. Monensin blocks formation of complex oligosaccharides in lysosomal enzymes. In the presence of monensin, total phosphorylation of glycoproteins is partially inhibited, whereas the secreted glycoproteins are enriched in the phosphorylated species. The suggested inhibition by monensin of the transport within the Golgi apparatus [Tartakoff (1980) *Int. Rev. Exp. Pathol.* **22**, 227–250] may be the cause of some of the effects observed in the present study (iv). Other effects (i, ii) are rather explained by interference by monensin with the acidification in the lysosomal and pre-lysosomal compartments, which appears to be necessary for the transport of endocytosed and of newly synthesized lysosomal enzymes.

The pathway of lysosomal enzymes has several features in common with the secretory pathway. Lysosomal enzymes are synthesized on membrane-bound ribosomes, translocated across the membrane of the endoplasmic reticulum, glycosylated and subjected to carbohydrate-modifying reactions along their passage through the endoplasmic reticulum and the Golgi apparatus (Erickson & Blobel, 1979; Hasilik & von Figura, 1981; Rosenfeld *et al.*, 1982). The Golgi apparatus, Golgi-endoplasmic reticulum-lysosomes and the cell surface have been suggested as sites of the segregation of lysosomal enzymes from the secretory products (Novikoff *et al.*, 1964; Nichols *et al.*, 1971; von Figura & Weber, 1978; Sly & Stahl, 1979). The segregation depends on the binding of lysosomal enzymes to receptors specific for mannose 6-phosphate-containing oligosaccharides (for review, see Neufeld, 1981). The formation of mannose 6-phosphate residues represents an early modification of lysosomal-enzyme precursors in the Golgi apparatus, probably located in its *cis* (proximal) cisternae

(Pohlmann *et al.*, 1982; Goldberg & Kornfeld, 1983). These residues function as recognition markers (Kaplan *et al.*, 1977) for the receptors expressed on the cell surface and intracellular membranes (Rome *et al.*, 1979; Fischer *et al.*, 1980). After segregation from the secretory pathway by the receptor recognition-marker-dependent mechanism, lysosomal enzymes undergo a limited proteolysis, yielding the mature forms of the enzymes (for review see Hasilik, 1981). A minor fraction of lysosomal-enzyme precursors escapes the segregation and is secreted into the medium (Hasilik & Neufeld, 1980; Rosenfeld *et al.*, 1982).

Monensin, a carboxylic proton ionophore that exchanges preferentially Na^+ ions for protons (Pressman, 1976), is known to interfere with the transport of secretory products (reviewed by Tartakoff, 1980) and membrane proteins (Strous & Lodish, 1980) at the level of the Golgi apparatus. The carbohydrate in intracellularly accumulating secretory products suggests that monensin arrests transport before the distal Golgi elements (Tarta-

koff & Vassalli, 1979). Furthermore, monensin perturbs functions that are known to depend on the acidification of lysosomes and/or endosomes. Monensin blocks, for example, the recycling of low-density-lipoprotein receptors (Basu *et al.*, 1981) and the release of endocytosed diphtheria toxin (Marnell *et al.*, 1982) and Semliki Forest virus (Marsh *et al.*, 1982) into the cytosol. In view of the inhibitory effects of monensin on secretion and on endocytosis of various macromolecules on the one hand, and of the constitutive secretion of a portion of lysosomal-enzyme precursors (Hasilik & Neufeld, 1980) and of endocytosis of lysosomal enzymes by a receptor-mediated system (reviewed by Neufeld, 1981) on the other hand, we considered monensin to be useful in studies on the transport of lysosomal enzymes. We report below on distinctive effects of monensin on several steps in the transport and processing of lysosomal enzymes in cultured human fibroblasts.

