Effect of Tunicamycin on 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in C-6 Glial Cells*

Joseph J. Volpe and Richard I. Goldberg
From the Departments of Pediatrics, Neurology, and Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110

The effects of tunicamycin on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and cholesterol biosynthesis have been studied in cultured C-6 glial cells. Depending on culture conditions, exposure to tunicamycin caused either a marked inhibition of induction of HMG-CoA reductase activity or, under steady state conditions, a marked reduction in enzymatic activity. Incorporation of \[^{14}C\text{acetate}\] into sterols was affected similarly. After a 24-h exposure, a 50% reduction in reductase activity was observed with a concentration of 0.05 \( \mu \text{g/ml} \), and a maximal, 65–70% reduction occurred with 0.10 \( \mu \text{g/ml} \) of the drug. The effect of tunicamycin on reductase activity and on sterol synthesis was apparent 4 h after addition of the drug and nearly maximal after 6 h. The relative specificity of the effect of tunicamycin was indicated by the finding of no change in the activities of NADPH-cytochrome c reductase, acetyl-CoA carboxylase, or fatty acid synthetase, in incorporation of \[^{3}H\text{leucine}\] into total protein, or in the rate of increase in cellular protein and phospholipid at concentrations of tunicamycin that caused the marked effect on HMG-CoA reductase. The reversibility of the effect of tunicamycin was shown by observing total recovery of reductase activity within 24 h after removal of the drug following a 24-h exposure. That the effect of tunicamycin on reductase is related to the drug’s effect on glycoprotein synthesis was shown in two ways. First, the range of the drug led to a decrease in reductase activity was essentially identical with the range over which the drug led to a decrease in incorporation of \[^{3}H\text{leucine}\] into protein. Second, incubation of C-6 cells with \(^{N}\text{acetylglucosamine}\) simultaneously with tunicamycin was accompanied by prevention of the drug’s effect on both HMG-CoA reductase and glycoprotein synthesis. These data suggest that glycoprotein synthesis is necessary for the expression of HMG-CoA reductase activity and, thereby, cholesterol synthesis in glial cells. Moreover, a link between glycoprotein and cholesterol biosynthesis could play a role in the mediation of certain maturational events in cells of neural origin.

**Experimental Procedures**

Regulation of the principal regulatory enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase, is of particular interest in cultured C-6 glial cells, because this cell is a good model of the glial cell found in mammalian brain during development (1–3), when cholesterol deposition in glia and glial differentiation are particularly active processes (see Refs. 4 and 5 for reviews). Previous work has shown that HMG-CoA reductase in C-6 glia, as in other mammalian cells (see Refs. 6 and 7 for reviews), is actively regulated. Thus, the glial enzyme is responsive to low density lipoproteins, certain free sterols, membrane lipid composition, rates of cellular proliferation, and the integrity of microtubules and microfilaments (8–13). The latter observations, i.e., those regarding the relation of the reductase to the cytoskeletal system (12, 13), suggested the possibility of a relation between regulation of the enzyme and message transmitted into the cell from surface membranes.

Glycoproteins are critical components of surface membranes and, as such, have been shown to be involved in a variety of membrane-mediated events (see Refs. 14 and 15 for reviews). Such events include embryonic development (16–18), cellular differentiation (19–22), pinocytosis (23), cell-cell recognition (22), intercellular adhesion (24–27), membrane transport (28), and function of various receptors (29). The role of glycoproteins in such events has been probed in most instances by utilizing tunicamycin, an inhibitor of the synthesis of \(^{N}\text{acetylglucosaminylpyrophosphoryl isoprenol}\) from dolichol phosphate and UDP-\(^{N}\text{acylglucosamine}\) (see Refs. 30 for review).

In this study, we have explored the possibility of a relationship between glycoprotein synthesis and the expression of HMG-CoA reductase in C-6 glial cells. Our specific objectives were to determine: 1) whether exposure of C-6 glial cells to tunicamycin caused any alteration in HMG-CoA reductase activity and cholesterol synthesis; 2) the relative specificity and reversibility of any such effects; and 3) the relation of effects on the reductase to those on glycoprotein synthesis.

