Non-sterol regulation of low density lipoprotein receptor gene expression in T cells

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Abstract Non-sterol regulation of low density lipoprotein (LDL) receptor gene expression was examined in a mitogen-responsive human T cell line. Stimulation of the leukemic T cell line Jurkat with the phorbol ester phorbol 12-myristate 13-acetate (PMA) and the calcium ionophore ionomycin rapidly and transiently increased LDL receptor mRNA levels. Inhibition of protein synthesis with cycloheximide (CHX) or puromycin resulted in superinduction of LDL receptor mRNA levels by mitogenic stimulation. The increase in LDL receptor mRNA levels resulted from increased gene transcription rather than stabilization of mRNA half-life. Thus, similar results were obtained when reporter gene expression was assessed in Jurkat cells transfected with LDL receptor promoter constructs and mRNA half-life was not significantly altered by the stimuli. Neither mitogenic induction nor superinduction of LDL receptor mRNA levels in Jurkat cells was prevented by sterol down-regulation of LDL receptor gene expression. The protein synthesis inhibitors CHX and anisomycin, but not puromycin, also directly stimulated LDL receptor mRNA levels, suggesting that these compounds could provide a signal required for LDL receptor gene transcription. Taken together, these data indicate that various non-sterol stimuli, including activation of protein kinase C, increases in intracellular calcium, inhibition of protein synthesis, and signals generated by the protein synthesis inhibitors CHX and anisomycin, induce LDL receptor gene expression. Thus, transcription of the LDL receptor gene is not only regulated by ambient sterols but also by a variety of influences that govern the various primary response or immediate early genes. These stimuli may play an important role in normal regulation of LDL receptor gene expression.—**Makar, R. S. J., P. E. Lipsky, and J. A. Cuthbert.** Non-sterol regulation of low density lipoprotein receptor gene expression in T cells. *J. Lipid Res.* 1994. **35:** 1888–1895.

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Mitogenic activation of T lymphocytes initiates a complex program leading to entry into the cell cycle and cell division. Among the cellular events resulting from mitogenic stimulation are increases in LDL receptor mRNA levels and functional LDL receptor activity (1, 2). Of note, mitogen-induced increases in LDL receptor gene expression in human T cells were not prevented by incubation with sterols (1). These results suggested that LDL receptor expression was regulated by mitogenic signals as well as ambient sterol concentrations, but the precise nature of the regulatory events remained unknown.

Non-sterol regulation of LDL receptor expression does not appear to be unique to T lymphocytes. Thus, LDL receptor gene expression was induced in human dermal fibroblasts by platelet-derived growth factor (3–6). Similarly, the phorbol ester, phorbol 12-myristate 13-acetate (PMA), increased LDL receptor mRNA levels in the human monocytic leukemia cell line, THP-1, and the hepatocarcinoma cell line, HepG2 (7). The protein synthesis inhibitor cycloheximide (CHX) also increased LDL receptor mRNA levels in fibroblasts (4, 8) and THP-1 cells (7). In fibroblasts, platelet-derived growth factor and CHX superinduced LDL receptor gene expression (4) whereas PMA in combination with CHX resulted in superinduction of LDL receptor mRNA levels in THP-1 cells (7). Superinduction by the combination of mitogenic stimulation and CHX is a characteristic of the class of genes termed primary response or immediate early response genes (9), suggesting that LDL receptor gene expression may be induced as part of this response. The precise mechanisms of LDL receptor gene regulation by mitogens and the role of sterols in this process have not been clearly delineated. In the current study, mitogenic activation of LDL receptor gene expression in the human leukemic T cell line Jurkat was examined to address these issues.

MATERIALS AND METHODS

Cell preparation and culture

Jurkat cells were maintained in RPMI-1640 medium (Whittaker Bioproducts, Walkersville, MD) containing

Abbreviations: CAT, chloramphenicol acetyltransferase; CHX, cycloheximide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDL, low density lipoprotein; LPP, lipoprotein-poor plasma; PMA, phorbol 12-myristate 13-acetate; SRE-1, sterol regulatory element-1; SREBP, sterol regulatory element binding protein.

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L-glutamine (0.3 mg/ml), gentamicin (10 μg/ml), penicillin G (200 units/ml), and 10% iron-supplemented bovine calf serum (Sigma Chemical Co., St. Louis, MO). Cells in logarithmic growth were washed extensively and then cultured for 24 h in medium supplemented with either 10% human serum or 1% lipoprotein-poor plasma (LPP, d ≥ 1.230 g/ml) prepared as previously described (10). In some experiments, cultures were also supplemented with either 25-hydroxycholesterol (Steraloids, Inc., Wilton, NH) or human LDL (d 1.020–1.050 g/ml) isolated as detailed (10). Where indicated, PMA (Sigma), ionomycin (Calbiochem, San Diego, CA), CHX, puromycin, anisomycin (all purchased from Sigma), and actinomycin D (Boehringer Mannheim Biochemicals, Indianapolis, IN) were added for varying times before harvesting cells for RNA isolation.

