Microtubules and the Regulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase*

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SUMMARY

Cultured C-6 glial cells were utilized to evaluate the effect of antimicrotubular drugs on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and cholesterol synthesis. Colchicine, Colcemid, and vinblastine (1.0 μM) caused a marked reduction in HMG-CoA reductase activity and, as a consequence, the rate of cholesterol synthesis in these cells. No effect was observed with lumicolchicine, a mixture of colchicine isomers with no effect on microtubules. The effect of colchicine was apparent within 1 h after addition to the culture medium, and, after 6 h, HMG-CoA reductase activity in treated cells was only approximately 15 to 30% of that in untreated cells. Reductase activity was very sensitive to the concentration of drug added, i.e. cells treated with just 0.1 μM colchicine for 6 h exhibited a 50% lower enzymatic activity than did untreated cells. The lack of a generalized, nonspecific toxic effect on the cells was indicated by the finding of no change in the activities of fatty acid synthetase and NADPH-cytochrome c reductase and on a parameter of microtubular function, i.e. maintenance of glial cell shape. The data suggest that microtubules are involved in the regulation of HMG-CoA reductase and cholesterol synthesis in C-6 glial cells.

3-Hydroxy-3-methylglutaryl coenzyme A reductase is the microsomal, rate-limiting enzyme in cholesterol biosynthesis in C-6 glial and other mammalian cells (see Refs. 1 and 2 for reviews). The enzyme is subject to active regulation in the glial cells, and particularly important effectors include cholesterol, bound to low density lipoproteins, and certain free sterols (3). These data are of particular interest because the C-6 glial cell is a good model of the glial cell found in mammalian brain during development (4, 5), when cholesterol deposition in glia is a particularly active process (see Refs. 6 and 7 for reviews).

Microtubules are involved in a variety of cellular functions important for cell differentiation (see Ref. 8 for review). Moreover, these organelles are thought to play an integral role in the intracellular transmission of signals from cell surface receptors (see, for example, Refs. 9 to 12), as well as the transport and metabolism of intracellular materials that might serve as important effectors of critical enzymes (8). For these reasons, we undertook this investigation of the regulation of HMG-CoA reductase and cholesterol synthesis in cultured C-6 glial cells to determine: 1) whether antimicrotubular drugs caused any alteration in HMG-CoA reductase activity and the rate of cholesterol synthesis, and 2) whether any such effects correlated with apparent effects on microtubular function. The data indicate that microtubular disruption is associated with a distinct alteration in the regulation of HMG-CoA reductase.

EXPERIMENTAL PROCEDURES

Materials—[3-3H]HMG-CoA (5.2 Ci/mmol), dl-3-[14C]mevalonic acid (45.9 Ci/mmol), [1,2-3H]cholesterol (32.9 Ci/mmol), and L-[4,5-3H]leucine (33.1 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Sodium [1-3H]acetate (60.2 Ci/mol) was obtained from Amersham (Arlington Heights, IL), HMG-CoA, colchicine, Colcemid (N-deacetyl-N-methylcolchicine) (Sigma, St. Louis, MO), vinblastine (Eli Lilly, Indianapolis, IN), Dowex AG1-X8 (200 to 400 mesh, formate) (Bio-Rad Laboratories, Richmond, CA), and prolidid scintillation fluid (3470, Research Products International, Elk Grove Village, IL) were obtained from the designated sources. Lumicolchicine, prepared by ultraviolet irradiation of colchicine (13), was the generous gift of Dr. Richard E. Ostlund, Jr. (Washington University School of Medicine, St. Louis, MO). All sera, antibiotics, and other reagents for cell culture were obtained from Grand Island Biological Co. (Grand Island, NY) and tissue culture flasks from Falcon (Oxnard, CA).

Prior to addition to cultures, colchicine, Colcemid, vinblastine, and lumicolchicine were dissolved in ethanol and added to flasks to give a final concentration 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 the rate of cholesterol synthesis or HMG-CoA reductase activity when compared to values obtained from cells maintained in the absence of ethanol.)

Cell Cultures—C-6 glial cells, cloned originally from a rat glioma, were obtained from the American Type Culture Collection (Rockville, MD) and have been maintained in this laboratory for the past 7 years. The methods of cell culture have been described previously (14, 15). The medium was prepared in this laboratory (15). Lipoprotein-poor serum was prepared from fetal calf serum by the method of Kirsten and Watson (16), and final preparations were assayed for cholesterol content by the method of Wybenga et al. (17).

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. Inocula were adjusted to give a final concentration of 0.5 x 10⁶/ml. Photographs of cells in monolayer culture were performed with an inverted phase-contrast microscope (Nikon MS 76560) equipped with a microflex camera (Nikon PFM-B).