Our data indicate that tunicamycin causes a marked, reversible, specific reduction in HMG-CoA reductase, and that the effects are correlated closely with concomitant effects on glycoprotein synthesis.

*This investigation was supported by Grants HD-07464 and NS-14834 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be sent, at 500 S. Kingshighway Blvd., P.O. Box 14871, St. Louis, MO 63178.

1 The abbreviation used is: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.
Tunicamycin, Glycoprotein Synthesis, and HMG-CoA Reductase in Glia

Tunicamycin—Glycoprotein Synthesis, and HMG-CoA Reductase in Glia

national, Elk Grove Village, IL). AG 1-X8 (formate) ion exchange resin (Bio-Rad, Richmond, CA), fetal calf serum (Kansas City Biological, Lenexa, KS), cholesterol assay kits (Boehringer Mannheim, Indianapolis, IN), and plastic tissue culture flasks (Corning, Corning, NY) were obtained from the designated sources.

Cell Cultures—The cell type utilized in this study, C-6 glial cells, cloned from a rat glioma, was obtained from the American Type Culture Collection (Rockville, MD) and maintained in monolayer culture in Hagiuchi medium (31), modified as described previously (3).

For routine growth, stock cultures were supplemented with 10% (v/v) fetal calf serum and were subcultured 3 days after reaching confluence. Cultures used for the experiments described herein were between the 9th and 27th passages in our laboratory. The methods for subculturing and subcloning C-6 glial cells have been described previously (32, 33).

For each experiment, cells were derived from a single flask. Size of each inoculum was identical and based on cell number (determined on a gentian violet-stained aliquot and counted in a hemocytometer). Cells were adjusted to give a final concentration of cells transferred of 5 × 10⁶/ml.

Prior to addition to cell cultures, tunicamycin (isomer composition: 9% A, 27% B, 53% C, 9% D) was dissolved in ethanol and added to the flasks to yield a final concentration of ethanol of 0.1% (v/v). A similar quantity of ethanol was added to control flasks. This concentration of ethanol in the culture medium for 24 h did not alter HMG-CoA reductase specific activity or the incorporation of [1-¹⁴C]acetate into sterols, when compared to values obtained from cells maintained in the absence of ethanol.

Preparation of Lipoprotein-poor Serum—Lipoprotein-poor serum was prepared by centrifugation of the method of Kirsten and Watson (34), using amphyous KBr for the single adjustment of density. Final preparations were assayed for cholesterol content by the use of a commercially available colorimetric assay kit (Boehringer Mannheim). This method of preparation of lipoprotein-poor serum resulted in a >95% decrease in cholesterol content.

Cholesterol Synthesis—Synthesis of total cholesterol was determined in intact cells by measuring the incorporation of [¹⁴C]acetate or [³H]HMG-CoA into cholesterol in the absence of ethanol. Both of these precursors were used to determine that the enzyme systems responsible for cholesterol synthesis were present in the C-6 glial cells used in the experiments described in this communication.

Protein Synthesis—Synthesis of total cellular protein was evaluated by measurement of the incorporation of [¹⁴C]acetate or [³H]HMG-CoA. Separation of the product, [¹⁴C]acetate, from an extracted substrate, [¹⁴CHMG-CoA, was accomplished by using AG 1-X8 anion exchange resin by a modification (37) of the method of Avigan et al. (38). Specific activity is expressed as picomoles of mevalonate formed/min/mg of protein.

HMG-CoA Reductase Assay—This enzyme assay was assayed in cell homogenates by a modification of the method of Brown et al. (39), as described previously (8), except that [³H]HMG-CoA was used instead of [¹⁴C]HMG-CoA. Separation of the product, [³H]mevalonate, from an extracted substrate, [³H]HMG-CoA, was accomplished by using AG 1-X8 anion exchange resin by a modification (37) of the method of Avigan et al. (38). Specific activity is expressed as picomoles of mevalonate formed/min/mg of protein.

Protein Synthesis—Synthesis of total cellular protein was evaluated by measurement of the incorporation of radioactivity from [¹⁴C]acetate into protein. Specific activity is expressed as picomoles of acetate incorporated/min/mg of protein.