Materials and methods

Materials

Monensin was kindly provided by Dr. R. L. Hamill, Eli Lilly and Co., Indianapolis, IN, U.S.A. *p*-Nitrophenyl β -*N*-acetylglucosaminide was from Koch-Light Laboratories, Colnbrook, Bucks., U.K.; *p*-nitrocatechol sulphate was from Sigma, St. Louis, MO, U.S.A. Endo- β -*N*-acetylglucosaminidase H (EC 3.2.1.96; 30 units/mg of protein) was from Seikagaku, Kogyo, Tokyo, Japan. L-[³⁵S]Methionine (1800 Ci/mmol), L-[4,5-³H]leucine (59.8 Ci/mmol) and [2-³H]mannose (22 Ci/mmol) were from New England Nuclear, Dreieich, Germany.

Cultivation and labelling of cells

Human skin diploid fibroblasts were maintained at 37°C in CO₂/air (1:19) in Eagle's minimal essential medium supplemented with antibiotics, non-essential amino acids and 7.5% (v/v) foetal-calf serum (Boehringer Mannheim, Mannheim, Germany) as described by Cantz *et al.* (1972).

Conditions for pulse and pulse-chase labelling with L-[4,5-³H]leucine, L-[³⁵S]methionine and [2-³H]mannose were as described (Hasilik & Neufeld, 1980; Hasilik & von Figura, 1981; Waheed *et al.*, 1982). Cultures in 25 cm² and 75 cm² flasks were incubated for the time indicated with 2–5 ml of labelling medium containing 0.05–0.5 mCi of radioactive precursor. Stock solutions of monensin were prepared in dimethyl sulfoxide and, where indicated, were added to the medium at 1% (v/v) dimethyl sulfoxide. The medium of controls was supplemented with 1% dimethyl sulfoxide. Ex-

tracts of cells and media were prepared (Hasilik & von Figura, 1981) and subjected to immune precipitation (Hasilik & Neufeld, 1980). In some experiments the extracts were treated with endo- β -*N*-acetylglucosaminidase H for 40 h before immune precipitation (Hasilik & von Figura, 1981). The immunoprecipitated polypeptides were separated by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Laemmli, 1970) and detected by fluorography (Laskey & Mills, 1975). Where desired, radioactivity incorporated into polypeptides was measured after treatment of the gel with NCS tissue solubilizer (Amersham Buchler, Braunschweig, Germany) (Waheed *et al.*, 1982).

Characterization of oligosaccharides

[2-³H]Mannose-labelled oligosaccharides were released from the extracts of cells and media by endo- β -*N*-acetylglucosaminidase H and separated into neutral and acidic species by ion-exchange gel chromatography on a column of AG 1-X2 (Hasilik *et al.*, 1980). Analytical characterization of neutral and acidic oligosaccharides by gel filtration on Bio-Gel P-4, degradation with alkaline phosphatase (EC 3.1.3.1) from *Escherichia coli* before and after mild hydrolysis and paper electrophoresis in 1.9 M-formic acid were performed as described by Hasilik *et al.* (1980). The fraction of phosphorylated oligosaccharides was defined as the fraction of acidic oligosaccharides rendered neutral by alkaline phosphatase after mild acid hydrolysis. Mono- and di-phosphorylated oligosaccharides were separated by paper electrophoresis. Phosphorylated oligosaccharides containing phosphodiester groups were defined as the fraction of phosphorylated oligosaccharides rendered neutral by alkaline phosphatase only after mild acid hydrolysis (Hasilik *et al.*, 1980).

Endocytosis of lysosomal enzymes

Uptake of arylsulphatase A (EC 3.1.6.1) by arylsulphatase-A-deficient fibroblasts was determined (Waheed *et al.*, 1982) in media supplemented with 120 units of arylsulphatase A partially purified from human urine and up to 1 μ M-monensin and 0.1% dimethyl sulfoxide. β -Hexosaminidase (EC 3.2.1.52) was prepared from secretions of human skin fibroblasts grown in the presence of either 10 μ M-monensin and 1% dimethyl sulfoxide or 1% dimethyl sulfoxide alone. After concentration by ultrafiltration and dialysis overnight against Eagle's minimal essential medium, uptake by fibroblasts deficient in β -hexosaminidase was determined (Ullrich *et al.*, 1978). Uptake is expressed in μ l of medium cleared from enzyme/h per mg of cell protein.

