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EFFECT OF AN UNNATURAL PHOSPHOLIPID BASE ANALOG, N-ISOPROPYLETHANOLAMINE, ON 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE IN CULTURED GLIAL AND NEURONAL CELLS

Joseph J. Volpe

Departments of Pediatrics and Neurology and Neurosurgery (Neurology), Washington University School of Medicine, St. Louis, Missouri 63110

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SUMMARY

Cultured C-6 glial and neuroblastoma cells were utilized to study the effect of the unnatural amino alcohol, N-isopropylethanolamine, on the microsomal enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase. Growth of both cell types in the presence of the compound was accompanied in 24 hr by a decrease in reductase activity to 25-35% of activity in control cells. The effect was accompanied by a comparable decrease in the rate of cholesterol synthesis. However, no comparable change occurred in cell growth, fatty acid synthetase activity, or in total protein synthesis from [^{3}H]leucine. The data suggest that the polar head groups of microsomal membrane phospholipids play an important role in the regulation of reductase activity.

Alteration of specific lipid components of cellular membranes provides the opportunity to evaluate the roles of such compounds in the functional properties of these membranes. Unnatural amino alcohols can be incorporated into the polar head groups of membrane phospholipids in mammalian liver (1-5) and cultured cells (6-8). One such alcohol, N-isopropylethanolamine (IPE)*, has been shown to be incorporated into membrane phospholipids of rat liver (5) and cultured L-M cells (8), primarily at the expense of phosphatidyl choline, and to a lesser extent, phosphatidyl ethanolamine. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)* reductase (EC 1.1.1.34), the rate-limiting and major regulatory enzyme in cholesterol biosynthesis, is a membrane-bound, i.e. microsomal enzyme (see reference 9 for review). In view of the importance of this enzyme in the biosynthesis of cholesterol, a critical constituent of cell membranes, and the lack of information re: the relationship of membrane lipid composition and regulation of reductase, we determined the effects of IPE on the enzyme of cultured glial and neuronal cells.

The abbreviations used are: IPE, N-isopropylethanolamine; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

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MATERIALS AND METHODS

Materials. D,L-[3-^{14}C]HMG-CoA (18.9-25.1 Ci/mol), sodium [2-^{14}C]acetate (51.4 Ci/mol) and L-[4,5-^{3}H]leucine (42.8 Ci/mmol) were obtained from New England Nuclear. IPE was obtained from Aldrich. This material was analyzed by thin layer chromatography in chloroform:methanol:NH_4OH (65:35:10, v/v) and found to be free of detectable impurities. IPE was neutralized with dilute HCl and added as a 0.3 M solution to the culture medium (final concentrations are given in Results). Lipoprotein-poor serum was prepared by the method of Kirsten and Watson (10). The last step of the procedure includes an exhaustive dialysis, which would be expected to remove choline and related compounds.

Cell Culture. C-6 glial cells and neuroblastoma cells, purchased originally from the American Type Culture Collection, have been maintained in this laboratory for the past 5 years. All methods of cell culture have been described (11,12). The basal medium is prepared in this laboratory (11,12) and includes choline, 0.07 mM. Cells for each flask of an experiment were derived from identically-sized inocula and were studied during mid to late log phase.

HMG-CoA Reductase. This enzyme is assayed by a modification of the method of Brown et al (13), as previously described (14). Separation of product, [3-^{14}C]mevalonate, from substrate, [3-^{14}C]HMG-CoA, is accomplished by ion-exchange chromatography (14).

Cholesterol Synthesis. Synthesis of sterols was determined by measuring the incorporation of radioactivity into these products from sodium [2-^{14}C]-acetate, as described (14). The basic procedures for isolation of digitonin-precipitable sterols were modified from those described by Popjak (15).

Protein Synthesis. Synthesis of total cellular protein was measured with L-[4,5-^{3}H]leucine as precursor, as described (16).

Fatty Acid Synthetase. Fatty acid synthetase was determined by the spectrophotometric assay (17).

Protein Content. The amount of protein in cellular extracts was determined by the method of Lowry et al. (18).

Statistical Procedures. Student's t test was utilized to determine statistical significance. The data presented in the Tables and Figure were subjected to this analysis, and all differences discussed in the text are significant at the $P<0.01$ level or better.

