The Role of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Activity in the Regulation of Ubiquinone Synthesis in Human Fibroblasts*

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Ubiquinone biosynthesis in cultured human skin fibroblasts was studied using 4-hydroxy[U-14C]benzoic acid (4-[U-14C]HB) and [5-3H]mevalonolactone as markers. The incorporation of 4-[U-14C]HB was maximal in cells incubated in the presence of whole human serum but was inhibited to varying degrees when incubated in lipoprotein-deficient serum or in the presence of 25-hydroxycholesterol, 7-ketocholesterol, or ML-236B (compactin). Further inhibition was obtained when these substances, as well as low density lipoprotein (LDL), were incubated with cells in the lipoprotein-deficient medium. Comparison of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity of cells incubated in human serum and lipoprotein-deficient medium showed that it was increased 17-fold in the latter. Despite this, the incorporation of 4-HB into ubiquinone was inhibited by 35%, thereby indicating that some factor in whole serum is involved in the regulation of 4-HB incorporation into ubiquinone. The level of mevalonate, on the other hand, is also important in regulating ubiquinone synthesis. 7-Ketocholesterol, 25-hydroxycholesterol, and LDL were inhibitory to HMG-CoA reductase activity as expected. Addition of exogenous mevalonate relieved the inhibition by LDL and ML-236B of the incorporation of 4-[U-14C]HB into ubiquinone, thereby indicating that the effect was due to decreased levels of endogenous mevalonate related to reduced HMG-CoA reductase activity.

When [3H]mevalonolactone incorporation into ubiquinone and cholesterol was assayed in the presence and absence of LDL, it was found that LDL stimulated the incorporation of mevalonate into ubiquinone; the increase was 5-fold at lower concentrations of MVL compared to 1½-fold at higher concentrations. The incorporation into cholesterol was reduced significantly in the presence of LDL, indicating an effect of LDL on the pathway from mevalonate to cholesterol. ML-236B had the same general effect as LDL with respect to incorporation of mevalonate into ubiquinone but had little effect on incorporation into cholesterol when mevalonate concentration was not limiting.

Double labeling experiments revealed that LDL and ML-236B inhibited the incorporation 4-[U-14C]HB into ubiquinone while [3H]mevalonate incorporation was enhanced. This apparent increase in [3H]mevalonate incorporation under conditions where HMG-CoA reductase is inhibited is largely due to higher specific radioactivity of the intracellular pool of mevalonate resulting from the lack of endogenous mevalonate to dilute the label and does not represent increased synthesis of ubiquinone. The same experiments show that fibroblasts have a very large capacity to convert exogenous mevalonate to cholesterol, even in the presence of LDL.

Bioisynthetic pathways leading to the formation of cholesterol and ubiquinone share a partially common route with respect to the formation of the appropriate polyprenoid precursor (1). Although the exact point of divergence is not clearly identified, it is presumed to be at the farnesyl pyrophosphate level. It has been demonstrated in rat liver mitochondria (2) and in yeast (3) that isopentenyl pyrophosphate can serve as the side chain precursor for the isoprenoid chain of ubiquinone. Isopentenyl pyrophosphate is converted to cholesterol in the cytosol and microsomes (4), whereas the events leading to ubiquinone synthesis, beginning with prenylation of 4-hydroxybenzoate, take place in the mitochondria (5, 6), and various intermediates, formed in this phase of the biosynthetic pathway, have been characterized.

The pathway for ubiquinone synthesis in prokaryotic cells (7) is also presumed to be operative in eukaryotic systems, but with minor modifications (8-11). Very little is known about the overall regulation of ubiquinone synthesis in either cell type. The reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate catalyzed by the enzyme HMG-CoA reductase (EC 1.1.3.4) is considered to be the major rate-determining step in cholesterol biosynthesis. Even though it has been recognized that the supply of mevalonate would greatly affect the rate of ubiquinone biosynthesis (1), the regulatory role of HMG-CoA reductase is just beginning to be investigated. Recently, Faust et al. (11) reported that human fibroblasts synthesize ubiquinone-10, and that the relative rates of ubiquinone and cholesterol biosynthesis are regulated by the availability of exogenous cholesterol.

