Vol. 161, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1989 Pages 267-271

STEROL-MEDIATED SUPPRESSION OF HMG-CoA REDUCTASE mRNA LEVELS IN CULTURED CELLS REQUIRES PROTEIN SYNTHESIS

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Received March 15, 1989

SUMMARY The mRNA levels for HMG-CoA reductase were measured in Chinese hamster lung Dede cells or ovary CHO cells by Northern blot analysis. It was observed that 25-hydroxycholesterol decreased the level of reductase mRNA by 40 to 70% in a 6 hour treatment. Inclusion of cycloheximide in the culture prevented the decrease observed with 25-hydroxycholesterol alone. Pretreatment of cells with 25-hydroxycholesterol for 6 hours prior to cycloheximide addition reveals that the protein synthesis inhibitor can return the suppressed levels of reductase mRNA back to control levels. Thus, protein synthesis is required for 25-hydroxycholesterol dependent suppression of HMG-CoA reductase mRNA. © 1989 Academic Press, Inc.

3-Hydroxy-3-methylglutaryl Coenzyme A reductase (HMGR; EC 1.1.1.34) is the rate-controlling enzyme in the biosynthesis of cholesterol from acetyl-CoA in mammalian cells (1). This important enzyme in cellular cholesterol homeostasis has been very actively studied in recent years resulting in isolation of the hamster cDNA and corresponding genomic clone (2,3). The HMGR gene has been shown to be negatively regulated and HMGR activity very tightly controlled based upon cellular sterol demand (3). The sterol-mediated control of reductase activity is exerted at several molecular levels including transcription, translation, protein turnover, and catalytic efficiency of the enzyme (3-9).

Details of these molecular mechanisms responsible for sterol control are now starting to emerge. It has recently been demonstrated that an octanucleotide sequence in the reductase promoter region confers sterol-mediated repression of the HMGR gene (10). This nucleotide sequence is located in footprint 2 of the reductase promoter (10), and by analogy to bacterial systems, represents an operator sequence to which a sterol-sensitive repressor protein might bind. Candidates for this repressor protein might include the oxysterol binding protein whose sterol binding activity has been correlated with HMGR suppression (11). The possible involvement of such novel proteins in sterol-mediated suppression of HMGR

0006-291X/89 $1.50
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prompted us to study the effects of protein synthesis inhibitors upon 25-hydroxycholesterol-mediated suppression of HMGR mRNA levels in cultured cells. Our results show that protein synthesis is required for the active suppression of HMGR mRNA by 25-hydroxycholesterol. Additionally, suppressed levels of HMGR mRNA caused by pre-treatment of cells with 25-hydroxycholesterol can be reversed by inhibiting protein synthesis.

METHODS

Chinese hamster lung Dede cells or ovary CHO cells were cultured in McCoy's 5a medium (modified) supplemented with 1% lipoprotein deficient serum (LPDS), delipidized with Cab-O-sil by the procedure of Weinstein (12). Cells were seeded in 150 mm tissue culture dishes (Costar) at 0.9 x 10^6 to 1.2 x 10^6 cells per dish containing 25 ml of culture medium. Cells were allowed to grow for 3 to 4 days at 37°C in 5% CO_2. On the day of treatment medium was replaced with fresh medium and cells were treated with 25-hydroxycholesterol suspended in 1% bovine serum albumin (BSA) in a small amount of ethanol. Cells were treated such that the final ethanol concentration did not exceed 0.5% (v/v). Treatments were done in triplicate and were continued for the extent of time indicated in the legend to table and figures. Total RNA was isolated by the guanidine isothiocyanate extraction method and CsCl gradient centrifugation (13). Poly (A^+) RNA was isolated by oligo (dT)-cellulose chromatography, quantitated spectrophotometrically and was electrophoresised in 0.66 M formaldehyde-1% agarose gels (13). RNA was transferred to nitrocellulose filters and hybridizations were performed with nick translated (α-^{32}P)dATP-labelled cDNA probes (13). Plasmids were prepared according to the triton-lysozyme method and inserts were isolated following restriction enzyme digestion by electroelutions (13). The cDNA probes used in these studies were as follows: the BamH1 insert of the HMG-CoA reductase plasmid pRed-227 (obtained from ATCC); the Pst I insert of the β-actin cDNA (14) and the Pst I insert from the gluteraldehyde phosphate dehydrogenase pGAPDH plasmid (a kind gift from K. Hastings and C. P. Emerson, Jr. to P. Benfield at DuPont) (15). The latter two probes were used to quantitate mRNA recovery and served as internal standards. mRNA species were localized by audioradioagraphy at -70°C for 3 to 24 hours and quantitation was done by cutting individual lanes from the nitrocellulose filter and counting in a liquid scintillation counter.