Vector construction

The plasmid pML4 was constructed from pJB20 (11) by replacing a 1.9 kb *Bam*HI-*Sca*I fragment containing the cytomegalovirus promoter/enhancer and the polylinker with a 1.1 kb *Bam*HI-*Sca*I fragment containing the polylinker of the plasmid pSL5 (obtained from Dr. S. W. Lacey, UT Southwestern). The vector was further modified by inserting a *Hind*III-*Bam*HI fragment containing the gene encoding the bacterial enzyme chloramphenicol acetyltransferase (CAT) derived from pRSV-CAT (12) into the polylinker to generate the vector termed pML4. A 6.5 kb *Hind*III fragment containing the LDL receptor promoter was isolated from the plasmid pLDLR-CAT 6500 (13) and inserted into the *Hind*III site of pML4, generating the plasmid pML-LDLr-6500. The orientation of the promoter fragment was confirmed by restriction mapping.

Preparation of transfectants

Jurkat cells were transfected by electroporation, as previously described (14). Briefly, Jurkat cells were washed, resuspended in Dulbecco's phosphate-buffered saline, and mixed with 30 μg of linearized plasmid DNA in a Gene Pulser electroporation cuvette (Bio-Rad Laboratories, Melville, NY). Electroporations were conducted with a Gene Pulser apparatus (Bio-Rad Laboratories) at 0.2 kV and 960 μF. Forty-eight h after electroporation, G418 (Gibco Life Technologies, Grand Island, NY) was added to the transfected cells (final concentration 2 mg/ml for the first week, 1 mg/ml thereafter) and cultures were maintained in G418 until resistant cells grew out, usually after 2–3 weeks.

Measurement of mRNA by nuclease protection

Total RNA was isolated from Jurkat cells solubilized in a solution of guanidinium thiocyanate (Fluka Chemical Corp., Ronkonkoma, NY). The guanidinium thiocyanate cell lysate was either subjected to ultracentrifugation over a cesium chloride cushion, as previously described (15) or acid phenol–chloroform extraction as detailed by Chomczynski and Sacchi (16). Single-stranded cDNA probes for human β-actin and LDL receptor were prepared as previously described (15). For measurement of CAT mRNA, a 272 nt *Bam*HI-*Eco*RI fragment containing 251 nt from the 5' coding region of the CAT gene derived from the plasmid pBLCAT3 (17) was inserted into M13. A 549 nt *Hind*III-*Xba*I fragment from the coding region of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the plasmid pHcGAP (American Type Culture Collection) was inserted into M13 for measurement of GAPDH mRNA.

Single-stranded ^{32}P-labeled probes were synthesized with an oligonucleotide primer in the presence of 0.75 μM [α-^{32}P]dCTP (~3000 Ci/mmol, Amersham Co., Arlington Heights, IL), unlabeled dCTP (0.75 μM for CAT, 1.5 μM for LDL receptor, 270 μM for β-actin and GAPDH probes), 0.1 mM dATP, dTTP, dGTP, and the Klenow fragment of *E. coli* DNA polymerase, as previously reported. The sizes of the probes (including M13 sequences) were: β-actin = 477 nucleotides, LDL receptor = 337 nucleotides, CAT = 319 nucleotides. Total RNA (10 μg) was hybridized with ^{32}P-labeled probes as described. Hybridization was carried out overnight at 37°C (CAT) or 48°C (LDL receptor), the samples were then digested with 5 units of mung bean nuclease (Bethesda Research Laboratories) and analyzed as detailed (15). The ^{32}P cDNA content was quantified by liquid scintillation spectroscopy as detailed (15). In addition, gels were also analyzed with an AMBIS 100 radioanalytical scanner, yielding comparable data. In the experiments described, results are presented as relative LDL receptor mRNA levels, normalized using the amount of β-actin mRNA to correct for procedural losses, and expressed as fold induction compared to control cells calculated by the following formula: β-actin-normalized LDL receptor mRNA levels in experimental cells/β-actin-normalized LDL receptor mRNA levels in control cells × 100.

Protein synthesis assays

Incorporation of [^{3}H]leucine was used to determine the potency of different protein synthesis inhibitors. Jurkat cells were washed in leucine-free minimal essential medium (Gibco) and then cultured in microtiter wells in the presence of anisomycin, cycloheximide, or puromycin (Sigma). [^{3}H]leucine (152 Ci/mmol, Amersham) was added to each well and the microtiter cultures were incubated for 6 h prior to harvest. Cells were harvested onto glass fiber filter paper with a semi-automated microharvesting device (MASH II, Microbiological Associates), and the incorporation of [^{3}H]leucine was determined by liquid scintillation spectroscopy.

