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Dexamethasone modulates lipoprotein metabolism in cultured human monocyte-derived macrophages. Stimulation of scavenger receptor activity.

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Research Article

Human monocyte-derived macrophages (HMM) play a key role in the formation of atherosclerotic plaques by accumulating cholesteryl ester (CE) to become foam cells. HMM have receptors for native low density lipoprotein (LDL) and acetylated-LDL (ALDL), and uptake of ALDL can promote substantial cellular CE accumulation. Furthermore, macrophages specifically and saturably bind glucocorticoids, which in turn modulate numerous macrophage functions. Preincubating HMM in dexamethasone-inhibited LDL degradation (230 +/- 12 vs. 515 +/- 21 ng/mg cell protein X 18 h, P less than 0.001) but stimulated ALDL degradation (5.3 +/- 0.5 vs. 2.5 +/- 0.3 micrograms/mg X 18 h, P less than 0.01). These effects were time- and dose-dependent, occurring maximally by 24 h and with 2.5 X 10(-8) M dexamethasone. Dexamethasone increased the maximum velocity for ALDL degradation (16.2 vs. 12.0 micrograms/mg X 18 h, P less than 0.01) without changing the apparent Michaelis constant. Progesterone, 11 alpha-epicortisol, and 17 alpha-OH progesterone (a competitive antagonist of the glucocorticoid receptor) had no effect on HMM ALDL degradation, but 17 alpha-OH progesterone abolished the stimulatory action of dexamethasone. In he presence of ALDL, incorporation of [14C]oleic acid into CE was enhanced over fourfold by dexamethasone (4015 +/- 586 vs. 943 +/- 91 cpm/mg X 2 h, P less than 0.01), and HMM incubated with ALDL and dexamethasone accumulated more free cholesterol (34.6 +/- […]

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Dexamethasone Modulates Lipoprotein Metabolism in Cultured Human Monocyte-derived Macrophages

Stimulation of Scavenger Receptor Activity

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Abstract

Human monocyte-derived macrophages (HMM) play a key role in the formation of atherosclerotic plaques by accumulating cholesteryl ester (CE) to become foam cells. HMM have receptors for native low density lipoprotein (LDL) and acetylated-LDL (ALDL), and uptake of ALDL can promote substantial cellular CE accumulation. Furthermore, macrophages specifically and saturably bind glucocorticoids, which in turn modulate numerous macrophage functions. Preincubating HMM in dexamethasone-inhibited LDL degradation (230±12 vs. 515±21 ng/mg cell protein · 18 h, $P < 0.001$) but stimulated ALDL degradation (5.3±0.5 vs. 2.5±0.3 μg/mg · 18 h, $P < 0.01$). These effects were time- and dose-dependent, occurring maximally by 24 h and with 2.5×10^{-8} M dexamethasone. Dexamethasone increased the maximum velocity for ALDL degradation (16.2 vs. 12.0 μg/mg · 18 h, $P < 0.01$) without changing the apparent Michaelis constant. Progesterone, 11α-epicortisol, and 17α-OH progesterone (a competitive antagonist of the glucocorticoid receptor) had no effect on HMM ALDL degradation, but 17α-OH progesterone abolished the stimulatory action of dexamethasone. In the presence of ALDL, incorporation of [^{14}C]oleic acid into CE was enhanced over fourfold by dexamethasone (4015±586 vs. 943±91 cpm/mg · 2 h, $P < 0.01$), and HMM incubated with ALDL and dexamethasone accumulated more free cholesterol (34.6±1.9 vs. 26.2±0.8 μg/mg, $P < 0.02$) and CE (32.8±2.3 vs. 14.8±0.8 μg/mg, $P < 0.002$) than did macrophages without dexamethasone. In cultured human umbilical vein endothelial cells, dexamethasone did not change ALDL degradation, but reduced LDL degradation by 30% ($P < 0.001$). In summary, dexamethasone inhibits LDL receptor activity by both macrophages and endothelial cells, but stimulates ALDL receptor activity only in macrophages. These observations provide evidence for the regulation of macrophage endocytic receptors by glucocorticoid hormones.