The studies we report here were designed to investigate the regulation of ubiquinone biosynthesis in human fibroblasts using 4-[U-14C]HB and [3H]MVL, which are the precursors for the quinone ring and the isoprenoid side chain, respectively, to determine the rate of synthesis. The effects of modifiers of HMG-CoA reductase activity on these processes were also examined and compared with their well known effects on sterol synthesis. We have also examined the relative flux of mevalonate into cholesterol and ubiquinone under these conditions.

Our results show that the level of HMG-CoA reductase

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The abbreviations used are: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low density lipoprotein; MVL, mevalonolactone; 4-HB, 4-hydroxybenzoic acid.
activity plays a role in determining the rate of ubiquinone synthesis in these cells.

MATERIALS AND METHODS

Chemicals—Mevalonolactone, (RS)-[5-3H] (78,000 mCi/mmol) and 4-hydroxy-3-methyl[14C]glutaric acid, enzyme A (51.3 mCi/mmol) were purchased from New England Nuclear Corp. 4-Hydroxy[14C]benzoic acid (460 mCi/mmol) was synthesized from L-[U-14C]tyrosine by alkaline infusion (12). Compound ML-236B was a gift from Dr. Akira Endo of Sankyo Co., Ltd., Tokyo, Japan. The lactone form of ML-236B was converted to the acid form by saponification (4) in 0.1 N NaOH at 50°C for 1 h. Multiple aliquots were stored at -20°C after adjusting the pH to 7.8 with HCl. 25-Hydroxycholesterol and 7-ketocholesterol solutions were prepared fresh in ethanol prior to the experiment. Eagle’s minimum essential medium was purchased from Grand Island Biological Co. All other chemicals, tissue culture, and chromatographic materials were of fine grade, commercially available.

Cell Cultures—Human fibroblast cell line GM0043 was obtained from the Human Genetic Mutant Cell Depository, Institute for Medical Research, Camden, N.J. Cells were cultured in monolayers and used between the 5th and 20th passage. Stock cultures were maintained on 10 ml of Eagle’s minimum essential medium (Catalog No. 330-145) supplemented with penicillin (100 μg/ml), streptomycin (100 μg/ml), 20 mM N-tris(hydroxymethyl)methylglycine (pH 7.4), 25 mM NaHCO3, 1% (v/v) nonessential amino acid, and 10% fetal bovine serum, in Petri dishes (100 mm). Cells from each dish were removed by trypsinization (2 min at 37°C) to dissociate from the plate using 0.04% trypsin, 0.02% EDTA in saline containing 0.8% NaCl, 0.04% KCl, 0.1% glucose, and 0.035% NaHCO3. Cells from each dish were suspended in 4 ml of growth medium containing 10% fetal bovine serum and were plated on dishes (60 × 15 mm), each dish receiving 3 ml of cell suspension. The medium was replaced on alternate days with fresh growth medium. On the 5th day of growth, the growth medium was replaced with fresh medium containing 10% human serum or delipidated human serum as indicated in the legend for each experiment. The protein content per dish ranged from 0.3 to 0.45 mg.

LDL and Lipoprotein-deficient Serum—Human LDL and lipoprotein-deficient serum were prepared by differential ultracentrifugation using KBr for density adjustments (13). The 10% human serum was dialyzed to the same extent as described in Ref. 13.