RESULTS

When Jurkat cells were cultured in the absence of exogenous cholesterol and stimulated with the T cell mitogens PMA and ionomycin, LDL receptor mRNA levels increased significantly (2.0- ± 0.1-fold, mean ± SEM, n = 3; $P < 0.007$) (**Fig. 1**). The increase in LDL receptor mRNA levels peaked 2 h after mitogenic stimulation, and declined rapidly thereafter (data not shown). A similar transient increase in LDL receptor mRNA levels was observed when Jurkat cells were stimulated with the lectin phytohemagglutinin and PMA (data not shown). This pattern of rapid, transitory induction of gene expression following mitogenic stimulation is observed in the immediate early response genes (9). A second characteristic of this group of genes is that inhibition of protein synthesis with CHX during mitogenic stimulation results in superinduction of gene expression (9). The effect of CHX on LDL receptor mRNA levels was therefore examined.

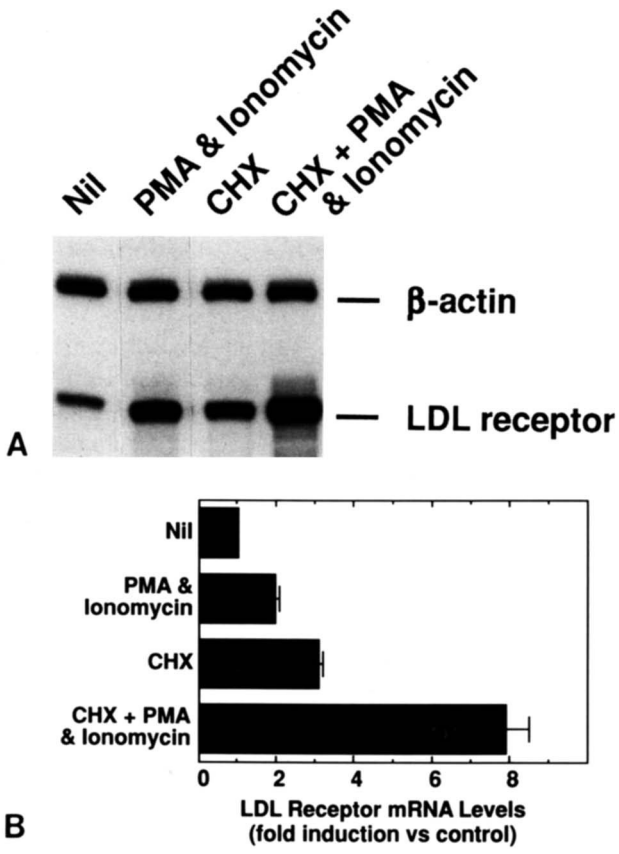

Fig. 1. PMA, ionomycin and CHX, either alone or in combination, increase LDL receptor mRNA levels in the Jurkat T cell line. Jurkat cells were cultured in lipoprotein-deficient medium (1% LPP) for 24 h before stimulation with either PMA (10 ng/ml) and ionomycin (2.5 μM), CHX (10 μg/ml), or the combination. Two h after stimulation, the cells were harvested. Total RNA (10 μg) was isolated and specific mRNAs were quantitated by nuclease protection assay. The relative abundance of LDL receptor mRNA was calculated as described in Materials and Methods. (A) Representative autoradiogram of nuclease protection assay gel. (B) Results are mean ± SEM of 3 experiments.

CHX alone increased LDL receptor mRNA levels (3.1- ± 0.1-fold; $P < 0.003$) (Fig. 1). When CHX was added to Jurkat cells stimulated with PMA and ionomycin, the increase in LDL receptor mRNA levels was significantly greater than that predicted by simple addition (observed = 7.9- ± 0.6-fold, expected = 4.0- ± 0.1-fold, $P < 0.04$). CHX thus superinduced LDL receptor mRNA levels in Jurkat cells stimulated with PMA and ionomycin. Levels of β-actin and GAPDH mRNA were not significantly increased by mitogenic stimulation, CHX, or the combination of both (data not shown). Of importance, these experiments were carried out with cells that were cultured in the absence of exogenous cholesterol and thus should have manifested maximally increased LDL receptor gene expression. These results imply that mitogenic stimulation and/or inhibition of protein synthesis induced LDL receptor gene expression over and above that stimulated by sterol deprivation alone. Furthermore, the findings indicate that the LDL receptor gene responds in a manner similar to that of the immediate early class of genes.