Introduction

Human monocyte-derived macrophages (HMM)[1] express the classic low density lipoprotein (LDL) receptor first described in human fibroblasts and subsequently found in most mammalian cells (1, 2). In addition, macrophages express a functionally and structurally distinct binding site for certain classes of chemically modified lipoproteins, including acetylated-LDL (ALDL) (3–5), malondialdehyde-LDL generated by aggregating platelets (6, 7), and LDL preincubated with cultured endothelial (8, 9) or arterial smooth muscle cells (9). This binding site, termed the scavenger receptor, can mediate substantial intracellular cholesteryl ester (CE) accumulation in the presence of modified lipoproteins (4). In contrast, the LDL receptor is effectively regulated to prevent excess cellular cholesterol accumulation (1). Such observations have led to the suggestion that in vivo modifications of native LDL may be responsible for the accumulation of CE in macrophages and the formation of macrophage-derived foam cells (4, 6, 8).

The suggestion that monocyte-derived macrophages are a major cellular progenitor of the atherosclerotic plaque foam cell (10–12) has led to intense interest in factors that regulate lipoprotein receptor activity in these cells. In HMM, LDL receptor activity is suppressed by increased cell cholesterol content, increased plating density, or lymphocyte-conditioned medium, while preincubation in lipoprotein-deficient serum promotes LDL degradation (1, 7, 13, 14). Macrophage scavenger receptor activity is inhibited by lymphocyte-conditioned medium (14) and bacterial endotoxin (15), but is stimulated by poorly characterized factors in serum, increased plating density, and macrophage-conditioned medium (13, 16). Insulin and platelet-derived growth factor, which enhance native LDL catabolism by fibroblasts and aortic smooth muscle cells (17, 18), have been shown to be without effect on the activity of either receptor in HMM (16).

While peptide hormones and growth factors have shown no regulatory activity for macrophage lipoprotein metabolism, the role of glucocorticoid hormones has not been investigated. A macrophage receptor for glucocorticoid hormones has been well characterized with a dissociation constant for dexamethasone between 10^{-9} and 10^{-8} M (19). Furthermore, glucocorticoid hormones have already been shown to regulate a number of macrophage immune functions including the expression of certain surface receptors, and neutral proteinase secretion (20–22). In this report we describe the results of studies designed to investigate the effects of dexamethasone on HMM lipoprotein metabolism and to address questions relating to the mechanisms of these effects.

Methods

Materials. Sodium [^{125}I]iodide (carrier free in 0.1 M NaOH) was obtained from Amersham Corp. (Arlington Heights, IL); [^{14}C]oleic acid from New England Nuclear (Boston, MA); Plasmagel from Roger Bellon Laboratories (Neuilly, France); Ficoll-Hypaque from Pharmacia Fine Chemicals (Piscataway, NJ); Dulbecco's modified Eagle's medium (DME) from Gibco (Grand Island, NY); bovine serum albumin (BSA) from Pentax Chemicals (Elkhart, IN); dexamethasone, progesterone, and 17α-OH

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1. *Abbreviations used in this paper:* ALDL, acetylated low density lipoprotein; CE, cholesteryl ester; DME, Dulbecco's modified Eagle's medium; HMM, human monocyte-derived macrophages.

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progesterone from Sigma Chemical Co. (St. Louis, MO); and 11α-epicortisol from Research Plus Laboratories (Denville, NJ).

Cell isolation and culture. Blood was obtained from healthy, normolipemic donors after a 12–14 h fast, and monocytes were prepared by elutriation as previously described in detail (23). Monocyte fractions were confirmed to be >90% pure by cell volume analysis on a Channelyzer (Coulter Electronics Inc., Hialeah, FL) and by stain morphology (Diff-Quik, Scientific Products, McGraw Park, IL) (16, 23).

Cells were plated at 0.5×10^{-6} cells per 16-mm well in 20% autologous serum in DME and incubated at 37°C in 5% CO_2. The medium was changed twice weekly. Experiments were begun after 17–22 d when the macrophages were washed twice with cold DME and placed in 1 ml of DME-0.2% BSA, plus or minus steroid hormones dissolved in ethanol. Steroid-treated and control wells contained a final concentration of 0.1% ethanol (vol/vol). Unless otherwise stated, HMM were incubated for 48 h, at which time 10 μg/ml of ^{125}I-lipoprotein was added to the cells. Lipoprotein degradation products were measured in the media following an additional 18-h incubation (see below).