Other methods

Protein was determined by the method of Lowry *et al.* (1951), and β -hexosaminidase and lactate dehydrogenase were assayed as described by von Figura (1978).

Results

Effect of monensin on the maturation and transport of lysosomal enzymes

In the presence of monensin (3–10 μ M), the processing of the precursors of cathepsin D (EC 3.4.23.5) and β -hexosaminidase was nearly abolished and the precursors accumulated within the cells (Figs. 1 and 2). At the concentrations used, monensin did not significantly affect the labelling of cathepsin D and β -hexosaminidase. After 24 h the labelling of the material precipitable with trichloroacetic acid was inhibited by 15–25%.

Attempting to characterize the site(s) of the accumulation of the precursors, we used two fractionation procedures. In a Percoll gradient it was not possible to separate lysosomes from other membranous material when cells treated with

monensin were examined. In differential centrifugation (Remacle *et al.*, 1980) of homogenates prepared from monensin-treated cells, the accumulated precursors sedimented less readily than did mature lysosomal enzymes, and more extensively than did the Golgi-apparatus marker enzymes galactosyltransferase (EC 2.4.1.38) and α -D-mannosidase (results not shown). The site(s) of the accumulation could donate the precursors to lysosomes and to the secretory pathway. When cells labelled for 13.5 h in the presence of monensin were transferred for 36 h to a medium without the drug, about half of the precursors was released to the medium (Fig. 3). The intracellular enzymes were converted into the mature lysosomal forms.

Effects of monensin on secretion of lysosomal enzymes

Surprisingly, monensin affected differentially the secretion of the precursors of the two enzymes studied in Figs. 1 and 2. Cells incubated with monensin secreted 6 times as much cathepsin D precursor as did the control (Fig. 1). In contrast, the secretion of β -hexosaminidase precursor was approxi-

Fig. 1. *Effect of 3 μ M-monensin on synthesis, distribution and processing of cathepsin D*

Confluent cultures in 25 cm² flasks were incubated for 3–24 h (see numbers above the lanes) with 2.5 ml of medium containing 0.25 mCi of L-[4,5-³H]leucine, 1% dimethyl sulphoxide and 3 μ M-monensin where indicated. Cathepsin D was immune-precipitated from extracts of cells and media, separated by polyacrylamide-gel electrophoresis and detected by fluorography. The migration positions of the precursor, intermediate and mature forms of cathepsin D are indicated by arrows. The cathepsin D precursor of monensin-treated cells migrated slightly faster (see the dashed arrow). In controls 6, 4, 5, 5 and 6% of labelled cathepsin D was recovered in the media after 3, 6, 9, 12 and 24 h respectively. In monensin-treated cells cathepsin D secreted after 3, 6, 9, 12 and 24 h accounted for 12, 22, 31, 28 and 33% of total ³H-labelled cathepsin D. Standards of *M*, 92500, 69000, 46000, 30000 and 12300 were used. (In the control of cells labelled for 24 h, the immune precipitate contains two heavy contaminants in the vicinity of the precursor and two additional ones at the bottom of the gel; for processing of cathepsin D see Hasilik & Neufeld, 1980.)

