THE HYPERACTIVITY OF HAMSTER FIBROBLAST LYSOSOMAL ENZYMES AFTER ENDOCYTOSIS OF SUCROSE

Michael J. Warburton and Colin H. Wynn

Department of Biochemistry, University of Manchester, Manchester, M13 9PL, U.K.

Received February 24, 1976

The uptake of intralysosomal storage of sucrose by hamster fibroblasts results in increases in the activities of several types of lysosomal enzymes. The increases are not restricted to glycosidases. The activities of plasma membrane and Golgi apparatus enzymes are also increased whereas enzymes of other organelles are not affected. Uptake of sucrose causes the cessation of endocytosis within 5 hours. The time course of increases in enzyme activities demonstrates that the initial changes occur in the plasma membrane.

Many cultured mammalian cells take up sucrose into the lysosomal system by pinocytosis (1-4) and since sucrose cannot be degraded by lysosomal enzymes it persists within the lysosomes (5). To maintain the osmotic balance between the interior of the lysosomes and the cytoplasm, there is an influx of water resulting in greatly enlarged lysosomes (6). Overloading of the lysosomal system with non-digestible material and the resulting vacuolation often leads to increased activities of lysosomal enzymes (1-3) and it has been suggested that there is a specific increase in glycosidase levels in response to the storage of sucrose (3).

We have investigated the effects of sucrose storage on several types of hamster fibroblast lysosomal enzymes and also on enzymes which are constituents of other components of the vacuolar apparatus (the plasma membrane and Golgi apparatus) to determine the specificity and extent of the response to sucrose storage.

MATERIALS AND METHODS

Monolayers of Chinese hamster CH-23 fibroblasts were maintained with Dulbecco's modified Eagle's medium supplemented with 10% calf serum (Flow) in 75 cm^2 Falcon flasks. To investigate the effects of sucrose on enzyme activities, approximately 10^5 cells were inoculated into 15ml of medium containing 0.08M sucrose. Control cultures were run concurrently. The cells were harvested by trypsinisation after 72h while still in exponential growth. The cell pellets were washed with 0.25M sucrose (3ml) and homogenised in 50mM sodium acetate buffer pH 5.0 (2ml) with a Potter-Elvejholm homogeniser. The homogenate was assayed for a variety of enzymes as described in references 7-10 in the presence of 0.1% Triton X-100. Protein was estimated by the method of Lowry *et al* (11).

The uptake of [U-^{14}C]-sucrose (Amersham) was investigated by adding 1μCi of [^{14}C]-sucrose to a series of flasks containing confluent cells in 15ml of medium. The cells were harvested at various times after washing the cell sheet with 0.25M sucrose (2 x 10ml) to remove free radioactive sucrose. After trypsinisation of the cell pellet was further washed with 0.25M sucrose (3 x 5ml) and homogenised in 2ml of water. Triton X-100 was added to the homogenate to a final concentration of 0.2% (v/v). The homogenate was centrifuged at 5 000 x g for 15 min to remove cellular debris and the radioactivity of a 0.2ml aliquot of the supernatant was determined by liquid scintillation counting using a xylene-Triton-X-100 based scintillator.

RESULTS AND DISCUSSION

Growth of Chinese hamster fibroblasts in the presence of 0.08M sucrose produces small but statistically significant increases in the activities of the four lysosomal enzymes, arylsulphatase B, cathepsin D, β-glucuronidase and N-acetyl-β-glucosaminidase (table 1). Contrary to the results obtained by Bernacki and Bosmann (1) with mouse L5178Y cells, the increases are not restricted to glycosidases but include proteases and sulphatases. Thus sucrose does not specifically induce the synthesis of enzymes involved in polysaccharide degradation.

The increase in the activity of the Golgi apparatus enzyme, UDP galactose, N-acetylglucosamine galactosyl transferase is comparable with that of the lysosomal enzymes. Dingle *et al* (2)

TABLE 1. The specific activities of hamster fibroblast enzymes in the presence and absence of 0.08M sucrose. Cells were grown in the presence of sucrose for 72h. The numbers in parenthesis refer to the number of determinations. Statistics refer to ± standard deviation, t for the difference between the means was calculated using the student t test and P was determined from tables, N.S. = not significant.