4-[U-14C]HB Incorporation into Ubiquinone—Monolayers of fibroblasts (60 × 15 mm dishes) were incubated in 3 ml of growth medium containing 10% human serum or lipoprotein-deficient medium with oxygenated derivatives of cholesterol, cholesterol, or ML-236B as indicated at 37°C. After 24 h, 0.1 μCi of 4-[U-14C]HB was added to the medium and incubated further for 24 h at 37°C. The medium was then removed and the monolayer was washed twice with 3 ml of saline. The cells were then lysed for 20 min with 1 ml of 0.1 N NaOH. After transferring the lysate to 30-ml test tubes, the dishes were washed twice with 1 ml each of water. The combined washings and lysate were taken in a 3% volume of chloroform/methanol (2:1 v/v) and allowed to stand at room temperature for 1 h. The lipids were extracted according to the method of Galliard et al. (14) and washed as described by Momose and Rudney (2). Unlabeled ubiquinone (200 μg) was added to the extraction mixture as carrier. The extracts were subjected to chromatographic analysis.

Incorporation of [3H]MVL into Ubiquinone and Cholesterol—Conditions for the incubations for the incorporation of [3H]MVL into ubiquinone and cholesterol were the same as described for 4-[U-14C]HB incorporation. The lipids were extracted in the same manner except that unlabeled cholesterol (400 μg) was also added as a carrier to the reaction mixture.

Thin Layer Chromatography—Lipid fractions were reduced to dryness under a stream of nitrogen and taken in a small volume of petroleum ether. The samples were subjected to thin layer chromatography using 10% petroleum ether (v/v) as solvent. In this solvent system, ubiquinone and cholesterol had Rf values of 0.54 and 0.36, respectively. Cholesterol was visualized by exposing the plates to iodine vapor.

Radioactivity Measurements—Areas corresponding to ubiquinone and cholesterol on the thin layer plates were scraped off and the radioactivity determined using Beckman LS 1845P liquid scintillation counter.

Measurement of HMG-CoA Reductase Activity—HMG-CoA reductase activity in fibroblasts was determined by measuring the rate of conversion of 3-hydroxy-3-methyl[3,4C]glutaric-CoA to [14C]mevalonate in detergent-solubilized cell-free extracts according to the method of Brown et al. (15).

Protein Determinations—The protein content of whole cells and extracts was determined by the method of Sedmak and Grossberg (16) using bovine serum albumin as standard.

RESULTS

Time Course of Incorporation of 4-[U-14C]HB into Ubiquinone in Fibroblasts—Fig. 1 illustrates the incorporation of 4-[U-14C]HB into ubiquinone in cultured human fibroblasts. The rate of incorporation was linear, at least up to 72 h.

Effect of LDL and Oxygenated Derivatives of Cholesterol on Ubiquinone Synthesis from 4-[U-14C]HB—It is well known that LDL cholesterol and oxygenated derivatives of cholesterol play a role in regulating HMG-CoA reductase levels in fibroblasts and, consequently, cholesterol biosynthesis. Table I shows the effect of LDL, 25-hydroxycholesterol, and 7-ketocholesterol on ubiquinone synthesis from 4-[U-14C]HB. The data indicated that these compounds do inhibit by 35 to 50% the incorporation of radioactivity from 4-[U-14C]HB into ubiquinone. The same inhibitory effects were also evident when the cells were incubated in the presence of lipoprotein-deficient medium. Further, it is of interest to note that cells incubated on serum lipoprotein-deficient medium were about 35% less efficient in synthesizing ubiquinone as compared to cells incubated in medium containing whole human serum. As expected, removal of lipoproteins from the medium resulted in a 17-fold increase in the HMG-CoA reductase level. Addition of LDL to the lipoprotein-deficient medium lowers the enzyme to a level comparable to that of cells incubated in the presence of total human serum. Oxygenated derivatives of cholesterol, 25-hydroxycholesterol, and 7-ketocholesterol were also inhibitory, approximately to the same extent in cells incubated in whole human serum or lipoprotein-deficient serum. Thus, if one compares cells incubated in LDL added to lipoprotein-deficient serum with whole human serum, an inhibition of 59% is observed.