To determine whether increased LDL receptor gene expression induced by mitogenic stimulation and protein synthesis inhibition was independent of sterols, the effect of exogenous sterols on LDL receptor mRNA levels in mitogen-stimulated Jurkat cells was examined. The oxygenated sterol, 25-hydroxycholesterol, suppressed LDL receptor mRNA levels by 64 ± 3% (n = 3) in unstimulated cells and by 54 ± 5% in Jurkat cells stimulated with PMA and ionomycin (data not shown). However, stimulation of oxysterol-treated Jurkat cells with PMA and ionomycin increased LDL receptor mRNA levels by 2.1- ± 0.3-fold (n = 3) above the level in the unstimulated oxysterol-treated control (**Fig. 2**). Furthermore, stimulation of oxysterol-treated cells with PMA, ionomycin, and CHX synergistically increased LDL receptor mRNA levels by 5.1- ± 0.8-fold. In the absence of 25-hydroxycholesterol, PMA, ionomycin, and CHX induced a 5.5- ± 0.6-fold increase in LDL receptor mRNA levels in these experiments (Fig. 2), indicating that mitogenic stimulation of LDL receptor gene expression was comparable regardless of the presence of exogenous sterols. Similar results were obtained in experiments using saturating concentrations of human LDL to suppress LDL receptor mRNA levels (data not shown). These data indicate that mitogenic stimulation of LDL receptor gene expression was not prevented by sterol-mediated down-regulation.

The next experiments examined the separate contributions of PMA and ionomycin to mitogenic induction of LDL receptor mRNA levels. PMA alone did not significantly induce LDL receptor mRNA levels (1.2- ± 0.1-fold, n = 8; $P > 0.1$) (**Fig. 3**). In contrast, ionomycin was able to increase LDL receptor mRNA levels by 1.6- ± 0.2-fold ($P < 0.02$). However, this level of

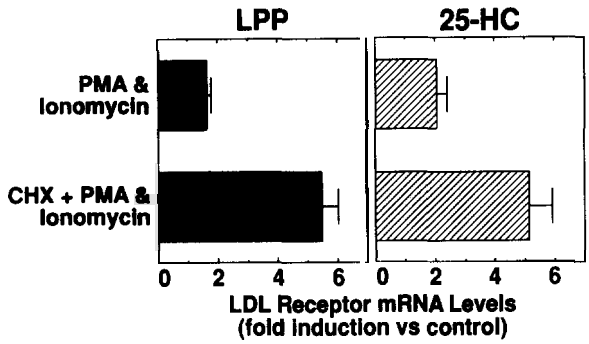

Fig. 2. 25-Hydroxycholesterol (25-HC) does not prevent either induction or superinduction of LDL receptor mRNA levels. Jurkat cells were cultured in medium supplemented with lipoprotein-poor plasma (1% LPP) with or without 25-hydroxycholesterol (25-HC, 0.1 μg/ml) for 24 h before stimulation with PMA and ionomycin, with or without CHX. Two h after stimulation, the cells were harvested. Total RNA (10 μg) was assayed and analyzed. 25-HC suppressed LDL receptor mRNA levels by 64 ± 3% in unstimulated Jurkat cells, by 54 ± 5% in cells stimulated with PMA and ionomycin, and by 67 ± 4% in mitogen-stimulated cells treated with CHX. Results are mean LDL receptor mRNA levels compared with control unstimulated cells cultured in LPP (left panel) or control unstimulated cells cultured with 25-HC (right panel), ± SEM of 3 experiments.

induction was less than that observed with the combination of PMA and ionomycin (2.2- ± 0.4-fold; $P < 0.03$ compared with ionomycin alone). The results indicated that LDL receptor mRNA levels were increased by changes in intracellular calcium concentrations induced by ionomycin whereas activation of protein kinase C alone, by PMA, failed to stimulate increased LDL receptor mRNA levels without another signal.

The nature of the second signal involved in the induction of LDL receptor mRNA levels in PMA-stimulated Jurkat cells was explored in greater detail. Addition of CHX with PMA led to a 6.4- ± 0.4-fold (n = 8) increase in LDL receptor mRNA levels (Fig. 3), similar in magnitude to that induced by the combination of PMA, ionomycin, and CHX (7.7- ± 0.5-fold). In contrast, CHX and ionomycin only resulted in a 3.1- ± 0.3-fold increase in LDL receptor mRNA levels, not significantly different from the increase induced by CHX alone (2.6- ± 0.4-fold; $P > 0.3$). Taken together, these data indicated that PMA induced LDL receptor mRNA levels in Jurkat cells only when another signal was present. A second signal could be provided by either ionomycin or CHX. In contrast, ionomycin and CHX were separately able to increase LDL receptor mRNA levels in Jurkat cells, but were not additive.

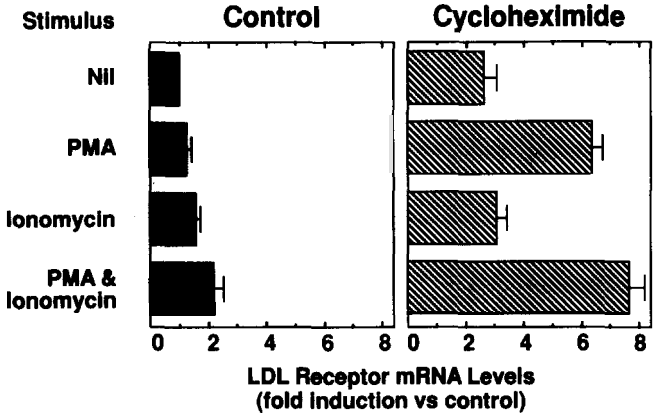

Fig. 3. Ionomycin alone induces LDL receptor mRNA levels, but PMA induces LDL receptor mRNA levels only in the presence of either ionomycin or cycloheximide. Jurkat cells were cultured in lipoprotein-deficient medium (1% LPP) 24 h before stimulation with PMA, ionomycin, cycloheximide, and different combinations of these agents. Two h after stimulation, the cells were harvested. Total RNA (10 μg) was analyzed and specific mRNA species were assayed. Results are mean ± SEM of 8 experiments.