Enyzme	Specific Activity (units/mg protein)		Specific Activity in presence of 0.08M sucrose		% change	significance (P)
β-glucuronidase	0.164 ±0.014	(16)	0.213 ±0.017	(10)	+29.9	<0.05
N-acetyl-β-glucosaminidase	0.626 ±0.041	(10)	0.890 ±0.069	(10)	+42.2	<0.05
arylsulphatase B	2.45×10^{-2} $\pm 0.020 \times 10^{-2}$	(21)	3.75×10^{-2} $\pm 0.28 \times 10^{-2}$	(15)	+46.1	<0.05
cathepsin D	6.53 ±0.92	(10)	8.36 ±0.87	(12)	+28.0	<0.05
5'-nucleotidase	0.94×10^{-2} $\pm 0.08 \times 10^{-2}$	(10)	1.42×10^{-2} $\pm 0.09 \times 10^{-2}$	(12)	+51.1	<0.05
UDP-galactose, N-acetyl-glucosamine galactosyl transferase	9.30×10^{-4} $\pm 0.77 \times 10^{-4}$	(14)	1.20×10^{-3} $\pm 0.13 \times 10^{-3}$	(12)	+28.6	<0.05
fumarase	0.126 ±0.015	(8)	0.117 ±0.016	(6)	-7.1	N.S.
alkaline ribonuclease	0.144 ±0.011	(18)	0.154 ±0.012	(14)	+7.0	N.S.

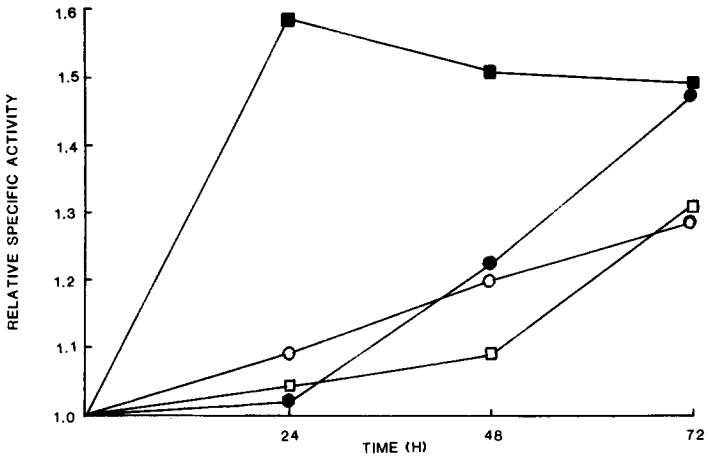

Fig. 1. The time course of the effect of sucrose on enzyme activities. Cells grown in the presence of 0.08M sucrose were harvested at various times, homogenised and assayed for several enzymes as described in the text. 5'-nucleotidase (■ - ■), galactosyl transferase (O - O), arylsulphatase B (● - ●), β-glucuronidase (□ - □).

noted a hypertrophy of the Golgi apparatus and an increased production of primary lysosomes during growth of chick embryonic cartilage in the presence of sucrose. The plasma membrane enzyme, 5'-nucleotidase, shows a relatively large increase after growth in medium containing sucrose.

The changes in the activities of mitochondrial and soluble cytoplasmic marker enzymes (fumarase and alkaline ribonuclease respectively) are not statistically significant. Endoplasmic reticulum enzymes (8) could not be detected in this cell line (12). The effects of sucrose, consequently, appear to be limited to those organelles which participate in the vacuolar apparatus (13) namely the Golgi apparatus, lysosomes and plasma membrane.

The time courses of the increases in enzyme activities induced by growth in the presence of sucrose are shown in fig. 1. There is a lag of about 24h before the activity of arylsulphatase B begins to increase and a 48h lag in the case of β-glucuron-

idase. Galactosyl transferase shows a linear rate of increase over the entire time period. The plasma membrane enzyme, 5'-nucleotidase, shows a rapid increase in activity reaching its maximum specific activity within 24h.

Wagner et al (14) have shown that the uptake of sucrose by Chang liver cells results in the rapid cessation of endocytosis, presumably due to the irreversible internalisation of plasma membrane. The persistence of the sucrose valuoles prevents the internalised membrane from being reutilised for plasma membrane synthesis. Hamster fibroblasts take up [^{14}C]-sucrose linearly after a lag phase (fig. 2). The lag phase may represent the time necessary for [^{14}C]-sucrose to penetrate the space between the confluent cells. When 0.08M sucrose is added to the culture medium at the same time as the [^{14}C]-sucrose, the lag phase is abolished and [^{14}C]-sucrose is taken up at a rate which is comparable to the maximum rate in the absence of 0.08M sucrose.

In the presence of 0.08M sucrose, uptake of [^{14}C]-sucrose stops after about 5h. The intracellular concentration of sucrose at this time is 65µg per mg of cell protein. When [^{14}C]-sucrose is added to the cells which have been cultured in the presence of 0.08M sucrose for 48h, little uptake of [^{14}C]-sucrose was observed, indicating that the cessation of endocytosis is permanent. Endocytosis may constitute a mechanism whereby certain plasma membrane enzymes are degraded (15,16). Inhibition of endocytosis should therefore give rise to increased levels of these enzymes. Our observations on 5'-nucleotidase are in agreement with this suggestion.