In order to ascertain whether the inhibitory effect of LDL on the incorporation of 4-[U-14C]HB into ubiquinone could be relieved by mevalonate, cells were supplemented with increasing concentrations of mevalonate in the presence of LDL and tested for their ability to synthesize ubiquinone. The results

![FIG. 1. Time course of incorporation of 4-hydroxy[U-14C]benzoic acid (4-HB) into ubiquinone in monolayers of human skin fibroblasts. On the 5th day of growth, each monolayer received 3 ml of fresh growth medium containing 0.1 μCi of 4-[U-14C]HB. After incubation for the indicated time intervals at 37°C, cells from three plates (60 × 15 mm) were harvested (ordinate = dpm/mg of protein × 10⁻³).](image-url)
HMG-CoA Reductase and Ubiquinone Synthesis in Fibroblasts

### Table I

| Incubation medium | RS incorporation into ubiquinone | HMG-CoA reductase |
|-------------------|---------------------------------|-------------------|
|                   | cpm/mg protein                  | %                 | mmol/mg protein/2 h |
| HS                | 11,460                          | 100.0             | 154.6               | 1.60               |
| HS + 25-hydroxycholesterol | 7,520 | 65.6 | 101.4 | 0.49 |
| HS + 7-ketocolesterol | 8,250 | 71.9 | 111.3 | 0.67 |
| LPDS              | 7,410                           | 64.6              | 100.0               | 27.40              |
| LPDS + 25-hydroxycholesterol | 4,200 | 38.6 | 56.6 | 0.51 |
| LPDS + 7-ketocolesterol | 4,220 | 38.8 | 56.9 | 0.64 |
| LPDS + LDL        | 4,730                           | 41.2              | 63.8                | 1.50               |

### Table II

| Mevalonate concentration | Incorporation of 4-[U-14C]HB into ubiquinone | Inhibition by LDL |
|--------------------------|----------------------------------------------|-------------------|
| mM                       | cpm/mg protein                               | %                 |
| 0.0                      | 11,730                                       | 8,760             | 25.4               |
| 0.1                      | 11,520                                       | 9,960             | 12.1               |
| 0.5                      | 11,400                                       | 11,020            | 3.4                |
| 1.0                      | 12,140                                       | 11,930            | 1.8                |

shown in Table II demonstrate that at 1 mM concentration of exogenous mevalonate, LDL does not inhibit 4-HB incorporation into ubiquinone. It is evident from the data in Tables I and II that LDL limits the production of mevalonate needed for ubiquinone synthesis, most probably by inhibiting HMG-CoA reductase.

**Effect of exogenous mevalonate added to the medium on the synthesis of ubiquinone from 4-[U-14C]CHB in the presence of LDL in human fibroblasts**

Cells were grown as described under "Materials and Methods." On the 5th day of growth, each monolayer received 3 ml of growth medium containing 10% HS (whole human serum) or LPDS (lipoprotein-deficient human serum) and 25-hydroxysterol, 7-ketosterol (5 mg/ml each), or LDL (25 mg of protein/ml), as indicated. After incubation for 24 h at 37°C, 0.1 μCi of 4-[U-14C]HB was added. After further incubation for 24 h, the cells were harvested and radioactivity incorporated into ubiquinone was determined.

**Effect of LDL and oxygenated derivatives of cholesterol on ubiquinone synthesis from 4-[U-14C]HB in fibroblasts**

Cells were grown as described under "Materials and Methods." On the 5th day of growth, each monolayer received 3 ml of growth medium containing 10% HS (whole human serum) or LPDS (lipoprotein-deficient human serum) and 25-hydroxysterol, 7-ketosterol (5 mg/ml each), or LDL (25 mg of protein/ml), as indicated. After incubation for 24 h at 37°C, 0.1 μCi of 4-[U-14C]HB was added. After further incubation for 24 h, the cells were harvested and radioactivity incorporated into ubiquinone was determined.