The next series of experiments sought to determine the mechanism by which these various regulatory influences increased LDL receptor mRNA levels. To determine whether increases in LDL receptor mRNA levels resulted from enhanced LDL receptor gene transcription or from increased LDL receptor mRNA stability, the mRNA half-life in stimulated and unstimulated Jurkat cells was compared. Actinomycin D was used to prevent RNA synthesis and the half-life of LDL receptor mRNA was estimated by measuring residual mRNA levels. In preliminary experiments residual mRNA levels were quantitated at 0.5-h intervals and the kinetics were similar to those observed in peripheral blood mononuclear cells (1, 15). Thus, decay was linear for 3 h, after which the amount of residual LDL receptor mRNA remained stable (data not shown). The mean half-life of LDL receptor mRNA in unstimulated cells was 1.5 h (**Table 1**). Stimulation with PMA and ionomycin modestly increased the half-life to 2.0 h. Of importance, CHX had no effect on the half-life of the LDL receptor mRNA in Jurkat cells stimulated with PMA and ionomycin (2.1 h; $P > 0.3$). Therefore, mitogen-mediated increases and CHX-mediated superinduction of LDL receptor mRNA did not result from increased mRNA stability, but rather are more likely to

TABLE 1. CHX does not alter LDL receptor mRNA stability in mitogen-stimulated Jurkat cells

Addition	LDL Receptor mRNA Half-Life
	hours
Control	1.5 ± 0.1
PMA and ionomycin	2.0 ± 0.2
PMA and ionomycin + CHX	2.1 ± 0.2

Jurkat cells were cultured in lipoprotein-deficient medium (1% LPP) for 24 h. Actinomycin D (10 μg/ml) was added 30 min before stimulation with PMA and ionomycin, with or without CHX, as indicated, and cells were harvested 2 h later. Total RNA was isolated and specific RNA species were assayed. Results are mean ± SEM of three separate experiments.

result from activation of LDL receptor gene transcription.

To confirm that the effects of PMA, ionomycin, and CHX upon LDL receptor gene expression resulted from induction of transcription, transfection studies were undertaken using an LDL receptor promoter construct. Unlike nuclear run-on measurements, these studies permitted both direct and quantitative measurements of LDL receptor promoter-regulated gene transcription. Jurkat cells were transfected with a plasmid bearing 6.5 kb of DNA from the LDL receptor promoter region fused to the coding region of the bacterial gene for chloramphenicol acetyltransferase (CAT). The transfectants were stimulated with PMA, ionomycin, and CHX, alone or in combination. Individually, neither PMA nor ionomycin reproducibly induced CAT mRNA levels (**Fig. 4**). However, the combination of PMA and ionomycin induced a 1.9- ± 0.1-fold (n = 3) increase in CAT mRNA levels and CHX alone increased CAT mRNA levels by 2.0- ± 0.3-fold. The combination of CHX and PMA stimulated CAT mRNA levels by 4.1- ± 0.1-fold, similar to that induced by PMA, ionomycin, and CHX (4.8- ± 0.5-fold). In contrast, addition of ionomycin with CHX did not increase CAT mRNA levels above those induced by CHX alone. The increase in CAT mRNA levels with mitogenic stimulation was not prevented by sterol down-regulation (data not shown). These findings indicated that changes in LDL receptor mRNA levels in response to PMA, ionomycin, and CHX resulted from activation of LDL receptor gene transcription. The combination of PMA and ionomycin or CHX induced gene transcription,

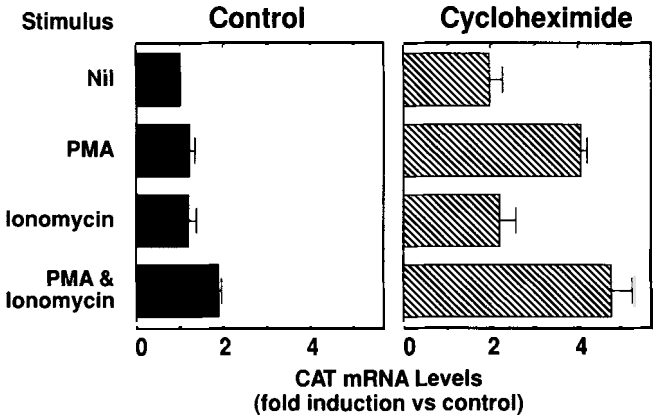

Fig. 4. PMA, ionomycin, and cycloheximide, either alone or in combination, induce CAT mRNA levels in Jurkat cells transfected with the LDL receptor promoter-CAT construct. Jurkat cells transfected with the LDL receptor promoter construct were cultured in lipoprotein-deficient medium (1% LPP) for 24 h before stimulation with PMA, ionomycin, cycloheximide, and different combinations of these agents. Two h after stimulation, the cells were harvested. Total RNA (10 μg) was hybridized with probes for β-actin and CAT, and bands resistant to digestion by mung bean nuclease were identified and assayed. The relative abundance of CAT mRNA was calculated as described. Results are mean ± SEM of 3 experiments (each experiment used a different transfectant).