Fig. 2. Effect of monensin on synthesis, distribution and processing of β -hexosaminidase

For labelling conditions, isolation of labelled polypeptides and standards see the legend to Fig. 1. Migration positions of the precursor (Pro α and Pro β) and the main mature (Mature α , Mature β) forms of the α - and β -chains are indicated by arrows. The minor polypeptides smaller than the β -chain are fragments of the β -precursor. The low intensity of bands in samples of the 9 h control cells and 6 h monensin-treated cells is due to a loss of material during the isolation. In controls 5, 14, 27 and 28% of total β -hexosaminidase were recovered in the media after 3, 6, 12 and 24 h, whereas in monensin-treated cells 3, 10 and 12% of total β -hexosaminidase were present in the media after 3, 9 and 12 h.

mately halved (Fig. 2) under the same conditions. This was not due to degradation, since polypeptides of β -hexosaminidase were stable in the medium incubated for 24 h at 37°C (results not shown). It had been reported, however, that monensin increased the secretion of β -hexosaminidase (Vladutiu & Rattazzi, 1980). Therefore we also determined the activity of β -hexosaminidase in secretions of cells treated with monensin. After 24 h incubation in the presence of 1% dimethyl sulfoxide and 0, 0.1 μ M-, 0.3 μ M-, 1 μ M-, 3 μ M- and 10 μ M-monensin, 10, 13, 14, 18, 24 and 33% of total β -hexosaminidase were found in the medium, respectively. This suggested that monensin induces secretion of mature lysosomal enzymes. In order to test such a possibility, fibroblasts with labelled mature lysosomal enzymes and depleted of radioactive precursors were prepared and incubated with or without monensin (Fig. 4). In the absence of the drug, no radioactive β -hexosaminidase or cathepsin D was released into the medium, whereas in its presence mature forms of both lysosomal enzymes were released from the cells. Incubation with 10 μ M-monensin for 24 h caused the release of 9.7 and 10.3% of the mature forms of β -hexosaminidase and cathepsin D, respectively, whereas

in controls the release of mature forms was below 1%. Monensin had no effect on release of lactate dehydrogenase. The mixture of precursor and mature β -hexosaminidase secreted in the presence of monensin contained a considerable proportion of the phosphorylated 'high uptake' enzyme; the endocytosis in fibroblasts was sensitive to mannose 6-phosphate, and the clearance rate was about 2-fold higher for β -hexosaminidase from monensin-stimulated secretion than with the control (Table 1).

Effect of monensin on processing of carbohydrate in lysosomal enzymes

The extent of conversion of high-mannose oligosaccharides in lysosomal enzymes into complex oligosaccharides was probed with endo- β -N-acetylglucosaminidase H. As reported previously (Hasilik & von Figura, 1981), the oligosaccharide side chains in cathepsin D precursors secreted by controls were only partially sensitive to treatment with endo- β -N-acetylglucosaminidase H. In contrast, in cathepsin D precursors synthesized in the presence of monensin, virtually all oligosaccharide side chains were of the high-mannose or hybrid type, as judged from sensitivity to endo- β -N-acetyl-

Fig. 3. Secretion and processing of accumulated lysosomal enzyme precursors after removal of monensin
Cultures in 25cm² flasks were incubated with 2ml of medium containing 0.1 mCi of L-[4,5-³H]leucine, 1% dimethyl sulphoxide and 10µM-monensin. After incubation for 13.5h the medium was removed (Pulse). The cells were washed twice with 5ml of Eagle's minimal essential medium containing 7.5% foetal-calf serum and then fed with 3ml of the same medium. After 36h the cells (Cells) and the medium (Chase) were collected and analysed for ³H-labelled β-hexosaminidase and cathepsin D.

Fig. 4. Effect of monensin on the secretions of mature forms of β-hexosaminidase and cathepsin
Confluent cultures in 25cm² flasks were incubated for 24h with 3ml of medium in the presence of 0.25mCi of L-[4,5-³H]leucine. Then 1.25µmol of unlabelled leucine was added and the media were collected after 12.5h (Pulse). The cultures were incubated with 3ml of medium containing 4% of inactivated and dialysed foetal-calf serum (Hasilik & von Figura, 1981), 1% dimethyl sulphoxide and 10µM-monensin where indicated. After 24h the media (Chase) and cells (results not shown) were collected and analysed for ³H-labelled β-hexosaminidase and cathepsin D. Extracellular lactate dehydrogenase activity in the chase medium accounted for 2.4% of intracellular activity in controls and 2.1% in monensin-treated cells respectively; β-hexosaminidase activity secreted was 3.8-fold higher in monensin-treated cells than in controls.

