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SELECTIVE MODIFICATION OF CELL SURFACE PROTEINS AND THYMIDINE TRANSPORT IN HAMSTER CELLS EXPOSED TO CHOLERA TOXIN

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The increased adherence and morphological response which occurs in Chinese hamster ovary cells as a result of exposure to cholera toxin is paralleled by modification in the relative exposure of outer proteins. Mild proteolysis treatment of the cells prelabeled with [^{3}H] glucosamine reveals a markedly different kinetics of release of external glycopeptides as a result of exposure to cholera toxin. Selective alterations in external tyrosyl-rich proteins can also be detected by lactoperoxidase-catalyzed radioiodination. The above modifications are accompanied by a decrease in the rate of thymidine uptake by toxin-treated cells.

INTRODUCTION

Several groups have clearly shown that cholera toxin can markedly alter the morphology and regulation of mammalian cells (1–5) presumably by causing an alteration at the plasma membrane level leading to an activation of the adenyl cyclase (2–5). We have now confirmed that low levels of cholera toxin indeed cause clear morphological changes in Chinese hamster ovary (CHO) cells (2) resembling those reported to occur at much higher molar levels of dibutyryl cyclic AMP (6). In such a system, we have further studied whether the morphological changes are accompanied by alterations in the relative exposure of external proteins. We have also begun a preliminary study on the transport properties of cells exposed to cholera toxin.

MATERIALS AND METHODS

Materials

[^{125}I] sodium iodide carrier-free for protein iodination, L-[^{3}H] leucine (30–50 Ci/mM), D-[^{3}H] glucosamine hydrochloride (5–15 Ci/mM), and [^{3}H] thymidine methyl (20 Ci/mM) were obtained from New England Nuclear, Waltham, Mass.; twice-crystallized trypsin (98.000 BAEE U/mg) was purchased from Sigma Chemical Co., St. Louis, Mo.;

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lactoperoxidase was obtained from Calbiochem, San Diego, Calif., and glucose oxidase was purchased from Worthington Biochemicals, Freehold, N.J.

Purified cholera toxin lot no. 0673 was kindly supplied by Drs. Richard Finkelstein and Carl E. Miller through the Cholera Program, National Institutes of Health.

Cell Cultures

CHO cells checked for the absence of mycoplasma (7) were seeded at a concentration of $2.5 \times 10^4/cm^2$ in 9-cm petri dishes containing F-12 medium (Gibco catalog no. H-17).

Enzymatic Radioiodination

This was carried out in the presence of lactoperoxidase and glucose oxidase (7) in cultures seeded 16 hr prior to the experiment with or without 1 μg/ml toxin. The reaction was carried out by a 10-min exposure to NaI^{125} (100 μCi/ml). Cells were washed in PBS in which NaCl was substituted by sodium iodide to stop the iodination, and then resuspended in PBS for proteolysis as indicated elsewhere (7).

SDS-Polyacrylamide Gel Electrophoresis

This was carried out in the discontinuous slab gels by the high resolution procedure of Laemmli (8). Prior to electrophoresis, samples were exposed to 1% sodium dodecyl sulphate and 1% β-mercaptoethanol in electrophoresis buffer containing 0.002 M phenyl methyl sulphonyl fluoride to prevent proteolytic degradation and heated to 90°C for 3 min to promote dissociation. Samples were run to within 5 mm of the end of the gels and then fixed overnight in 7% CH_3COOH, 3% ethanol.

Autoradiography

This was carried out for the iodinated samples by drying the gels onto filter paper under vacuum at a temperature of about 60°C for 1 hr. Subsequently the dried gels were placed in contact with medical X-ray film (RP-X-Omat obtained from the Eastman-Kodak Co., Rochester, N.Y.).

RESULTS AND DISCUSSION

Exposure of CHO cells to cholera toxin led to marked morphological alterations, in that most cells became increasingly elongated and flattened (Fig. 1), exhibiting an increased adherence like that recently reported in the same cells by Guerrant et al. (2). As the increased adherence and flattening suggested to us a change in cell surface components, we decided to investigate the nature of the toxin-mediated alterations in the periphery of the cells.