RESULTS

Our first experiments were designed to test the hypothesis that cycloheximide could prevent the 25-hydroxycholesterol-dependent suppression of HMG-CoA reductase mRNA. Results are presented in Table I. As can be seen, 25-hydroxycholesterol causes a suppression in the level of HMG-CoA reductase mRNA relative to media controls. The extent of suppression in two experiments varied between 30 and 60%. Relative to media controls, cycloheximide prevented the suppression in the level of HMG-CoA reductase mRNA seen in 25-hydroxycholesterol treated samples by as much as 30%. To determine whether this effect of cycloheximide was due to a blocking of the decrease in the level of HMG-CoA reductase mRNA caused by 25-hydroxy-

TABLE I

Cycloheximide Inhibition of 25-Hydroxycholesterol-dependent Suppression of HMGR mRNA

Treatment	mRNA LEVEL		HMGR GAPDH RATIO	% of CONTROL
	HMGR	GAPDH		
Exp I	CPM/Band			
media control	1135	203	5.59	100
25-hydroxycholesterol	233	135	1.73	31
25-hydroxycholesterol plus cycloheximide	764	212	3.60	64
Exp II				
media control	3514	1748	2.01	100
25-hydroxycholesterol	3010	2391	1.26	63
25-hydroxycholesterol plus cycloheximide	3471	2082	1.67	83

On day zero hamster lung Dede cells were seeded at 0.9 x 106 cells in 150 mm cell culture dishes in 25 nl of McCoy's 5a medium (modified) containing 1% lipoprotein deficient serum (LPDS). On day three when cultures were actively growing, but subconfluent, cells were treated with 25-hydroxycholesterol (1 μg/ml final) or cycloheximide (2 μg/ml final) plus 25-hydroxycholesterol. The cycloheximide was added 10 min prior to the 25-hydroxycholesterol addition. Treated cells were incubated for an additional 6 hrs and then harvested for poly (A^+) RNA by procedures outlined in Methods. Following Northern blot analysis, the amount of HMGR mRNA relative to GAPDH mRNA was determined by scintillation counting of isolated bands detected by autoradiography also as detailed in Methods.

cholesterol or was actually a reversal of the decrease in reductase message caused by 25-hydroxycholesterol, we performed the following experiment. Cells were treated with 25-hydroxycholesterol for 6 hours prior to the addition of cycloheximide. We then monitored the ability of cycloheximide to cause an increase in the level of HMG-CoA reductase mRNA that was initially suppressed by the addition of 25-hydroxycholesterol. The results of this study are shown in Figure 1. As expected, 25-hydroxycholesterol caused a decrease in the level of reductase mRNA to approximately 60% of media control values. Addition of cycloheximide produced a time-dependent increase in reductase mRNA up to the level seen in non-treated cells. The increase occurred over the first 3 hours of the experiment and then plateaued at controlled values. Thus, it appears that cycloheximide can reverse the 25-hydroxycholesterol-dependent suppression of HMG-CoA reductase mRNA, as well as, block the initial decrease produced by the sterol.

DISCUSSION

The results presented in this manuscript are intruiging since they indicate that the level of HMG-CoA reductase mRNA as mediated by

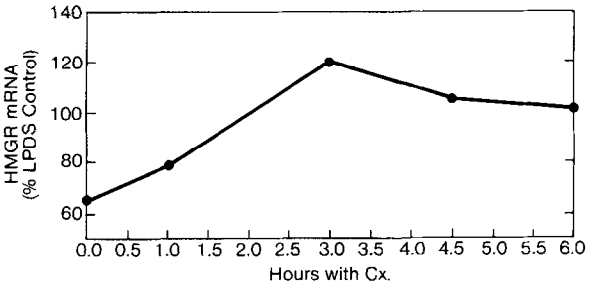

Figure 1. Time-course of Cycloheximide Reversal of 25-Hydroxycholesterol-dependent Suppression of HMG-CoA reductase mRNA. Chinese hamster ovary cells (CHO) were seeded in 25 ml of McCoy's 5a medium (modified) containing 1% lipoprotein deficient serum (LPDS) in 150 mm culture dishes (Costar) at 0.9 x 10^6 cells/dish. Cells were grown for 4 days and then treated with 25-hydroxycholesterol (1 μg/ml) 6 h prior to the addition of cycloheximide (2 μg/ml). Cells were harvested for RNA isolation at the indicated time following cycloheximide addition as described in Methods. HMG-CoA reductase and β-actin mRNA were detected by Northern blot analysis of poly (A^+) RNA and quantitated by liquid scintillation counting. The ratio of HMGR mRNA relative to β-actin mRNA was calculated and the results are expressed relative to LPDS controls.