Table 1. Effect of monensin on uptake properties of β-hexosaminidase

β-Hexosaminidase was prepared from secretions from cells incubated in the absence or presence of 10µM-monensin and added to the medium of Sandhoff fibroblasts that are deficient in β-hexosaminidase. After incubation for 24h in the absence or presence of 5mM-mannose 6-phosphate, the cells were harvested and assayed for β-hexosaminidase activity and protein. All values are corrected for the endogenous residual β-hexosaminidase activity in Sandhoff fibroblasts. The specific activities of the precursor and mature forms of β-hexosaminidase do not differ from each other (Hasilik *et al.*, 1982).

β-Hexosaminidase added (50 munits) from secretions of:	Mannose 6-phosphate (mM)	β-Hexosaminidase clearance rate (µl/h per mg of protein)
Control cells	0	6.2
	5	0.03
Cells treated with 10µM-monensin for 48h	0	12.0
	5	0.02

Fig. 6. Effect of monensin in size distribution of neutral high-mannose oligosaccharides

Oligosaccharides were prepared as described in the legend of Table 2 and in the Materials and methods section. Neutral oligosaccharides were applied to Bio-Gel P-4 columns (1 cm $\times$ 200 cm) in 50 mM-acetic acid. The ratio of the elution and total volumes (V_e/V_t) is based on the elution of $^3\text{H}_2\text{O}$, which served as an internal standard. The elution of $\text{Man}_x\text{GlcNAc}$ standards with x from 4–8 is indicated. (a) Cellular fraction; (b) secreted fraction. O, Radioactivity in the oligosaccharides from monensin-treated cells (1 unit on the ordinate corresponds to 4000 c.p.m. for the cellular and to 1000 c.p.m. for the secreted material); ●, radioactivity in controls (1 unit on the ordinate corresponds to 2000 c.p.m. for the cellular and to 1000 c.p.m. for the secreted material).

The alterations caused by monensin in the synthesis of carbohydrate moieties in total cellular and secreted material were as follows. Monensin stimulated the incorporation of $[2\text{-}^3\text{H}]\text{mannose}$ into total protein 3-fold and into oligosaccharides sensitive to endo- β -*N*-acetylglucosaminidase H 3.6-fold (Table 2). In the presence of monensin the abundance of oligosaccharides releasable with endo- β -*N*-acetylglucosaminidase in the total product, and especially in the secreted glycoproteins, was increased. The ionophore also caused a partial decrease in phosphorylation of high-mannose oligosaccharides. The relative amount of radioactivity present in phosphorylated oligosaccharides was about halved in monensin-treated cultures. The percentage of intracellular phosphorylated oligosaccharides with two phosphates or with phosphomonoesters was also decreased in the presence of monensin. Interestingly, secretions of monensin-treated cells were relatively enriched in phosphorylated oligosaccharides, especially in species with two phosphate groups and those with uncovered phosphate groups. Further, monensin changed the size distribution of neutral oligosaccharides, as shown in Fig. 6. The relative abun-

dances of oligosaccharides separated according to their size were differentially changed in the cellular and the secreted material. In the latter, monensin caused an increase in the relative abundance of shorter oligosaccharides.

Effect of monensin on mannose 6-phosphate-sensitive endocytosis

Arylsulphatase A was incubated with fibroblasts for 6 h (see the Materials and methods section). In the control the rate of endocytosis was calculated as 0.093 ml/h per mg of cell protein. In the presence of 10 mM-mannose 6-phosphate it was inhibited by 93%. In the presence of 0.03 μM -, 0.1 μM -, 0.3 μM - and 1 μM -monensin the rate was diminished by 9, 35, 40 and 53% (mean values from two determinations) respectively.