RESULTS

Growth of C-6 glial cells in the presence of 10 mM IPE was associated with a marked decrease in specific activity of HMG-CoA reductase (Fig. 1). An approximately 40% fall in enzymatic activity occurred after 12 hr, and an essentially maximal 75% decrease was observed after 16 hr. This marked change in activity was not accompanied by a change in cell growth, estimated by measuring total protein in the culture flask. (Both control and IPE-treated flasks exhibited a 2-fold increase in protein content during the 24 hr.) Use

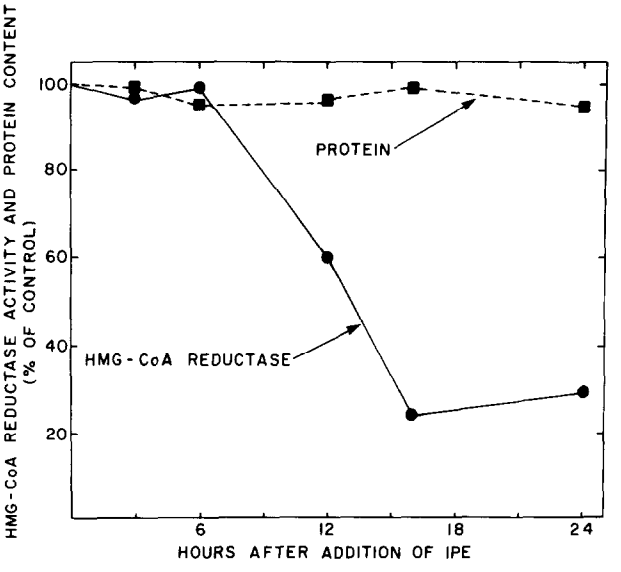

Figure 1. Effect of IPE on HMG-CoA reductase and growth of C-6 glial cells. C-6 glial cells were transferred to a series of 25-cm^2 flasks and grown in 10% fetal calf serum for 48 hr. At that time the medium was changed so that all of the flasks contained 10% lipoprotein-poor serum. IPE was added to one-half of the flasks in a final concentration of 10 mM. At the indicated times thereafter HMG-CoA reductase specific activity (●—●) and protein content per flask (■---■) were determined. Values are means, obtained from separate determinations on each of 3 flasks and expressed as % of control, i.e. flasks with no added IPE, and did not vary more than 5-10%. The 100% values at 24 hr were 58.9 pmol/min/mg protein for HMG-CoA reductase and 9.08 mg for protein. Essentially identical results were obtained in two separate experiments.

of concentrations of IPE of 20 mM or greater caused more marked depressions of reductase activity than did 10 mM, but cell growth was also disturbed at the higher concentrations.

We next asked whether the effect of IPE was a nonspecific toxic effect and whether the inhibition of HMG-CoA reductase caused a disturbance in cholesterol synthesis. Fatty acid synthetase activity, protein synthesis from [^{3}H]leucine, cholesterol synthesis from [^{14}C]acetate and HMG-CoA reductase activity were compared in the glial cells grown in 10 mM IPE for 12 and 24 hr (Table 1). No change in fatty acid synthetase activity was observed at either time period, and a modest decrease in the incorporation of radioactivity

TABLE 1

EFFECT OF IPE ON FATTY ACID SYNTHETASE ACTIVITY, PROTEIN SYNTHESIS, CHOLESTEROL SYNTHESIS AND HMG-CoA REDUCTASE ACTIVITY IN C-6 GLIA*

Hours in 10mM IPE	PERCENTAGE OF CONTROL			
	Fatty Acid Synthetase Specific Activity	Protein Synthesis	Cholesterol Synthesis	HMG-CoA Reductase Specific Activity
12	110	98.2	61.4	57.7
24	105	82.4	38.5	33.1

* C-6 glial cells were grown in the presence of IPE, as described in the legend to Figure 1. Values are means obtained from separate determinations on each of 3 flasks, are expressed as % of control, i.e. flasks with no added IPE, and did not vary more than 5-10%. The 100% values at 24 hr were 5.35 nmol NADPH oxidized/min/mg protein for fatty acid synthetase, 7312 cpm/mg protein for protein synthesis, 7777 cpm/mg protein for cholesterol synthesis, and 53.2 pmol/min/mg protein for HMG-CoA reductase. Essentially identical results were obtained in a separate experiment.

from [^{3}H]leucine into protein was noted only after 24 hr. In contrast, cholesterol synthesis was inhibited by approximately 40% after 12 hr and by nearly 65% after 24 hr. These effects were accompanied by and apparently caused by the comparable changes in activity of HMG-CoA reductase.