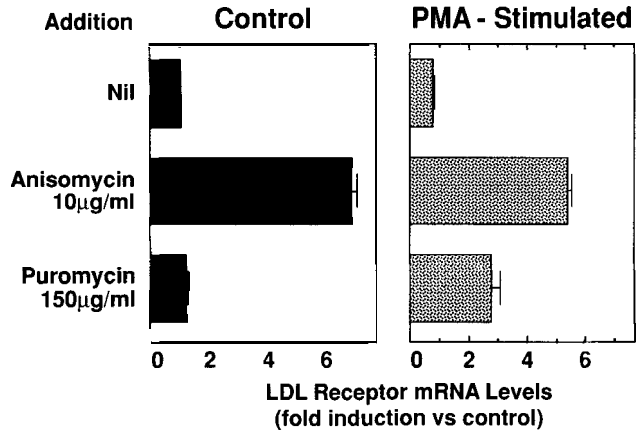

Fig. 5. Concentrations of puromycin that completely block protein synthesis co-stimulate LDL receptor mRNA levels with PMA. Jurkat cells were cultured in lipoprotein-deficient medium (1% LPP) for 24 h before the addition of either anisomycin or puromycin, with or without PMA. Two h after the additions, the cells were harvested. Total RNA (10 μg) was analyzed and specific mRNA species were assayed. Results are mean ± SEM of 3 experiments.

whereas synergistic effects on transcription were noted with the combination of CHX and PMA.

The final experiments explored the mechanism of CHX-dependent induction of LDL receptor gene transcription in more detail. To determine whether this effect resulted entirely from inhibition of protein synthesis, experiments were carried out using two additional protein synthesis inhibitors, anisomycin and puromycin. Initial experiments determined the concentrations of each inhibitor required to block incorporation of [^{3}H]leucine into newly synthesized proteins. Anisomycin was the most potent of the three inhibitors (IC_{50} = 22 ng/ml), followed by CHX (IC_{50} = 68 ng/ml) and then puromycin (IC_{50} = 1 μg/ml). Concentrations of anisomycin (10 μg/ml) and puromycin (150 μg/ml) that blocked protein synthesis comparably to CHX (≥96% inhibition with 10 μg/ml) were selected. Anisomycin (10 μg/ml) alone increased LDL receptor mRNA levels by 6.9- ± 0.2-fold (n = 3) (**Fig. 5**). Addition of anisomycin to PMA-stimulated Jurkat cells did not further increase LDL receptor mRNA levels above those observed with anisomycin alone. In contrast, puromycin (150 μg/ml) alone did not alter LDL receptor mRNA levels. Whereas neither puromycin nor PMA alone was effective at increasing LDL receptor mRNA levels, the combination of puromycin and PMA increased LDL receptor mRNA levels by 2.8- ± 0.3-fold ($P < 0.04$). Concentrations of anisomycin and puromycin that comparably blocked total cellular protein synthesis thus behaved quite differently in their capacity to induce LDL receptor mRNA levels. Inhibition of protein synthesis alone by puromycin was not sufficient to induce LDL receptor mRNA levels, but apparently required an additional regulatory influence, provided by either

anisomycin or CHX or co-stimulation with PMA.

Enhancement of LDL receptor mRNA levels was only observed with concentrations of anisomycin that inhibited protein synthesis to some degree. Thus, 10 ng/ml anisomycin, that did not inhibit protein synthesis in Jurkat cells (data not shown) failed to induce LDL receptor mRNA levels (**Fig. 6**). However, higher concentrations of anisomycin that only partially blocked protein synthesis were able to induce LDL receptor mRNA levels. Thus, anisomycin (25 ng/ml and 50 ng/ml) increased LDL receptor mRNA levels by 1.9- and 2.7-fold, respectively. PMA stimulation did not further increase LDL receptor mRNA levels induced by these low concentrations of anisomycin.