Acetyl-CoA Carboxylase Assay—This enzyme was assayed in 12,000 g supernatant fractions by the radiochemical method, as described by Alberts et al. (40). Specific activity is expressed as nanomoles of bicarbonate fixed/min/mg of protein.

Fatty Acid Synthetase Assay—This enzyme was assayed in 12,000 g supernatant fractions by the radiochemical method, as described by Alberts et al. (40). Specific activity is expressed as nanomoles of [¹⁴C]acetate converted/min/mg of protein.

Protein Determination—Protein was determined by the method of Lowry et al. (44) or by a microbiuret procedure (45).

RESULTS

Effect of Tunicamycin on HMG-CoA Reductase Activity and Cholesterol Synthesis as a Function of Concentration—We first investigated the effect of a 24-h exposure of C-6 glial cells to tunicamycin on HMG-CoA reductase activity as a function of the concentration of the drug (Fig. 1). A 50% lower reductase activity was observed in the tunicamycin-treated versus the control cells at a concentration of only 0.05 µg/ml. The effect was essentially maximal with a concentration of 0.1 µg/ml, which caused about a 65–70% lower reductase activity in the treated versus the untreated cells. It should be noted that during the 24 h in 10% lipoprotein-poor serum, HMG-CoA reductase in untreated cells increased nearly 4-fold; whereas in the tunicamycin-treated cells this induction was prevented.

The changes in HMG-CoA reductase activities caused by tunicamycin resulted in comparable alterations in rates of cholesterol synthesis (Fig. 1). Thus, incorporation of [¹⁴C]acetate into sterols in cells treated with tunicamycin, 0.05 µg/ml, was approximately 50% of that in the untreated cells, and a maximal, approximately 70% reduction in incorporation was observed with 0.1 µg/ml of tunicamycin.

To ensure that the effect of tunicamycin on incorporation of [¹⁴C]acetate into sterol reflected the effect on HMG-CoA reductase and not an effect on transport or pool sizes of acetate or conversion of acetate to acetyl-CoA,sterol synthesis also was estimated by measuring the incorporation of [²H]O into sterols. Changes in sterol synthesis essentially identical with those depicted in Fig. 1 for [¹⁴C]acetate incorporation were observed with [²H]O as precursor. Thus, after 24 h in 10% lipoprotein-poor serum, incorporation of [²H]O into sterols in the untreated cells was 1122 ± 94 cpm/h/mg of protein, and in the tunicamycin-treated cells, incorporations were 934 ± 50, 732 ± 64, 410 ± 53, and 361 ± 30 cpm/h/mg of protein at tunicamycin concentrations of 0.02, 0.05, 0.10, and 0.50 µg/ml, respectively.

To determine whether the effect of tunicamycin was solely to prevent the induction of HMG-CoA reductase activity induced by change of medium from fetal calf serum to lipoprotein-poor serum, C-6 glial cells were exposed to the antibiotic for 24 h after change of medium to serum-free conditions. 10% fetal calf serum, or 10% lipoprotein-poor serum (Table I). The 24-h exposures to tunicamycin were carried out after 24 h of initial growth in 10% fetal calf serum for the cells studied subsequently in serum-free medium or fresh 10% fetal calf serum, and after 24 h of initial growth in 10% lipoprotein-poor serum for the cells studied subsequently in fresh 10% lipoprotein-poor serum. (Note that the cells described in Fig. 1, although also exposed to tunicamycia in 10% lipoprotein-poor serum, had been grown initially for 24 h in 10% fetal calf serum.) Despite these differences in culture conditions, the effects of tunicamycin on HMG-CoA reductase activity were very similar to those described in Fig. 1, i.e., a maximal 50% lower reductase activity with 0.05 µg/ml and a maximal, 65–75% lower activity with 0.1–0.5 µg/ml.

Effect of Tunicamycin on HMG-CoA Reductase Activity and Cholesterol Synthesis as a Function of Time of Exposure—We next evaluated the temporal features of the effect of a 0.5 µg/ml concentration of tunicamycin on HMG-CoA reductase activity and cholesterol synthesis (Fig. 2). A distinct difference between treated and untreated cells was apparent within 4 h, and approximately 90% of the maximal effect of tunicamycin on both HMG-CoA reductase and the incorporation of [¹⁴C]acetate into sterols occurred by 6 h. Although the data
Activity was apparent. By the drug, and recovery was essentially complete by removal of tunicamycin, beginning recovery of reductase activity was evaluated (Fig. 4). Thus, after decrease in reductase activity, the drug was removed, and reversibility of the effect was studied first (Fig. 3). Thus, after decrease in reductase activity, the drug was removed, and reversibility of the effect was studied first (Fig. 3). Thus, after decrease in reductase activity, the drug was removed, and reversibility of the effect was studied first (Fig. 3).