Cholesterol Synthesis—Synthesis of sterols was determined in intact cells by measuring the incorporation of sodium [3-3H]acetate into digitonin-precipitable sterols by the method of Popjak (18) as modified and described previously (3). Specific radioactivity of the precursor in the culture medium was 10 μCi/μmol, and duration of the pulse was 60 min.

HMG-CoA Reductase Assay—This enzyme was assayed by a modification of the method of Brown et al. (19), as previously described (3). Separation of the product, [3-3H]mevalonate, from the sub-

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† The abbreviation used is: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.
The effect of a 1.0 μM concentration of colchicine on HMG-CoA reductase activity in relation to cholesterol synthesis was determined by adding 1.0 μM colchicine during the preincubation prior to assay and to the assay mixture as well, utilizing extracts prepared from cells grown in fetal calf serum or lipoprotein-poor serum for 24 h. No change in reductase activity was observed with either extract in the presence of colchicine. Moreover, the possibility of a dissociable inhibitor in the colchicine-treated cells was evaluated by mixing extracts of cells maintained in lipoprotein-poor serum for 6 h with or without 1.0 μM colchicine. The antimicrotubular effect of colchicine was evaluated by adding 1.0 μM colchicine to the reaction mixture instead of the reaction mixture was combined with 3 ml of packed wet resin, which is then suspended in 6 ml of distilled water and centrifuged at 500 × g. The supernatant solution is mixed with 13 ml of scintillation fluid and counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3375) with discriminators set to minimize spillover of counts from the 14C to the 3H channel. Unreacted HMG-CoA is retained by the resin with an efficiency of 99% or more, and recoveries of the internal standard, [3H]mevalonate, are approximately 60 to 65%, which compares favorably with previously published procedures. When representative samples of the AG1-X8 supernatant solutions were extracted with ether and the mevalonolactone was isolated by thin layer chromatography (21), the ratio of 3H to 14C in the lactone spot was found to be the same as that in the original AG1-X8 supernatant solutions.

Fatty Acid Synthetase Assay—This enzyme was assayed by the spectrophotometric method (14, 22), which is based on the malonyl-CoA-dependent oxidation of NADPH.

NADPH-Cytochrome c Reductase Assay—NADPH-dependent cytochrome c reductase was assayed as described previously (23).

Protein Synthesis—Synthesis of total cellular protein was evaluated by measurement of the incorporation of radioactivity from 1,4,5-3H)leucine (24). Specific radioactivity of the precursor in the culture medium was 6.3 μCi/μmol, and duration of the pulse was 60 min. The cells were then washed with 0.1 M sodium phosphate, pH 7.5, and the pool of radioactive 2-oxo acids was isolated by trichloroacetic acid precipitation.

Protein Content—The protein concentration in cellular extracts was determined by the method of Lowry et al. (25) or the microbiuret method (26).

Statistical Procedures—Statistical significance was determined by Student's t test. Differences discussed in this paper are significant at the p < 0.01 level or better.

RESULTS

Effect of Antimicrotubular Drugs on HMG-CoA Reductase Activity and Cholesterol Synthesis—The effect of colchicine, Colcemid, and vincristine, drugs known to disrupt the microtubules (27), on HMG-CoA reductase activity and cholesterol synthesis was determined after exposure of C-6 glial cells to 1.0 μM concentrations for 6 h (Table I). The antimicrotubular drugs resulted in markedly lower activities of HMG-CoA reductase; treated cells exhibited values 18 to 27% of those in control cells. This effect on HMG-CoA reductase was reflected in comparably lower rates of cholesterol synthesis. In contrast, cells exposed to a mixture of isomers of colchicine, i.e. lumicolchicine, that does not disrupt the microtubular apparatus (28), exhibited no difference in HMG-CoA reductase activity or cholesterol synthesis, as compared to control cells. Concentrations of lumicolchicine as high as 5.0 μM failed to cause any significant alteration in reductase activity (data not shown).

Effect of Colchicine on HMG-CoA Reductase Activity as a Function of Concentration—We next evaluated the effect of a 6-h exposure to colchicine on HMG-CoA reductase activity as a function of concentration of the drug (Fig. 1). A 50% lower reductase activity was observed in cells exposed to just a 0.1 μM concentration of colchicine. An essentially maximal effect was observed with 0.5 to 1.0 μM colchicine, which caused 75 to 80% lower reductase activities in the treated versus untreated cells.