A brief exposure of the cells to 1 μg/ml crystalline trypsin was carried out to define the relative exposure of external trypsin-sensitive glycoprotein components in toxin-treated and control cells prelabled with $[^3H]$ glucosamine. Mild tryptic treatment clearly reveals (Fig. 1) a differential kinetic release of ^3H label in cells seeded in the presence of cholera toxin as compared with control cells. From such observations, it seems apparent that toxin-treated cells are much more elongated and flat, and expose more trypsin-sensitive glycopeptides than the rounded control cells. Similar qualitative results with regard to the release of glycoproteins from flat CHO cells treated with dibutyryl cyclic AMP, as compared with control cells, have been observed following treatment of the cells with insolubilized papain (7).

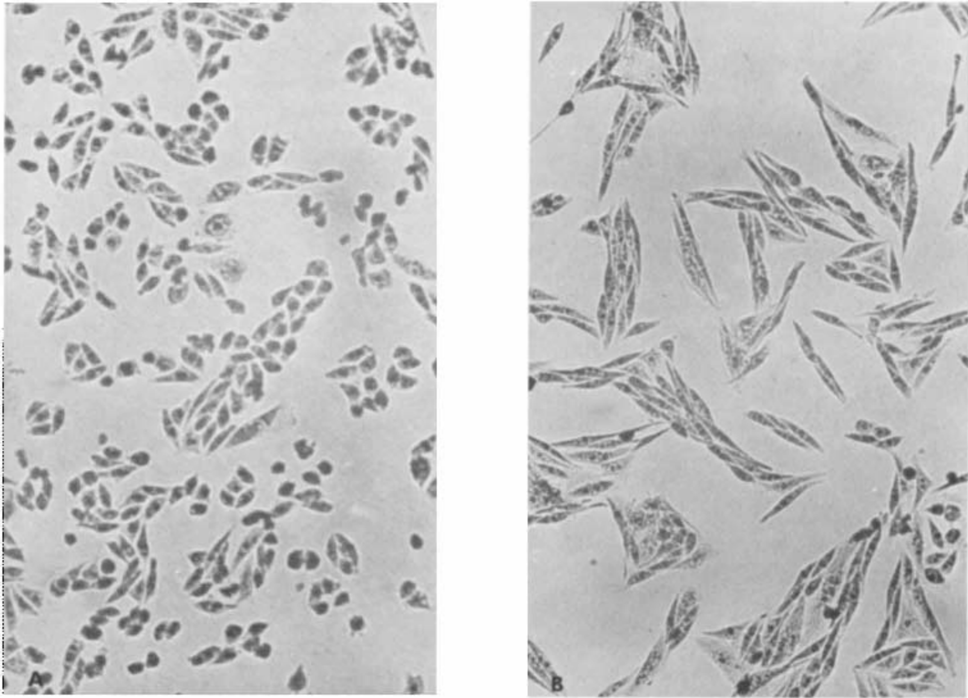

Fig. 1. Light micrograph of CHO cells. (A) Control culture after 24 hr of growth in complete medium supplemented with 10% fetal calf serum. (B) Cells after 24 hr of growth in complete medium with 10% serum plus the addition of 1 μg/ml cholera toxin. Magnification (× 50), phase contrast.

We also examined whether some specific external proteins of CHO cells became affected as a result of exposure to cholera toxin and colchicine. This was investigated by means of lactoperoxidase-catalyzed radioiodination of surface proteins. Figure 2 shows that several selective alterations became evident as a result of exposure of the cells to the drug.

We have also asked the question whether the toxin-mediated effect on growth and cell surface proteins occurs concurrently with an effect on nutrient uptake. Preliminary experiments (not shown) revealed that cholera toxin has no effect whatsoever on the uptake of leucine by the cells or in the incorporation of leucine into protein. Also, in agreement with observations in various cell systems showing that cholera toxin inhibits DNA synthesis (3, 4), we have been able to confirm an effect on the rate of DNA synthesis (Table I). However, our results in which we did bother to measure the acid-soluble pool clearly show that the toxin-mediated decrease in the incorporation of [^{3}H] thymidine into DNA is not just the result of inhibition of macromolecular synthesis (3, 4), but is also due to a decreased uptake of thymidine by the cells. This result of cholera toxin on the utilization of thymidine clearly differs from that of another inhibitor of DNA synthesis, hydroxyurea, which inhibits incorporation of thymidine into DNA without affecting its uptake by the cells (9). Moreover, sparse cultures of CHO cells seeded in the presence of cholera toxin are certainly not blocked in DNA synthesis because they tend to complete the monolayers