In contrast to these results, when the incorporation of MVL into ubiquinone (A) and cholesterol (B) in the presence of LDL (25 μg/ml) and no LDL is compared, there is a clear inhibition in the incorporation of MVL into ubiquinone in the presence of LDL at 50 μM concentration of mevalonate. At higher concentrations of MVL, the difference was relatively less. The increased incorporation of MVL into ubiquinone in the presence of LDL was 1.5-fold at 1 mM concentration of MVL.

**Effect of exogenous mevalonate added to the medium on the synthesis of ubiquinone from 4-[U-14C]HB in the presence of ML-236B in human fibroblasts**

Conditions were the same as described for Table II except that the cells were incubated with ML-236B (1 μM) instead of LDL.

**Table III**

| Mevalonate concentration | Incorporation of 4-[U-14C]-CHB into ubiquinone | Inhibition |
|--------------------------|-----------------------------------------------|------------|
| mM                       | cpm/mg protein                               | %          |
| 0.0                      | 11,730                                       | 8,760      | 25.4               |
| 0.05                     | 11,610                                       | 7,140      | 38.5               |
| 0.5                      | 11,760                                       | 9,220      | 21.6               |
| 1.0                      | 11,230                                       | 10,320     | 8.6                |

**Fig. 2. Incorporation of [3H]mevalonolactone into ubiquinone (A) and cholesterol (B) in the presence and absence of LDL in human skin fibroblasts.**

On the 5th day of growth, each monolayer received 2 ml of fresh growth medium containing 10% lipoprotein-deficient human serum and either 25 μg of protein/ml of LDL or no LDL. After incubation for 24 h at 37°C, 0.1 μCi of 4-[U-14C]HB was added. After further incubation for 24 h, the cells were harvested and radioactivity incorporation into ubiquinone and cholesterol determined.
into cholesterol was examined (Fig. 2B), there was significant reduction of incorporation in the presence of LDL. These calculated values for the \([^{3}H]\)MVL incorporation are not corrected for the dilution of the label by endogenously produced mevalonate. Thus, it seemed possible that the apparent increase in the incorporation of label from MVL into ubiquinone in the presence of LDL could be due to the absence of endogenous mevalonate to dilute the added \([^{3}H]\)mevalonate.

We repeated the experiments under the same conditions using compound ML-236B in place of LDL. ML-236B, being a competitive inhibitor of HMG-CoA reductase, is not known to inhibit cholesterol synthesis from mevalonate (19). The results are presented in Fig. 3, A and B. It is evident from the data that, like LDL, ML-236B also facilitated increased incorporation of the MVL into ubiquinone. If the apparent increased incorporation of the label into ubiquinone in the presence of ML-236B was due to lack of endogenous mevalonate to dilute the label, the same relative increase should have been observed in the cholesterol synthesis as well. Although not readily apparent because of the ordinate scale in Fig. 3B, there was, however, a moderate inhibition (0.57 and 0.87 nmol in the presence and absence of ML-236B, respectively) in the incorporation of the label into cholesterol in the presence of ML-236B, but as higher concentrations of MVL were reached, the inhibition almost disappeared.

The effects of LDL and ML-236B on ubiquinone and cholesterol synthesis from \([^{3}H]\)MVL were more pronounced when tracer concentrations (0.6 \(\mu\)M) were used while ubiquinone synthesis was assayed simultaneously with \([^{4}U\)C\]HB. Data presented in Table IV clearly indicate that formation of cholesterol from mevalonate was inhibited 67% by LDL and 43% by ML-236B. When the cells were exposed to both LDL and ML-236B, the incorporation of \([^{3}H]\)MVL into cholesterol was the lowest. The net synthesis of ubiquinone as measured by the incorporation of \([^{3}H]\)MVL into ubiquinone, on the other hand, was enhanced under the same conditions. Addition of unlabelled MVL to the medium diluted the label incorporation into ubiquinone, in cells grown in the presence of LDL or ML-236B. Surprisingly, the incorporation of radioactivity into cholesterol was increased by severalfold on addition of unlabelled mevalonate to the medium, suggesting an increased synthesis of cholesterol at higher concentrations of mevalonate.