Effect of Colchicine on HMG-CoA Reductase Activity as a Function of Time of Exposure—The effect of a 1.0 μM concentration of colchicine on reductase activity was determined as a function of time of exposure to the drug (Fig. 2). In these experiments, the effect of the drug was evaluated during the first hours after change of medium from 10% fetal calf serum to 10% lipoprotein-poor serum (Fig. 2A), as well as after the cells were induced to high levels of reductase activity by growth in the lipoprotein-poor serum for 24 h (Fig. 2B). In the former instance (Fig. 2A), control cells exhibited a nearly 2-fold increase in reductase activity between 1 and 6 h after the change of medium to lipoprotein-poor serum. This is the approximate onset of the marked induction of enzymatic activity caused by removal of low density lipoproteins from the medium, as previously described (3). In cells treated with colchicine, there was no induction of HMG-CoA reductase and, in fact, enzymatic activity decreased over the 6-h time period. The difference in enzymatic activity between treated and untreated cells was apparent approximately 40 to 60 min after exposure; after 2 and 6 h, activities in treated cells were 45% and 15%, respectively, of those in untreated cells (Fig. 2A). No further reduction in reductase activity occurred in the treated cells between 6 and 24 h of exposure to the drug (data not shown).

When colchicine was added to the medium of cells that had been maintained in lipoprotein-poor serum for 24 h (Fig. 2B), we observed an effect on reductase activity similar to that just described (compare Fig. 2A). In treated cells, a decrease in enzymatic activity was apparent after 1 to 2 h of exposure to colchicine, and, after 3 h, reductase activity was reduced by approximately 70%.

The rapidity of the effect of colchicine raised the possibility of a direct effect of the drug on the enzyme. This possibility was evaluated by adding 1.0 μM colchicine during the preincubation prior to assay and to the assay mixture as well, utilizing extracts prepared from cells grown in fetal calf serum or lipoprotein-poor serum for 24 h. No change in reductase activity was observed with either extract in the presence of the colchicine. Moreover, the possibility of a dissociable inhibitor in the colchicine-treated cells was evaluated by mixing experiments. Thus, extracts of cells maintained in lipoprotein-poor serum for 6 h with or without 1.0 μM colchicine were assayed separately and after mixing. Specific activities were 24.3 for the extracts from the colchicine-treated cells, 91.2 for those from the untreated cells, and 55.2 for the mixed extracts. Thus, the enzymatic activity with the mixed extracts was additive.

Specificity of the Effect of Colchicine on HMG-CoA Reductase and Cholesterol Synthesis—We next asked whether the effect of colchicine on HMG-CoA reductase and cholesterol synthesis was part of a generalized, nonspecific toxic effect on the cells. The impact of the drug was determined on the activities of fatty acid synthetase, a critical enzyme for nonsteroidal lipid biosynthesis (29), and NADPH-cytochrome
Fig. 1 (left). Effect of colchicine on HMG-CoA reductase activity as a function of the concentration of the drug. C-6 glial cells were transferred to a series of flasks and grown in 10% fetal calf serum for 48 h. At that time, the medium was changed so that all flasks contained 10% lipoprotein-poor serum. To all but three control flasks, colchicine was added in the indicated concentrations. After 6 h HMG-CoA reductase specific activity was determined. See legend to Table I.

Fig. 2 (center and right). Effect of 1.0 μM colchicine on HMG-CoA reductase activity as a function of time of exposure to the drug. C-6 glial cells were transferred to a series of flasks and grown in 10% fetal calf serum for 48 h. In A, three flasks were utilized to determine HMG-CoA reductase specific activity at zero time, and the medium in the remainder was changed so that all flasks contained 10% lipoprotein-poor serum, and one-half, 1.0 μM colchicine in addition. In B, all flasks contained 10% lipoprotein-poor serum for the 24-h prior to the experiment. At that time, three flasks were utilized to determine reductase specific activity at zero time, and the medium in the remainder was changed so that all flasks contained fresh 10% lipoprotein-poor serum, and one-half, 1.0 μM colchicine in addition. At the indicated times, HMG-CoA reductase specific activity was determined. Values were means obtained from separate determinations on each of three flasks, and standard deviations did not exceed 5 to 10% of the mean. Essentially identical results were obtained in two separate experiments.

**Table II**

<table>
<thead>
<tr>
<th>Colchicine concentration (μM)</th>
<th>Fatty acid synthetase activity (%)</th>
<th>NADPH-cytochrome c reductase activity (%)</th>
<th>Protein synthesis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>102</td>
<td>94.7</td>
<td>93.4</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>96.2</td>
<td>92.7</td>
</tr>
</tbody>
</table>

Colchicine, a microsomal enzyme, and on the rate of protein synthesis (Table II). Each parameter was studied after 6 h of exposure to 0.5 and 1.0 μM concentrations of colchicine. No change in the activities of fatty acid synthetase or NADPH-cytochrome c reductase or in the rate of protein synthesis was observed.