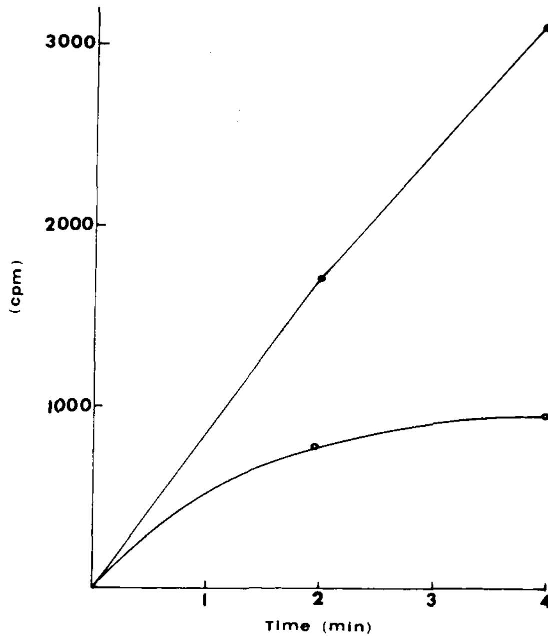

Fig. 2. Effect of cholera toxin on the trypsin-mediated release of glycopeptides. Cells seeded in complete medium containing [^{3}H] glucosamine (20 μCi) and cholera toxin as indicated below were grown at 37°C for 16 hr. Prior assay monolayers were washed three times in medium without serum and three times in phosphate-buffered saline. Subsequently cells were exposed to 1 μg/ml crystalline trypsin in the same isotonic saline. Aliquots were taken at the intervals indicated for the determination of radioactivity precipitable by 10% trichloroacetic acid (7). o—o control; •—• 1 μg/ml cholera toxin.

TABLE I. Effect of Cholera Toxin on the Incorporation and Utilization of Th''midine

Cholera toxin concentration (μg/ml)	Incorporation of ^{3}H-thymidine: Acid-soluble fraction (cpm)	Incorporation of ^{3}H-thymidine: Acid-insoluble fraction (cpm)
–	3,465	7,786
	3,828	8,182
1	1,424	3,917
	1,680	4,294
0.1	2,536	4,597
	2,485	4,696

CHO cells were partially synchronized by seeding them in duplicate at 2.5 × 10^{4} cells/cm^{2} in F-12 medium containing only 0.5% serum for 48 hr at 37°C. Subsequently, cells were stimulated to undergo DNA synthesis by adding fresh medium containing 10% serum. To test the effect of cholera toxin at the concentrations described above, the drug was added at the time of medium change. Incorporation of thymidine was tested in each case by adding 10 μc of isotope for a 30-min period chosen 16 hr after serum supplementation. Aliquots were taken for measurement of the incorporation as described elsewhere (9). The cpm referred to above are related to identical protein concentrations as evident from the Lowry procedure.

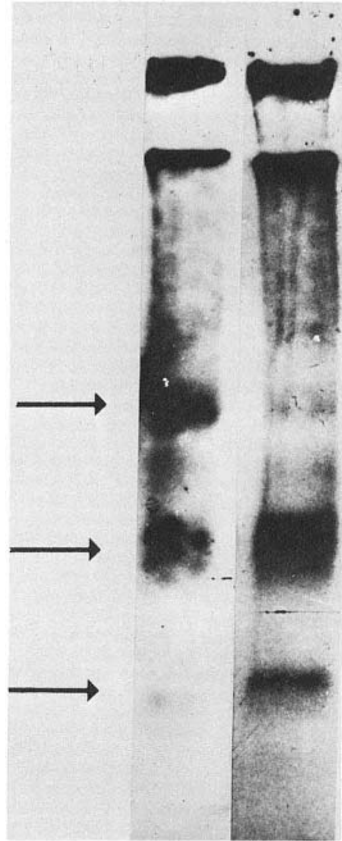

Fig. 3. Differential exposure of external proteins as a result of exposure to cholera toxin. Duplicate monolayers consisting of control cells or cells seeded in the presence of 1 μg/ml cholera toxin were grown for 16 hr in complete medium as indicated in Materials and Methods. Subsequently, cells were externally labeled by iodination (7) and examined by polyacrylamide gel electrophoresis in 6% gels. From left to right, the samples correspond to control cells and to cells seeded in the presence of 1 μg/ml cholera toxin.

after a 72-hr period, although even then they certainly show a completely different morphology from control cells as indicated in Fig. 1.

ACKNOWLEDGMENTS

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