DISCUSSION

The current studies demonstrate that stimulation of the human leukemic T cell line Jurkat with the combination of the calcium ionophore ionomycin and the phorbol ester PMA induced LDL receptor mRNA levels. Raising intracellular calcium concentrations alone, with ionomycin, increased LDL receptor mRNA levels. Activation of protein kinase C with PMA, however, only increased LDL receptor mRNA levels when an additional regulatory influence was present. Co-stimulation with ionomycin or the protein synthesis inhibitors CHX and puromycin superinduced LDL receptor mRNA levels in PMA-stimulated cells. This synergistic increase in LDL receptor mRNA levels was the result of increased gene transcription and not merely secondary to stabilization of mRNA as mRNA turnover was unaltered. Moreover, when CAT mRNA levels were measured in Jurkat cells transfected with LDL receptor promoter-CAT constructs, the effects of mitogenic stimulation and CHX on CAT gene expression were similar to those on the native LDL receptor gene. Of note, induction and superinduction of LDL receptor and CAT mRNA levels were not prevented by sterol down-regulation of gene transcription. Like PMA, some but not all protein synthesis inhibitors also directly increased transcription of the LDL receptor gene, suggesting that they might provide a signal necessary for induction of LDL receptor gene expression, beyond their capacity to suppress protein synthesis. As Jurkat cells provide a reliable model system to study mitogen-stimulated gene transcription in T cells, these studies are consistent with the conclusion that LDL receptor gene expression in human T cells is not dependent merely on the ambient level of regulatory sterols but is also modulated by mitogenic activation and other regulatory signals.

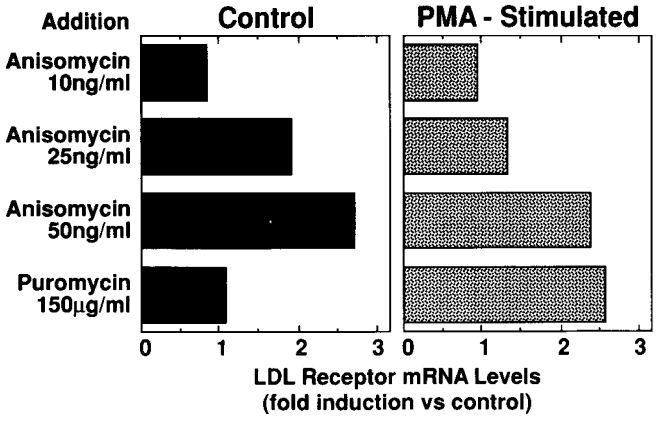

Fig. 6. Low concentrations of anisomycin induce LDL receptor mRNA levels. Jurkat cells were cultured in lipoprotein-deficient medium (1% LPP) for 24 h before the addition of either anisomycin or puromycin, with or without PMA. Two h after the additions, the cells were harvested. Total RNA (10 μg) was analyzed and specific mRNA species were assayed.

Primary response genes, also called immediate early response genes, are transcribed after stimulation of cells with mitogens, growth factors, and tumor promoters (9). The characteristic pattern of response includes rapid and transient increases in mRNA levels and superinduction when ongoing protein synthesis is inhibited during stimulation (9). A similar pattern is observed in various cells activated by many different stimuli, such as serum-stimulated murine fibroblasts (18) and mitogen-induced human T cells (19). Mitogen-inducible primary response genes include transcription factors, cytoskeletal and extracellular matrix proteins, transmembrane proteins, and cytokines (9). As the expression of the LDL receptor gene is rapidly and transiently increased by mitogenic stimulation in Jurkat T cells and mRNA levels are superinduced by CHX, the LDL receptor gene can be considered a primary response or immediate early gene. The regulatory region of the LDL receptor gene may contain *cis*-acting element(s) in common with other, similarly responsive genes. In contrast, neither β-actin nor GAPDH mRNA levels were significantly induced by any of the stimuli, demonstrating that the increase in LDL receptor gene expression was not merely part of a nonspecific or general increase in gene transcription.

The calcium ionophore ionomycin induced LDL receptor mRNA levels in Jurkat cells, indicating a role for intracellular calcium in the regulation of LDL receptor mRNA levels. Similarly, A23187, a less specific calcium ionophore than ionomycin, induced transcription of the LDL receptor gene in human monocytic THP-1 cells and increased LDL receptor mRNA levels in human hepatocarcinoma HepG2 cells (20). In Jurkat cells transfected with LDL receptor promoter-CAT constructs, ionomycin was found to be insufficient to induce transcription. The data suggest that the increase in native LDL receptor mRNA levels resulting from stimulation of Jurkat cells with ionomycin alone cannot be accounted for by an effect on transcription and therefore regulation of this gene may differ in various cells.

PMA increased LDL receptor mRNA levels in Jurkat cells only when other regulatory influences were present.