Under these conditions of decreased HMG-CoA reductase activity, the incorporation of \([^{4}U\)C\]HB is inhibited, indicating reduced synthesis of ubiquinone. Addition of large amounts of mevalonate restored the rate of synthesis to the normal level. Therefore, these data show that the apparent increase in \([^{3}H]\)mevalonate incorporation into ubiquinone is primarily due to less dilution of radioactivity of mevalonate by the endogenous mevalonate pool.

**DISCUSSION**

The regulatory role of HMG-CoA reductase in cellular cholesterol synthesis is well documented (19). In cultured human fibroblasts this enzyme is regulated by the low density lipoprotein levels in the incubation medium (15). A number of oxygenated derivatives of cholesterol are also known to inhibit HMG-CoA reductase activity in intact cells grown in tissue culture (20–23).

It would be expected that inhibition of HMG-CoA reductase would limit the supply of endogenously produced mevalonic acid.
acid needed for the synthesis of sterols, ubiquinone, dolichols, and other mevalonate-derived compounds (24). Recent work on dolichol synthesis bears this out in some cases but not in others. Thus, Mills and Adamanly (25) found that the synthesis of dolichol in endothelial smooth muscle cells was inhibited by 25-hydroxycholesterol. Also, James and Kandutsch (26) indicated that under certain conditions, the dolichol synthetic pathway may be affected by treatments causing variations in cholesterol synthesis in L-cell cultures while other conditions existed where large fluctuations in sterol synthesis had little effect on dolichol synthesis. Koller et al. (27) concluded that in rat liver, the rate of dolichol phosphate biosynthesis was not regulated by the activity of HMG-CoA reductase. More recently, Carson and Lennarz (28) show that compactin, an HMG-CoA reductase inhibitor used in these studies, specifically affected dolichol synthesis in sea urchin embryos with inhibition of glycoprotein synthesis and resultant abnormal embryonic development. Faust et al. (11) from their recent studies concluded that in human skin fibroblasts, ubiquinone synthesis proceeds at near-maximal levels in the presence of whole human serum and lipoprotein-deficient sera on ubiquinone synthesis is currently under investigation.

It is evident from our results that, in fibroblasts, the net synthesis of ubiquinone, as measured by incorporation of 4-[U-14C]HB into ubiquinone, is dependent on the modulation of mevalonate levels in cells. Suppression of HMG-CoA reductase activity by LDL and ML-236B inhibits the incorporation of 4-[U-14C]HB up to 36% and 40%, respectively. Addition of saturating levels of exogenous mevalonate increases the radioactivity incorporation from 4-[U-14C]HB to control levels, indicating that the inhibition is due to reduced production of mevalonate. A similar effect was observed by Faust et al. (11) with the incorporation of [methyl-3H]methionine into the ubiquinone in fibroblasts. Similarly, Aiyar and Olson found that the biosynthesis of ubiquinone from [1-14C]benzoate by rat liver slices was decreased in cholesterol-fed rats and this inhibitory effect was overcome by the addition of mevalonate to the liver slices (29).

Different effects are noted when variable concentrations of [3H]MVL are incubated with cells in lipoprotein-deficient serum in the presence or absence of LDL and ML-236B and the incorporation of mevalonate into ubiquinone and cholesterol is compared. LDL stimulated the incorporation of MVL into ubiquinone 5-fold at the 50 µM level of MVL, but only 1½-fold at the 1 mM level (Fig. 2). Generally, similar results were observed with ML-236B, but when incorporation of [3H]MVL into cholesterol was assayed in the presence of LDL, an inhibition was noted (Fig. 2). Reduction in the incorporation of mevalonate into cholesterol in cholesterol-fed animals has been observed by others (29, 30) and has been ascribed to an inhibition by cholesterol of some step in the mevalonate to cholesterol pathway. When ML-236B is present, however, incorporation at the 50 µM level of MVL was inhibited by 30%, but to a lesser degree when the MVL concentrations were raised from 0.25 to 1.0 mM. It seems reasonable to conclude, therefore, that LDL cholesterol stimulates the incorporation of MVL into ubiquinone because the conversion of MVL to cholesterol is inhibited by LDL (11). However, this cannot be the sole explanation since ML-236B, which stimulates MVL incorporation into ubiquinone, does not inhibit sterol synthesis from MVL except at rather low concentrations. Furthermore, the data can be readily explained by analyzing the consequences of the observed inhibition of HMG-CoA reductase and its effect on the endogenous pool of MVL.