HMG-CoA Reductase Activity—We next asked whether the intact cells were necessary for the effect of tunicamycin, we incubated the drug with extracts of C-6 glial cells prior to assay of the enzyme. Thus, extracts of C-6 glia, which had been growing in 10% lipoprotein-poor serum, were determined. Values are expressed as a percentage of control and were derived from separate determinations on each of three flasks. The mean 100% values (±SD) after 24 h in lipoprotein-serum were 98.4 ± 6.1 pmol/min/mg of protein for HMG-CoA reductase 522 ± 402 cpm/h/mg of protein for sterol synthesis. (At zero time, the mean values for HMG-CoA reductase and sterol synthesis were 98.2 ± 2.0 pmol/min/mg of protein and 1310 ± 110 cpm/mg of protein, respectively.) Values shown were derived from means obtained from separate determinations on each of three flasks, and standard deviations did not exceed 10% of the mean. Essentially identical results were obtained in three separate experiments.

FIG. 2 (center). Effect of tunicamycin on HMG-CoA reductase activity and sterol synthesis as a function of time of exposure to the drug. The initial conditions of the experiment were similar to those described in Fig. 1. Six flasks were utilized to determine HMG-CoA reductase activity (O) and the rate of sterol synthesis (●) at zero time; to one-half of the remaining flasks, 0.5 μg/ml of tunicamycin was added and to the other one-half, vehicle only. At the indicated times, reductase specific activity and the rate of sterol synthesis were determined in three tunicamycin-treated and three control flasks. Values are expressed as a percentage of control and were derived from means obtained from separate determinations on each of three flasks. The mean 100% values at zero time and at 2, 4, 6, and 24 h, respectively, were: for HMG-CoA reductase, 21.3 ± 1.7, 23.2 ± 2.1, 31.1 ± 2.2, 61.6 ± 4.9, and 84.7 ± 7.2 pmol/min/mg of protein; and for sterol synthesis, 1147 ± 97, 1223 ± 104, 1603 ± 92, 4741 ± 210, and 4792 ± 422 cpm/h/mg of protein.

FIG. 3 (right). Reversibility of the effect of tunicamycin on HMG-CoA reductase activity. The initial conditions of the experiment were similar to those described in Fig. 1, except that only a concentration of 0.1 μg/ml of tunicamycin (or vehicle only) was utilized. After the 24-h exposure to the drug (or vehicle), the cultures were washed and the medium was changed to fresh 10% lipoprotein-poor serum without tunicamycin (time 0 in the figure). At the indicated times, HMG-CoA reductase activity was determined. Values are expressed as a percentage of control and were derived from means obtained from separate determinations on each of three flasks. The 100% values were obtained from cells grown in the absence of tunicamycin from the start of the experiment and were: at zero time, 92.3 ± 8.1 pmol/min/mg of protein, and at 24 h, 98.9 ± 7.2 pmol/min/mg of protein.

Reversibility and Specificity of the Effect of Tunicamycin—We next asked whether the effect of tunicamycin on HMG-CoA reductase activity was a part of a generalized, nonspecific toxic effect on the cells. The reversibility of the effect was studied first (Fig. 3). Thus, after growth in tunicamycin, 0.1 μg/ml, for 24 h caused a 75% decrease in reductase activity, the drug was removed, and recovery of activity was evaluated (Fig. 3). Within 6 h after removal of tunicamycin, beginning recovery of reductase activity was apparent. By 12 h after removal of the antibiotic, reductase activity was 70% of that in cells never exposed to the drug, and recovery was essentially complete by 24 h.