25-hydroxycholesterol can be blocked or reversed by the inclusion of protein synthesis inhibitors. These results are in keeping with previous findings by Chen et al. (7) and Chang et al. (16) which demonstrated that the decrease in HMG-CoA reductase activity caused by 25-hydroxycholesterol could also be blocked by the inclusion of cycloheximide in 25-hydroxycholesterol treated cultures. Such findings suggest that the action of 25-hydroxycholesterol is dependent upon the presence of proteins which are either induced by the presence of 25-hydroxycholesterol, or are rapidly turning over and whose synthesis is prevented by the inclusion of cycloheximide in treated cultures. Alternatively, inclusion of cycloheximide in these experiments may result in stabilization of reductase mRNA which is reflected in an increase in detectable levels during the course of these investigations. Specific studies are needed to measure RNA turnover to determine whether the increase in reductase mRNA is due to new synthesis of HMG-CoA reductase mRNA or simply a stabilization of preexisting message. Recently it has been shown that another gene under sterol control namely the LDL receptor gene may also require protein synthesis-dependent mechanisms to regulate mRNA expression (17). Cuthbert et al. (17) have demonstrated that negative feedback of LDL receptor mRNA by sterols is lost in early cultures of freshly isolated human lymphocytes, whereas longer culture times bring about a sterol-dependent regulation of receptor gene expression. These same authors (17) have shown that cycloheximide causes a decrease in receptor mRNA in 12 to 24 hour cultures, and indicate that sterols do not further decrease the level of receptor mRNA. One could speculate from these results that the synthesis of either the same or a

family of related proteins may be controlled in concert by the action of sterols to function in the coordinate regulation of HMG-CoA reductase, as well as, LDL receptor gene expression. Support for this latter hypothesis has appeared (18).

ACKNOWLEDGMENT

Special thanks are given to Claire Stecher for typing and preparation of the manuscript.

REFERENCES

1. Brown, M.S. and Goldstein, J.L. (1980) J. Lipid. Res. 21, 505-517.
2. Chin, D.L., Gil, G., Russell, D.W., Liscum, L., Luskey, K.L., Basu, S.K., Okayama, H., Berg, P., Goldstein, J.L. and Brown, M.S. (1984) Nature 308, 613-617.
3. Reynolds, G.A., Basu, S.K., Osborne, T.F., Chin, D.L., Gil, G. Brown, M.S., Goldstein, J.L., and Luskey, K.L. (1984) Cell 38, 275-285.
4. Luskey, K.L., Faust, J.R., Chin, D.J., Brown, M.S. and Goldstein, J.L. (1983) J. Biol. Chem. 258, 8462-8469.
5. Faust, J.R., Luskey, K.L., Chin, D.J., Goldstein, J.L., and Brown, M.S. (1982) Proc. Natl. Acad. Sci. USA 79, 5202-5209.
6. Chin, D.L., Faust, J.R., Goldstein, J.L., Brown, M.S., and Luskey, K.L. (1985) Mol. Cell. Biol. 5, 634-641.
7. Chen, H.W., Richards, B.A., and Kandutsch, A.A. (1981) Biochim. Biophy. Acta. 712, 484-489.
8. Tanaka, R.D., Edwards, P.A., Lan, S.-F., and Fogleman, A.M. (1983) J. Biol. Chem. 258, 13331-13339.
9. Von Gunten, C.F. and Sinensky, N. (1989) Biochim. Biophys. Acta. 1001, 218-224.
10. Osborne, T.F., Gil, F., Goldstein, J.L., and Brown, M.S. (1988) J. Biol. Chem. 263, 3380-3387.
11. Taylor, F.R., Saucier, S.E., Shown, E.P., Parish, E.L., and Kandutsch, A.A. (1984) J. Biol. Chem. 259, 12382-12387.
12. Weinstein, D.B. (1979) Circulation, 59 & 60, Suppl II, 54.
13. Davis, L.G., Dibner, M.D., and Battey, J.E. (1986) Basic Methods in Molecular Biology, Elsevier Science Publishing Co., Inc., New York, N.Y.
14. Katcoff, D., Nudel, V., Zevin-Sonkin, D., Carmon, Y. Shani, J., Lehrach, H., Frischauf, A.M., Yaffe, D. (1988) Proc. Natl. Acad. Sci. USA 77, 960-964.
15. Manning, R.W., Reid, C.M., Lampe, R.A., and Davis, L.G. (1988) Mol. Brain Res. 3, 293-298.
16. Chang, T.Y., Limaneck, J.S. and Chang, C.C.Y. (1981) J. Biol. Chem. 256. 6174-6180.
17. Cuthbert, J.A., Russell, D.W., and Lipsky, P.E. (1989) J. Biol. Chem. 264, 1298-1304.
18. Auwerx, J.H., Chait, A., and Deeb, S.S. (1989) Proc. Natl. Acad. Sci. USA 86, 1133-1137.