Human umbilical vein endothelial cells were kindly provided by F. Booyse (Michael Reese Hospital) and were isolated and cultured by previously described methods (24).

Lipoprotein preparation. Human LDL (d = 1.019–1.063 g/ml) was prepared by sequential ultracentrifugation from normal donors' fresh plasma as previously described (23) and acetylated by the method of Basu et al. (25). Both native and acetylated-LDL were iodinated with ^{125}I by the iodine monochloride method as modified for lipoproteins (26) to yield specific activities of 80–210 cpm/ng protein. The ^{125}I-lipoprotein was sterilized by passage through a filter (Millipore Corp., Bedford, MA) and used within 3 wk of preparation.

Assays. Lipoprotein and cell protein content were measured by the method of Lowry et al. using BSA as standard (27). The lipoprotein degradation assay was used as an integrated measure of receptor activity as previously reported (4, 17, 18, 28). In brief, after incubation of 10 μg/ml of ^{125}I-lipoprotein with cells, the media was removed and trichloroacetic acid-soluble counts were treated with H_2O_2 and KI and extracted with $CHCl_3$. Aqueous soluble radioactivity was measured and, using lipoprotein-specific activity, was corrected to the mass of lipoprotein protein degraded. Non–cell-associated degradation was subtracted from total degradation in all studies. At the end of most experiments the cells were washed twice with cold phosphate-buffered saline and dissolved in 0.1 N NaOH for protein determination. Gas-liquid chromatography was used to measure cellular free and esterified cholesterol (esterified cholesterol equals total minus free cholesterol) after extraction in hexane-isopropanol (29). ^{14}C-labeled cholesteryl ester was quantified by separation of extracted lipid samples on silica gel G plastic thin-layer chromatography plates developed with petroleum ether/ethyl ether/acetic acid (75:25:1). An internal standard (1,2-[^{3}H]cholesterol in chloroform) was added during extraction and the results corrected for recovery. Data are expressed as mean±SEM for triplicate or quadruplicate determinations with statistical significance analyzed by Student's t test (30).

Results

Preincubation of macrophages with 2.5×10^{-9}–2.5×10^{-7} M dexamethasone significantly inhibited ^{125}I-LDL degradation and stimulated ^{125}I-ALDL degradation as shown in Fig. 1. Effects were seen at 2.5×10^{-9} M dexamethasone, and were maximal at 2.5×10^{-8} M. At this concentration dexamethasone reduced LDL degradation by 55% ($P < 0.001$) but stimulated ALDL degradation by 108% ($P < 0.01$). In other experiments (see below), dexamethasone stimulated ALDL degradation by 32–65%. For further studies, cells were routinely treated with 2.5×10^{-8} M dexamethasone. Cell protein content per well usually varied by less than 12% within each experiment. Values for nonspecific lipoprotein degradation in these 2–3-wk-old HMM (as measured by the degradation that occurs in the presence of a 20-fold excess of unlabeled ligand) averaged 10% for ALDL and 21% for LDL.