The relative specificity of the effect of tunicamycin was evaluated by comparing the effect not only on HMG-CoA reductase, but, in parallel flasks, on NADPH-cytochrome c reductase, another microsomal enzyme, on acetyl-CoA carboxylase and fatty acid synthetase, important cytosolic enzymes for nonsterolipid biosynthesis (46), on the rate of incorporation of [3H]leucine into total protein, and on the increase in total cellular protein and phospholipid per flask (Table II). Each parameter was studied after a 24-h exposure to 0.1 μg/ml tunicamycin. Despite the marked effect on HMG-CoA reductase, no significant change in the specific activities of the other enzymes, on total protein synthesis or on the increase in total cellular protein and phospholipid was detected.

Mechanism of the Effect of Tunicamycin on HMG-CoA Reductase Activity—To determine whether intact cells were necessary for the effect of tunicamycin, we incubated the drug with extracts of C-6 glial cells prior to assay of the enzyme. Thus, extracts of C-6 glia, which had been growing in 10% lipoprotein-poor serum for 24 h, were preincubated for 3 h either in the absence of tunicamycin or in the presence of concentrations of 0.1 and 0.5 μg/ml. Assays were then carried out with the standard 2-h incubation, still in the presence or absence of the drug. HMG-CoA reductase specific activities were 91.2 ± 2.3, 87.4 ± 4.5, and 88.5 ± 5.2 pmol/min/mg of protein in extracts exposed to 0, 0.1, and 0.5 μg/ml of tunicamycin, respectively. Thus, no diminution of reductase activity was caused by exposure to tunicamycin in vitro.

Because a fraction of HMG-CoA reductase of several tissues and cultured cells has been shown to exist in an inactive phosphorylated form (47-49), we asked next whether the decrease in reductase activity caused by tunicamycin was related to a relative increase in the inactive form. Because the...
TABLE I
Effect of tunicamycin on HMG-CoA reductase activity in C-6 glial cells grown in serum-free medium, fetal calf serum, or lipoprotein-poor serum

<table>
<thead>
<tr>
<th>Tunicamycin concentration (µg/ml)</th>
<th>HMG-CoA reductase activity (pmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum-free</td>
</tr>
<tr>
<td>0.05</td>
<td>40.7</td>
</tr>
<tr>
<td>0.10</td>
<td>35.7</td>
</tr>
<tr>
<td>0.50</td>
<td>32.0</td>
</tr>
</tbody>
</table>

The conditions of the experiment were similar to those described in Fig. 1, except that only a concentration of 0.1 µg/ml of tunicamycin was utilized. After the 24-h exposure to the drug, the activities of several enzymes, the rate of incorporation of [3H]leucine into total protein, and the total cellular protein and lipid phosphorus per flask were determined. Values are expressed as a percentage of control (i.e., no tunicamycin) for two separate experiments. The mean 100% values were 92.4 ± 7.2 pmol/min/mg of protein for HMG-CoA reductase activity, 3.02 ± 0.40 nmol/min/mg of protein for acetyl-CoA carboxylase activity, 4.31 ± 0.38 nmol/min/mg of protein for fatty acid synthase activity, 1.85 ± 0.12 nmol/min/mg of protein for NADPH-cytochrome c reductase activity, 15.142 ± 504 cpm/h/mg of protein for protein synthesis, 0.42 ± 0.02 mg of protein flask (25-cm² surface area), and 0.17 ± 0.02 µmol of lipid phosphorus flask (25-cm² surface area). (The values per flask for protein and lipid phosphorus at the zero-time were, for the cells in 10% fetal calf serum, 35.7 ± 3.4 pmol/min/mg of protein, and for the cells in 10% lipoprotein-poor serum, 117.7 ± 11.0 pmol/min/mg of protein.)

Conversion of inactive enzyme to the active form appears to proceed through the action of a phosphatase that can be blocked by fluoride, we determined the effect of tunicamycin (0.1 µg/ml, 24-h exposure) on reductase when extracts were isolated and assayed in the presence or absence of fluoride (Table III). Extracts prepared from both control and tunicamycin-treated cells exhibited approximately 60-65% of reductase activity in the inactive form. A decrease in reductase activity of comparable magnitude was observed in tunicamycin-treated cells, whether studied in the presence or absence of fluoride.