Discussion

Monensin is an ionophore that exchanges protons for Na^+ (and to some extent K^+) ions (Pressman, 1976; Marsh *et al.*, 1982). Perturbation of several gradients across cellular membranes may be the cause of its multiple effects on the transport

and processing of lysosomal enzymes, namely (a) inhibition of endocytosis of lysosomal enzymes, (b) stimulation of secretion of newly synthesized and stored lysosomal enzymes, (c) inhibition of the formation of complex oligosaccharides, and (d) inhibition of the proteolytic maturation of precursor polypeptide chains.

Concerning (a), monensin inhibits transport of a variety of ligands into mammalian cells (Vladutiu & Rattazzi, 1980; Basu *et al.*, 1981; Marnell *et al.*, 1982; Marsh *et al.*, 1982; Wilcox *et al.*, 1982; Dickson *et al.*, 1982). In fibroblasts the transport of lysosomal enzymes from the medium into the lysosomes depends on recognition of phosphorylated mannose residues in the oligosaccharide side chains in the enzymes by a specific receptor on the cell surface (Neufeld, 1981). The dissociation of the bound phosphorylated enzymes from the receptor appears to depend on acidification of an intracellular compartment (Gonzales-Noriega *et al.*, 1980). Since in fibroblasts the acidification of the endocytic vesicles is prevented in the presence of monensin (Maxfield, 1982), the drug is likely to block the endocytosis of lysosomal enzymes by interfering with the reutilization of the receptor. Merion & Sly (1983) reported that monensin inhibits the transfer of interiorized receptor-bound lysosomal enzymes and other ligands to secondary lysosomes.

Concerning (b), the above-mentioned receptor is believed to be involved in the segregation of newly synthesized lysosomal enzymes from the secretory products (Gonzales-Noriega *et al.*, 1980). Its function depends on persistence of acidic pH in the prelysosomal compartment. Therefore, if we were not aware that monensin inhibits transport to the plasma membrane (see below), we would expect monensin, in the context of its interference with the acidification of the endocytic vesicles (Maxfield, 1982), to promote secretion of newly synthesized lysosomal enzymes. Although monensin has previously been shown to stimulate the secretion of β -hexosaminidase in human fibroblasts (Vladutiu & Rattazzi, 1980), it has not been clear what forms of the enzyme are secreted by the treated cells. We show that, first, monensin stimulates secretion of the precursor of cathepsin D, but not of the precursor of β -hexosaminidase. A similar effect is exerted by cyanate (Hasilik *et al.*, 1983). Second, monensin induces a release of mature lysosomal enzymes (Fig. 5). Inasmuch as the unaffected release of lactate dehydrogenase can be taken as evidence for integrity of cells, the data suggest that monensin induces a (vesicular) transport of secondary lysosome contents to the exterior of the cell. This secretion is unlikely to be mediated by a rise in the intralysosomal pH, since weak bases cause negligible secretion of mature lysosomal enzymes as com-

pared with precursors (Hasilik & Neufeld, 1980). Third, monensin causes an accumulation of lysosomal enzymes, in a prelysosomal compartment, from which they are released into the medium in the precursor forms if monensin is withdrawn (see Fig. 3).

Concerning (c), the processing of the oligosaccharides occurs mainly in the Golgi apparatus, with the 'trimming' (Rothman, 1981) and the phosphorylation (Pohlmann *et al.*, 1982; Goldberg & Kornfeld, 1983) reactions being localized in the higher- and intermediate-density fractions and the terminal glycosylation reactions in the lower-density fractions, corresponding to the *trans* cisternae of the organelle (Tartakoff, 1980; Rothman, 1981; Roth & Berger, 1982; Griffith *et al.*, 1983). The effect of monensin on the Golgi apparatus is manifested in swelling of the organelle (Tartakoff & Vassalli, 1977; Nishimoto *et al.*, 1982; Wilcox *et al.*, 1982) and impairment of terminal glycosylation and of transport to the plasma membrane of secretory products (Tartakoff & Vassalli, 1979; Strous & Lodish, 1980; Ledger *et al.*, 1983) and of membrane proteins (Strous & Lodish, 1980; Pesonen & Kääriäinen, 1982; Quinn *et al.*, 1983). In a few cases the inhibition of the transport has been reported to occur after the terminal glycosylation reactions (Strous & Lodish, 1980; Crine & Dufour, 1982).