As with THP-1 cells and HepG2 cells (20), co-stimulation with an agent that increased intracellular calcium induced transcription of the LDL receptor gene in Jurkat cells. In contrast, PMA alone increased LDL receptor mRNA levels in THP-1 and HepG2 cells (7), as well as in fibroblasts and vascular smooth muscle cells (6). The requirement for an additional regulatory influence to increase LDL receptor mRNA levels in PMA-stimulated Jurkat cells may be explained by the incomplete mitogenic signal delivered by PMA activation of protein kinase C in T cells (21, 22). Thus, in both peripheral blood T cells and Jurkat cells, protein kinase C activation alone is not a sufficient signal for immunologically relevant stimulation as measured by interleukin-2 production. Provision of a second signal, by ionomycin for example, is also necessary (21, 22). Alternatively, Jurkat cells may contain an intrinsically active, or PMA-activated, negative repressor protein that prevents PMA stimulation from increasing LDL receptor gene transcription. The presence or activity of this regulator could be partially mitigated by co-stimulation.

The existence of a negative repressor protein that inhibits transcription of the LDL receptor gene in PMA-activated Jurkat cells is supported by the observation that protein synthesis inhibitors induced LDL receptor mRNA levels in PMA-stimulated cells. Of note, puromycin alone did not increase LDL receptor mRNA levels in Jurkat cells, suggesting that inhibition of protein synthesis in Jurkat cells was insufficient to induce transcription of the LDL receptor gene. In contrast to the present findings in Jurkat cells, puromycin alone induced LDL receptor mRNA levels as effectively as CHX in fibroblasts (3–5). These data suggest that the negative regulator or the LDL receptor gene may be constitutively active in fibroblasts, whereas in Jurkat cells activation of the repressor or the LDL receptor gene by PMA is necessary.

CHX and anisomycin appeared to provide regulatory influences beyond those of puromycin, inducing LDL receptor mRNA levels in the absence of other signals. These results imply that the mechanism whereby anisomycin and CHX increased LDL receptor mRNA levels was not solely the result of protein synthesis inhibition. Inhibition of protein synthesis, however, appears to be essential for this effect as concentrations of anisomycin that increased LDL receptor mRNA levels also inhibited protein synthesis whereas concentrations that did not increase mRNA levels also failed to inhibit protein synthesis. However, an additional influence of these compounds is implied by the finding that they directly induced LDL receptor gene transcription whereas puromycin did not, despite comparable degrees of inhibition of protein synthesis. Additional effects of anisomycin have been reported. Thus, anisomycin was found to induce phosphorylation of histone H3 and lead to increased expression of c-*fos* and c-*jun* in murine fibroblasts at concentrations that did not inhibit protein synthesis (23). The ability of anisomycin (and CHX) to induce LDL receptor mRNA levels in Jurkat cells may reflect a similar intrinsic signaling activity, the nature of which is currently unknown. Of note, direct induction of LDL receptor gene transcription is not uniformly observed in all cell types. Thus, CHX did not increase LDL receptor mRNA levels at all in freshly isolated T lymphocytes (1, 15) and in HepG2 cells the increase was less than one-tenth of that observed with PMA (7). Differences in signaling pathways after mitogenic activation or disparate patterns of intrinsically active transcriptional regulators may account for the diversity of responses.

Regulation of LDL receptor gene expression by sterol-mediated repression of transcription requires the DNA sequence termed sterol regulatory element-1 (SRE-1) (24). Induction of LDL receptor gene expression by PMA, ionomycin, and protein synthesis inhibitors might be explained by changes in the activity of protein(s) interacting with this element. Alternatively, mitogens and other factors may alter cellular sterol balance and thereby regulate LDL receptor gene expression. This latter explanation appears less likely as mitogen-induced induction of LDL receptor gene transcription is observed regardless of ambient and cellular sterols. Although absolute levels of LDL receptor mRNA were reduced as predicted from sterol-mediated regulation, exogenous sterols did not reduce the fold induction of LDL receptor mRNA levels stimulated by either PMA and ionomycin or PMA and CHX. These data suggest that PMA, ionomycin, and the different protein synthesis inhibitors induce LDL receptor gene expression by acting directly upon the LDL receptor promoter and not by altering the sterol balance of the cell. Support for this conclusion derives from preliminary experiments in which mutation of the SRE-1 in a reporter construct transfected into Jurkat cells totally prevented regulation but did not block mitogen-mediated induction (data not shown).

Two potential models can explain these observations. In the first, non-sterol agents such as mitogens or protein synthesis inhibitors interfere with sterol-mediated suppression of LDL receptor gene transcription by altering the activity of either of the two transcription factors thought to be involved in suppression of the gene, Sp1 or the sterol regulatory element binding protein (SREBP). Thus, either Sp1, SREBP, or both transcription factors might be reversibly phosphorylated after activation of protein kinase C, thereby increasing their transcriptional activity. Sp1 is known to be phosphorylated, although the biological significance of such a modification is uncertain (25). In the second model, mitogens or protein synthesis inhibitors activate gene expression through discrete transcriptional response elements that are separate from the SRE-1 and its flanking Sp1 binding sites. The observations that LDL receptor gene expression is induced by

ionophores, phorbol esters, protein synthesis inhibitors, growth factors, and cytokines in Jurkat and other cell lines (3–7, 20, 26–28) are consistent with the conclusion that the LDL receptor promoter contains additional response elements.

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