To determine whether new protein synthesis was required for recovery of reductase activity after exposure to tunicamycin, we studied the recovery of reductase activity, in an experiment similar to that illustrated in Fig. 3, but in the presence as well as absence of cycloheximide for 8 h. (The quantity of cycloheximide utilized, 5 µg/ml, was shown in parallel flask to cause a 96 to 98% inhibition of the rate of incorporation of [3H]leucine into total protein.) During the 8-h period after removal of tunicamycin, reductase activity in cells not exposed to cycloheximide increased by 2.1-fold, whereas activity in cells exposed to cycloheximide failed to increase and, in fact, decreased by approximately 65%. This rate of decrease in activity was essentially identical with that observed in cycloheximide-treated cells that were grown in lipoprotein-poor serum for a similar time but never exposed to tunicamycin. In a previous study, we have demonstrated a similar decline of reductase activity in cycloheximide-treated cells, i.e. t½ of 3-4 h (13).

Relationship of the Effects of Tunicamycin on HMG-CoA Reductase and on Glycoprotein Synthesis—To determine whether the effect of tunicamycin on HMG-CoA reductase was related to the effect of the antibiotic on dolichol-linked glycoprotein synthesis, we first measured the rate of incorporation of [3H]mannose into glycoprotein (Fig. 4) at concentrations of tunicamycin that cause the decrease in reductase activity (compare with Fig. 1). The effect of tunicamycin on glycoprotein synthesis was maximal at the same concentration (0.1 µg/ml) as the concentration that led to maximal effect on HMG-CoA reductase activity; the magnitude of the decrease in glycoprotein synthesis (approximately 95%) was somewhat greater than that of the decrease in reductase activity (approximately 65 to 70%). In contrast to the marked decrease in incorporation of [3H]mannose into glycoprotein caused by tunicamycin, only a slight decrease in incorporation of [3H]leucine into total protein was apparent, and this decrease occurred only at the highest concentration of tunicamycin utilized (0.5 µg/ml).

To demonstrate that the effect of tunicamycin on reductase was related directly to the drug's effect on the transfer of N-acetylglucosamine to phosphorylated dolichol, we evaluated whether exogenous N-acetylglucosamine could prevent the effect of tunicamycin on reductase and cholesterol synthesis (Fig. 5). Thus, C-6 glial cells were exposed to tunicamycin (0.1 µg/ml) for 24 h in 10% lipoprotein-poor serum, with the indicated concentrations of N-acetylglucosamine as well.

TABLE II
Relative specificity of the effect of tunicamycin on HMG-CoA reductase

<table>
<thead>
<tr>
<th>Enzyme, biosynthetic pathway, or cellular constituent</th>
<th>Specific activity, rate of synthesis, or cellular content (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMG-CoA reductase</td>
<td>32-37</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase</td>
<td>93-98</td>
</tr>
<tr>
<td>Fatty acid synthase</td>
<td>97-103</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase</td>
<td>94-102</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>91-94</td>
</tr>
<tr>
<td>Total protein</td>
<td>90-95</td>
</tr>
<tr>
<td>Total phospholipid</td>
<td>94-97</td>
</tr>
</tbody>
</table>

TABLE III
Effect of tunicamycin on HMG-CoA reductase in extracts isolated and assayed in the presence or absence of fluoride

<table>
<thead>
<tr>
<th>Tunicamycin concentration (µg/ml)</th>
<th>HMG-CoA reductase activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>103 ± 6.0</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>30.4 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>35.2 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>11.7 ± 1.7</td>
</tr>
</tbody>
</table>

The conditions of the experiment were identical with those described in Fig. 1, except that only a concentration of 0.1 µg/ml of tunicamycin was utilized. After the 24-h exposure, the cells from each culture were divided into two equal portions and pelleted by centrifugation. One cell pellet from each culture was homogenized, precipitated, and assayed for HMG-CoA reductase activity in the usual way, as described under "Experimental Procedures." The second cell pellet from each culture was treated identically, except that 50 mM NaF was included in all the buffers. Values are means (± SD) obtained from separate determinations on each of three flasks, containing either tunicamycin-treated or untreated cells.
Complete prevention of the effect of tunicamycin on both HMG-CoA reductase and cholesterol synthesis was apparent in tunicamycin-treated cells treated simultaneously with 25 mM N-acetylglucosamine. (N-Acetylglucosamine had no effect on HMG-CoA reductase in the absence of tunicamycin.) N-Acetylglucosamine also prevented the effect of tunicamycin on the incorporation of [3H]mannose into glycoprotein. Thus, in parallel flasks set up as described for Fig. 5, addition of 25 mM N-acetylglucosamine to the culture medium restored glycoprotein synthesis to 60% of control values, and total restoration of [3H]mannose incorporation into protein occurred with the addition of 50 mM N-acetylglucosamine (data not shown).