**Relation of the Antimicrotubular Effect of Colchicine to the Effect on HMG-CoA Reductase and Cholesterol Synthesis**—We next asked whether a temporal relation could be discerned between the effects of colchicine on microtubules and on HMG-CoA reductase. One measure of microtubular function, i.e. maintenance of cell shape, was studied as a function of time of exposure to 1.0 μM colchicine (Fig. 3). Marked changes in glial cell shape occurred shortly after the addition of colchicine. Untreated cells were primarily spindle-shaped with long processes (Fig. 3A). (No change in morphology occurred with change of medium from fetal calf serum to lipoprotein-poor serum.) After just 1 h in colchicine, the processes began to retract, and the cell margins became less smooth (Fig. 3B). These changes became more marked over the 6-h exposure period (Fig. 3, C and D). The time course and relative magnitudes of these effects on cell shape correlated closely with those defined above for HMG-CoA reductase activity and cholesterol synthesis (see Fig. 2A). Lumicolchicine (2.0 μM), which did not cause an alteration in reductase activity or cholesterol synthesis (see Table I), also failed to cause any change in glial cell shape.

**DISCUSSION**

This work has been concerned with the effect of antimicrotubular drugs on the regulation of HMG-CoA reductase and cholesterol synthesis in C-6 glial cells. Colchicine, Colcemid, and vinblastine caused a marked inhibitory effect on HMG-CoA reductase and, as a consequence, cholesterol synthesis in these cells. The effect of colchicine was studied in further detail and did not appear to relate to a nonspecific toxic effect on the cells. Despite marked and rapid effects on HMG-CoA reductase and cholesterol synthesis, the activities of fatty acid synthetase and NADPH-cytochrome c reductase and the rate of total protein synthesis were unaffected.

The present observations suggest that microtubules may be involved in the regulation of HMG-CoA reductase and cholesterol synthesis in mammalian cells. Disruption of microtubules is produced readily with the concentrations of colchicine,
Colcemid, and vinblastine utilized in this work (see Refs. 8, 27, and 30 for reviews). Colchicine causes a dissolution of microtubules by binding to a specific site on the microtubule subunit and causing a shift in the subunit-polymer equilibrium. Microtubules play a critical role in a variety of cellular functions, including chromosome movements in cell division, intracellular movements of various materials, cell motility, and development and maintenance of cell shape (8). The effect of colchicine on microtubules of C-6 glia was reflected in the marked changes in cell shape induced by exposure to the drug. Lumicolchicine, an isomeric mixture of colchicine that does not disturb microtubules, did not alter cell shape and was entirely ineffective in altering HMG-CoA reductase activity or cholesterol synthesis. This observation and the close temporal and quantitative correlation between the effects of colchicine on cell shape and HMG-CoA reductase suggest that microtubules may be involved in the regulation of this critical enzyme.

The mechanism by which microtubular disruption might lead to the effect of antimicrotubular drugs on HMG CoA reductase must occur at the level of enzyme synthesis, degradation, or catalytic efficiency. One possibility is that the alteration of the glial cell membrane disturbs surface receptors important for the regulation of HMG-CoA reductase activity. Specific cell surface receptors for low density lipoproteins have been shown to be critical for the regulation of HMG-CoA reductase (31, 32). The presence of such receptors in C-6 glia is suggested by the demonstration of active regulation of reductase in these cells by low density lipoproteins (3). Similarly, the inhibition of certain lectin induced responses by drugs that disrupt the microtubular network has provided support for the idea that signals originating from cell surface receptors may be transmitted intracellularly by the microtubular network (9). An example of this phenomenon in C-6 glia is the recent demonstration that the lectin induced synthesis of the S-100 protein is inhibited in the presence of colchicine (11). A second and related possibility is that the microtubular network is involved in the transport or metabolism, or both, of certain intracellular effectors to the transcriptional or translational machinery to alter reductase synthesis or degradation or to the site of the enzyme in the endoplasmic reticulum to alter reductase catalytic efficiency. For example, the binding of low density lipoprotein to the surface receptor is followed by endocytosis of the receptor-lipoprotein complex, transport of the endosome to the lysosome, fusion of these particles, hydrolysis of the lipoprotein cholesterol ester with release of free cholesterol, and degradation of the protein by lysosomal enzymes (1). It might be predicted that a number of sites in this pathway could be disturbed by microtubular disruption. Finally, the possibility could be raised that microtubules are directly or indirectly associated with the endoplasmic reticulum. Such an association has been demonstrated recently with mitochondria of several cultured cell lines (33). Thus, the current findings raise important topics for future study. Moreover, the data have important implications for the mechanisms of regulation of HMG-CoA reductase as well as for the roles of microtubules in mammalian cells.

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REFERENCES

Microtubules and the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase.
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