In fibroblasts monensin strongly inhibited secretion of mannose-labelled macromolecules, whereas it increased in the residual secretion the proportion of the oligosaccharides cleavable with endo- β -N-acetylglucosaminidase (Table 2). Normally, fibroblasts secrete a portion of cathepsin D precursor that is enriched in molecules containing one or two complex oligosaccharides (Hasilik & von Figura, 1981). As mentioned above, in the presence of monensin for 3–24 h the secretion of cathepsin D precursor is enhanced. The enhancement is accounted for by precursor polypeptides containing only high-mannose oligosaccharides. The block in the formation of complex oligosaccharides in cathepsin D and the shifts in the labelling of total oligosaccharides in favour of the cleavable high-mannose type and of shorter chains indicate that in fibroblasts, as in plasma cells, monensin impairs transport into the *trans* cisternae of the Golgi apparatus. It is not known which pathway is followed by the residually secreted glycoproteins containing high-mannose oligosaccharides. A proportion of the precursor of cathepsin D seems to by-pass the inhibited site and to avoid the terminal modifications, which normally render the oligosaccharides resistant to endo- β -N-acetylglucosaminidase H. In the presence of monensin, human fibroblasts secrete abnormally glycosylated fibronectin, which contains oligosaccharides sensitive

to endo- β -N-acetylglucosaminidase H (Ledger *et al.*, 1983). However, the secretion of fibronectin is severely decreased in the presence of monensin, whereas that of cathepsin D is increased. The phosphorylation and uncovering of phosphate groups, i.e. the typical modification reactions in lysosomal enzymes that are associated with Golgi fractions equilibrating at a higher density than those containing galactosyltransferase (Pohlmann *et al.*, 1982; Goldberg & Kornfeld, 1983), are little disturbed by monensin, as indicated by analysis of the phosphorylated oligosaccharides and of the uptake properties of β -hexosaminidase secreted in the presence of monensin. The partial decrease in the apparent extent of phosphorylation of oligosaccharides is probably secondary to change in the labelling of different oligosaccharides and/or processing in lysosomes. Occurrence of complex oligosaccharides in the mature enzymes within the lysosomes (Hasilik & von Figura, 1981) has been taken to signify packaging of the lysosomal enzymes after passage through the *trans* cisternae of the Golgi apparatus. The transient storage of the precursor of cathepsin D and the stimulation of the secretion of cathepsin D containing exclusively high-mannose type oligosaccharides by monensin leaves open the option of some lysosomal-enzyme precursor molecules being packaged before reaching the *trans* cisternae.

Concerning (d), monensin has been reported to inhibit proteolytic maturation of pro-opiomelanocortin in rat pituitary cells (Crine & Dufour, 1982) and of hormone receptors in rat pituitary cells (Jacobs *et al.*, 1983). Inhibition of the maturation of the precursors of lysosomal enzymes in fibroblasts by monensin resembles the effect of several other agents, including tunicamycin (von Figura *et al.*, 1981; Rosenfeld *et al.*, 1982), weak bases (Hasilik & Neufeld, 1980; Myerowitz & Neufeld, 1981), cyanate (Hasilik *et al.*, 1983) and concanavalin A (S. Krüger, unpublished work). It is known that lysosomal-enzyme precursors are subject to proteolytic maturation only after separation from the secretory products and transport into the lysosomes (Fischer *et al.*, 1980; Hasilik & Neufeld, 1980). The impairment of the maturation of the precursors of lysosomal enzymes in the presence of monensin is probably a result of an inhibition of the transport through the *trans* cisternae of the Golgi complex and from a prelysosomal compartment to lysosomes.

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