**DISCUSSION**

This work has dealt with the effect of tunicamycin on HMG-CoA reductase activity and cholesterol synthesis in C-6 glial cells. Depending on culture conditions, tunicamycin caused a marked inhibition of induction of HMG-CoA reductase activity or, under steady state conditions, a marked decrease in enzymatic activity. Rates of cholesterol synthesis were affected similarly. The effect of tunicamycin on the reductase was reversible by removal of the antibiotic and was not accompanied by a change in the activities of acetyl-CoA carboxylase, fatty acid synthetase, NADPH-cytochrome c reductase, or in the rate of total protein synthesis. Addition of N-acetylglucosamine in concentrations sufficient to overcome the effect of tunicamycin on incorporation of mannose into glycoprotein also overcame the effect of the drug on HMG-CoA reductase. These observations have implications for: 1) the regulation of HMG-CoA reductase and, thereby, cholesterol biosynthesis in these cells, and 2) the relationship between glycoprotein and cholesterol biosyntheses in developing glial cells.

In regard to the regulation of HMG-CoA reductase, the data suggest that glycoprotein synthesis is necessary for the expression of HMG-CoA reductase activity in C-6 glial cells. The evidence supporting this suggestion is primarily 2-fold. First, tunicamycin leads to a decrease in reductase activity in concentrations nearly identical with those that lead to a decrease in glycoprotein synthesis. Second, N-acetylglucosamine, added concomitantly with tunicamycin, prevents the effect of the antibiotic on both HMG-CoA reductase activity and glycoprotein synthesis. This prevention by N-acetylglucosamine of the effect of tunicamycin on glycoprotein synthesis, to our knowledge not previously shown in intact cells, is compatible with the demonstration that preincubation of the N-acetylglucosamine-1-phosphate transferase from hen oviduct with UDP-N-acetylglucosamine prevented the inhibitory effect of tunicamycin on the enzyme (50). Expansion of the intracellular UDP-N-acetylglucosamine pool presumably occurred in our cultured cells in a manner analogous to that shown by Kornfeld et al. (51) in studies of rat liver, i.e., the N-acetylglucosamine entered the pathway for biosynthesis of UDP-N-acetylglucosamine at a site distal to the site of feedback control, the L-glutamine:6-phosphate aminotransferase. Nevertheless, in the absence of delineation of the precise nature of the relationship between glycoprotein synthesis and HMG-CoA reductase, we cannot rule out the possibility that the effect of tunicamycin on reductase activity is unrelated to glycoprotein synthesis and, rather, is mediated through a “feedback” mechanism by causing an increase in intracellular dolichol and/or derivatives thereof. This possibility is rendered less likely by our observation that added dolichol (10 μM, 24-h exposure) did not influence HMG-CoA reductase activity, but the possibility is not ruled out unequivocally because uptake of dolichol was not proven.

The mechanism of the effect of tunicamycin on HMG-CoA reductase activity may involve a decrease in enzyme content rather than an alteration in catalytic efficiency of the enzyme. Thus, there is no effect of the drug added in vitro or on the phosphorylation-dephosphorylation system for activation-inactivation of the enzyme (52). An effect on enzyme content is supported, although not proven, by the demonstration that cycloheximide blocks the recovery of reductase activity after exposure to tunicamycin. Thus, new protein synthesis is nec-
Tunicamycin, Glycoprotein Synthesis, and HMG-CoA Reductase in Glia

...nessary for recovery. In addition, upon exposure to tunicamycin, after a 2-h delay, the rate of decline in reductase activity is similar to the rate of decline in activity in untreated cells exposed to cycloheximide. This suggests that the effect of tunicamycin ultimately is on reductase synthesis. Nevertheless, the observations with cycloheximide are indirect, and direct measurements of content of HMG-CoA reductase are necessary to determine the mechanism of the effect of tunicamycin. Currently, we are utilizing immunoochemical techniques to address these issues.