It is likely that the stimulatory effect of LDL and ML-236B on the incorporation of [3H]MVL is an apparent one because endogenous synthesis of mevalonic acid is inhibited via inactivation of HMG-CoA reductase; thus, the specific activity of the mevalonate pool in the cell would be much higher and give a false impression of increased synthesis of ubiquinone. To investigate this point, we performed double labeling experiments wherein the biosynthesis of ubiquinone from 4-[U-14C]HB and [5-3H]MVL was studied simultaneously.

The difference in the incorporation of 4-[U-14C]HB and [3H]MVL into ubiquinone is striking. Thus, although there is a strong inhibition of 4-HB incorporation into ubiquinone in the presence of LDL and ML-236B, an increase is seen in the radioactivity incorporated from MVL (Table IV). We conclude that the increased incorporation of [3H]MVL does not represent increased ubiquinone synthesis but is due in part to the high specific activity of the labeled intracellular mevalonate pool, resulting from the fact that endogenous mevalonate production is blocked by LDL and ML-236B. Actually, total biosynthesis of ubiquinone is inhibited.

Under the conditions of this experiment, exogenous unlabeled mevalonate added to the medium obviously dilutes the intracellular mevalonate pool. This is evidenced by the fact that although exogenous unlabeled MVL stimulates ubiquinone synthesis from 4-HB in the presence of LDL or ML-236B, there is a decrease in the radioactivity coming from MVL. If this is the case, it also shows that there must have been a large increase in the synthesis of cholesterol when more MVL
is available to the cell. Despite the dilution of radioactivity, there is enhanced incorporation of \(^{3}H\)MVL into cholesterol from 39,160 dpm/mg of protein to 59,620 dpm/mg of protein in the presence of LDL when unlabelled (1 mM) MVL is added to the system. The same effects are observed with ML-236B alone, and is even more pronounced when cells are exposed to both LDL and ML-236B together, i.e. in the presence of 1 mM unlabelled MVL, one observes a 5-fold increase in 4-HB incorporation, an almost 7-fold dilution of mevalonate radioactivity in ubiquinone, and yet a 7-fold increase in the incorporation of \(^{3}H\)MVL into cholesterol. Within the limitations of lack of knowledge of the precise role that dilution factors play, one interpretation is that when more mevalonate is available to the cell, the block in sterol synthesis exerted by LDL cholesterol is partially bypassed, either by activation of both LDL and ML-236B together, or by activation of the isoprenoid portion of the ubiquinone molecule is inhibited under these conditions.

Experiments were initiated to examine the lipoprotein effects on cells in which the isoprenoid portion of the ubiquinone molecule was monitored by the addition of \(^{14}\)Cacetate to the medium and incubated under similar conditions. Preliminary data show that compared to cells incubated in whole human serum, the cells in lipoprotein-deficient serum showed a 6-fold increase in incorporation of acetate into the nonsaponifiable lipid fraction, but the incorporation into ubiquinone was inhibited by about 15 to 40%. When LDL was added to the lipoprotein-deficient serum there was, as expected, a 90% inhibition of acetate incorporation into the nonsaponifiable fraction, but there was a further 35% drop in incorporation into ubiquinone. These data support the observations with 4-HB and indicate that the synthesis of the entire ubiquinone molecule is inhibited under these conditions.

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