In conclusion, sucrose produces elevated lysosomal and Golgi apparatus enzyme levels. The mechanism of this response

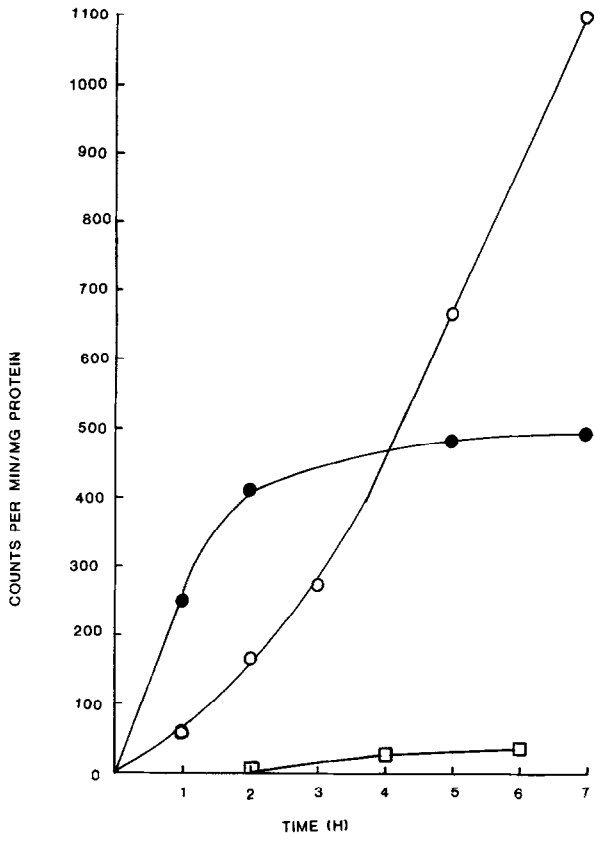

Fig. 2. The uptake of [^{14}C]-sucrose by hamster fibroblasts. Confluent cells were grown in the presence of [U-^{14}C]-sucrose (1μCi/ml) and harvested at time intervals. The experiment was carried out either in the absence of unlabelled sucrose (O - O) or with 0.08M sucrose added at zero time (● - ●) or with cells which had been grown in the presence of 0.08M sucrose for 48h before the addition of [^{14}C]-sucrose (□ - □).

is unclear although Dingle (17) has suggested that membrane instability caused by vacuolation may be important. These changes are preceeded by the cessation of endocytosis and increases in the activities of plasma membrane enzymes.

ACKNOWLEDGEMENTS

M.J.W. thanks the Medical Research Council for a research studentship.

REFERENCES

1. Bernacki, R.J. and Bosmann, H.B. (1971) J. Cell Sci. 8, 399-406.
2. Dingle, J.T., Fell, H.B. and Glauert, A.M. (1969) J. Cell Sci. 4, 139-154.
3. Cohn, Z.A. and Ehrenreich, B.A. (1969) J. Exp. Med. 129, 201-225.
4. Munro, T.R. (1968) Exp. Cell Res. 52, 392-400.
5. Horvat, A. (1973) Biochim. Biophys. Acta 297, 413-422.
6. Wattiaux, R. (1966) Thesis, University of Louvain.
7. Peters, T.J., Muller, M. and De Duve, C. (1972) J. Exp. Med. 136, 1117-1139.
8. Beaufay, H., Amar-Costesec, A., Feytmans, E., Thines-Sempoux, D., Wibo, M., Robbi, M. and Berthet, J. (1974) J. Cell Biol. 61, 188-200.
9. Fleischer, B., Fleischer, S. and Ozawa, H. (1969) J. Cell Biol. 43, 59-79.
10. Racker, E. (1950) Biochim. Biophys. Acta 4, 211-216.
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
12. Warburton, M.J. (1975) Ph.D. Thesis, University of Manchester.
13. De Duve, C. (1969) in Lysosomes in Biology and Pathology (Dingle, J.T. and Fell, H.B., eds.), Vol. I, pp. 3-40, North-Holland Publ. Co., Amsterdam.
14. Wagner, R., Rosenberg, M. and Estensen, R. (1971) J. Cell Biol. 50, 804-817.
15. Werb, Z. and Cohn, Z.A. (1972) J. Biol. Chem. 247, 2439-2446
16. Edelson, P.J. and Cohn, Z.A. (1974) J. Exp. Med. 140, 1364-1386.
17. Dingle, J.T. (1968) Brit. Med. Bull. 24, 141-145.