The nature of the apparent link between glycoprotein synthesis and HMG-CoA reductase activity can only be speculated upon at present. The most direct link would occur if HMG-CoA reductase were a glycoprotein. Recent studies of purified reductase do not provide support for this possibility (53, 54). However, because detailed investigation of a carbohydrate component of the enzyme was not carried out in these studies of others, the possibility that the enzyme is a glycoprotein cannot be ruled out conclusively. Perhaps the leading possibility linking glycoprotein synthesis and HMG-CoA reductase is that a message originating at the cell surface, mediated by glycoprotein, is linked to regulation of HMG-CoA reductase. The importance of glycoproteins in the mediation of a variety of events emanating from the cell surface is widely recognized (14, 15). The possibility that the glycoprotein(s) involved is (are) related nonspecifically to regulation of the reductase, e.g. by modulating transport of specific nutrients (28), cannot be ruled out but seems unlikely in view of the relative specificity of the effect on HMG-CoA reductase. The best documented specific effectors of the enzyme, low density lipoproteins, are operative in C-6 glial (8) as in extra-neuronal cells (6). When cultured human fibroblasts are exposed to tunicamycin, the high affinity binding and subsequent intracellular degradation of low density lipoproteins are impaired (55). It was suggested that glycosylation of the receptor for low density lipoproteins is necessary for its intracellular migration or for its normal orientation on the cell surface (55, 56). Involvement of the receptors for low density lipoproteins in the effect of tunicamycin on HMG-CoA reductase of C-6 glial cells seems unlikely, since our experiments were carried out in the absence of lipoproteins. Involvement of other cell surface (or intracellular) glycoproteins of C-6 cells seems more likely. Transmission of message from such glycoproteins could be mediated by the cytoskeletal network, shown previously to be important for the expression of HMG-CoA reductase activity in these cells (12, 13). In this context, the data raise the possibility that regulation of HMG-CoA reductase may be mediated by a system that includes surface glycoprotein and the cytoskeletal network. Precedent for interrelation of surface glycoprotein and the cytoskeletal network in regulation of complex cellular functions is available (57, 58).

These data may have important implications for the developing nervous system. Thus, the C-6 glial cell is a good model of the glial stem cell found in developing brain (1-3). Both glycoproteins and sterols are critical for several aspects of brain development. For example, glycoproteins have been shown to be involved in such membrane-mediated events in developing neuronal cells as cell-cell recognition, synaptogenesis, and neurite outgrowth (21, 22). Although little is known concerning the role of glycoproteins in developing glial cells, such roles should be significant because membrane-mediated events are prominent during maturation in these cells as well. An important role for sterol in developing neurons for at least one event requiring glycoprotein synthesis, i.e. neurite outgrowth, has been demonstrated by our studies with cultured neuroblastoma cells subjected to prior sterol depletion (59). Moreover, we have shown recently a critical role for sterol in the biochemical and morphological expressions of oligodendroglial differentiation in cultured C-6 glia (60). In view of the importance of glycoproteins both for HMG-CoA reductase and sterol synthesis, as shown in this report, and for important maturational events also dependent on sterol, as noted above, it is reasonable to suggest that in glial cells of neural origin at least one mechanism by which glycoproteins may mediate such maturational events is modulation of the synthesis of the critical membrane lipid, cholesterol.

Acknowledgment—We express our appreciation to Dr. Luis Glaser for his critical review of the manuscript and helpful suggestions.

REFERENCES


Downloaded from http://www.jbc.org/ by guest on October 24, 2017
Tunicamycin, Glycoprotein Synthesis, and HMG-CoA Reductase in Glia

Effect of tunicamycin on 3-hydroxy-3-methylglutaryl coenzyme A reductase in C-6 glial cells.

J J Volpe and R I Goldberg


Access the most updated version of this article at http://www.jbc.org/content/258/15/9220

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/15/9220.full.html#ref-list-1