Since the formation of the unnatural phospholipid, phosphatidyl-N-isopropylethanolamine, from IPE occurs primarily at the expense of phosphatidyl choline (5,8), we asked whether choline could reverse the effect of IPE on HMG-CoA reductase (Table 2). A slight accentuation of the inhibitory effect of the analog was observed when choline was absent from the medium, but more important, nearly complete reversal of the effect of IPE occurred when the choline concentration was increased, though to a level still approximately 15-fold lower than that of IPE. It should be noted that the inhibitory effect of IPE, obtained in this experiment with medium that was free of lipoprotein-poor serum, is similar to that observed with medium containing 10% lipoprotein-poor serum.

Because regulation of HMG-CoA reductase is an active process in cultured neuronal as well as glial cells, we determined the effect of IPE on the enzyme

TABLE 2

EFFECT OF CHOLINE ON RESPONSE OF HMG-CoA REDUCTASE TO IPE IN C-6 GLIAL CELLS*

Choline Concentration (mM)	PERCENTAGE OF CONTROL	
	HMG-CoA Reductase Specific Activity	Protein Content
0	30.1	96.7
0.07	37.1	93.8
0.70	84.7	94.4
7.0	85.9	94.9

* C-6 glial cells were grown initially in fetal calf serum, as described in the legend to Figure 1. The medium then was changed so that all the flasks contained serum-free medium with the indicated concentrations of choline and one-half contained 10 mM IPE. After 24 hr HMG-CoA reductase specific activity and protein content per flask were determined. Values are means obtained from separate determinations on each of 3 flasks, are expressed as % of control, i.e., flasks with no added IPE, and did not vary more than 5-10%. The 100% values did not vary consistently as a function of the choline concentration and were between 43.4 and 48.4 pmol/min/mg protein for HMG-CoA reductase, and 8.5 and 9.1 mg for protein. Essentially identical results were obtained in a separate experiment.

of neuroblastoma cells. After growth in medium containing 10 and 20 mM IPE for 24 hr, the neuronal cells exhibited reductase activities that were 35 and 19%, respectively, of values in cells grown in the absence of the analog. As with the glial cells, there was no decrease in protein content of the culture flasks in the presence of 10 mM IPE.

To ensure that the inhibitory effect of IPE on HMG-CoA reductase of the glial and neuronal cells was not a direct effect of the free amino alcohol, extracts of both cell types were exposed to 10 and 20 mM IPE during the 60 min preincubation prior to assay of HMG-CoA reductase. No effect on reductase activity of either cell type was observed.

DISCUSSION

The data indicate a marked inhibition of HMG-CoA reductase of cultured glial and neuronal cells after growth in medium containing the unnatural

amino alcohol, IPE. This is the first demonstration of a decrease in activity of HMG-CoA reductase, a microsomal enzyme, by an alteration in membrane lipid composition, previously shown to occur in cultured cells when grown in the presence of IPE (8). The findings suggest that the polar head groups of membrane phospholipids are of major importance in the regulation of HMG-CoA reductase activity. The mechanism of this effect is not clear. The recent demonstration that purified HMG-CoA reductase has phospholipid associated with the protein (19) raises the possibility that the enzyme requires phospholipid for catalytic function in vivo. An alternative explanation for the effect of IPE is that the altered phospholipid composition causes a perturbation of the microsomal membrane and, secondarily, a disturbance of reductase activity. This latter explanation is supported by the recent observation that IPE leads to a decrease in activity of another microsomal enzyme, stearoyl-CoA desaturase, in cultured L-M cells (20). At any rate, the present observations indicate an important relationship between membrane lipid composition and the regulation of HMG-CoA reductase.

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