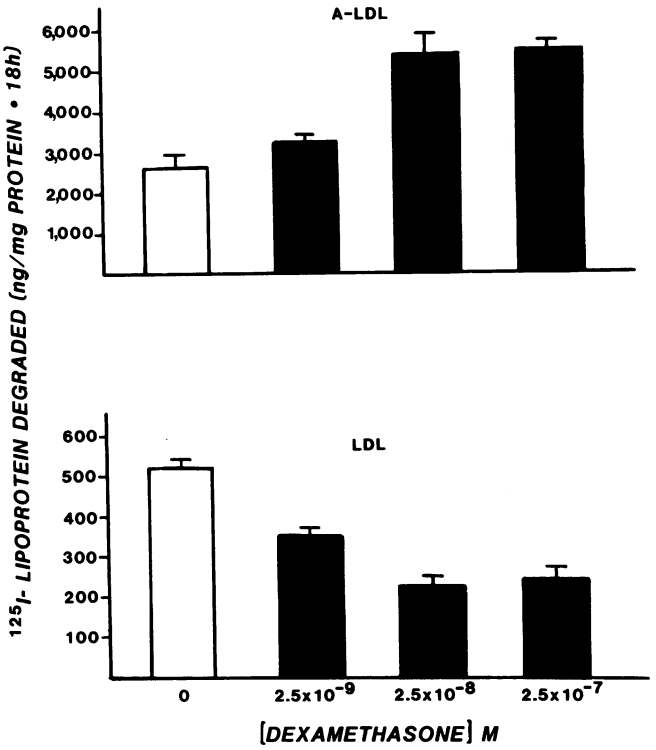
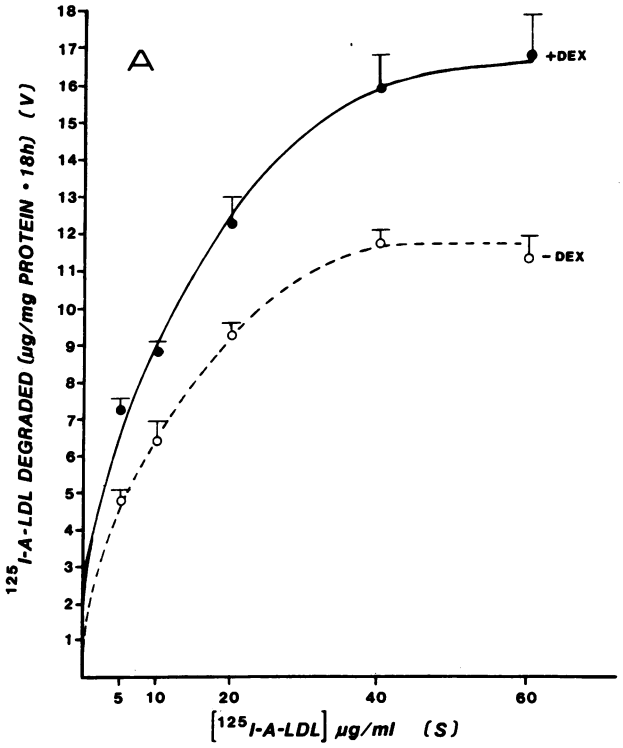


Figure 1. Dose response of dexamethasone effects on ^{125}I-lipoprotein degradation by human macrophages. Monocytes were obtained from healthy, fasting volunteers and cultured in DME-20% autologous serum at 0.5×10^{-6} cells/well. After 21 d, macrophages were washed with DME and placed in 1 ml of 0.2% BSA in DME, ±dexamethasone for 48 h. ^{125}I-ALDL (*upper*) or ^{125}I-LDL (*lower*) was added (10 μg/ml) and degradation products measured in the incubation media after 18 h. Values are expressed as mean±SEM of triplicate or quadruplicate dishes.

The effect of dexamethasone at 2.5×10^{-8} M on ALDL degradation as a function of ligand concentration is shown in Fig. 2 *A*. Saturation of ALDL degradation occurred at ~40 μg/ml, and dexamethasone enhanced ALDL catabolism at all ligand concentrations tested. Subtraction of nonspecific lipoprotein degradation and linearization of this data (31) yielded the results shown in Fig. 2 *B*. Dexamethasone increased the maximum velocity (V_{max}) for ALDL degradation from 12 to 16.2 μg/mg protein · 18 h ($P < 0.01$) without changing the Michaelis constant (K_m) (7.9 vs. 7.4 μg/ml, P = NS). These observations suggest that dexamethasone enhanced ALDL degradation by increasing the number of available ALDL binding sites without changing their affinity.

To further characterize the effects of dexamethasone on modified low density lipoprotein metabolism, macrophages were incubated with ALDL plus or minus dexamethasone, and their cholesterol esterification rates and free and esterified cholesterol mass were determined. Results are given in Table I. The incorporation of [^{14}C]oleic acid into CE in the presence of ALDL was enhanced over fourfold ($P < 0.01$) by dexamethasone. In addition, HMM incubated with dexamethasone and ALDL accumulated more free cholesterol ($P < 0.02$) and cholesteryl ester (CE) ($P < 0.002$) than cells incubated with ALDL alone.

To confirm that the effects of dexamethasone on HMM li-



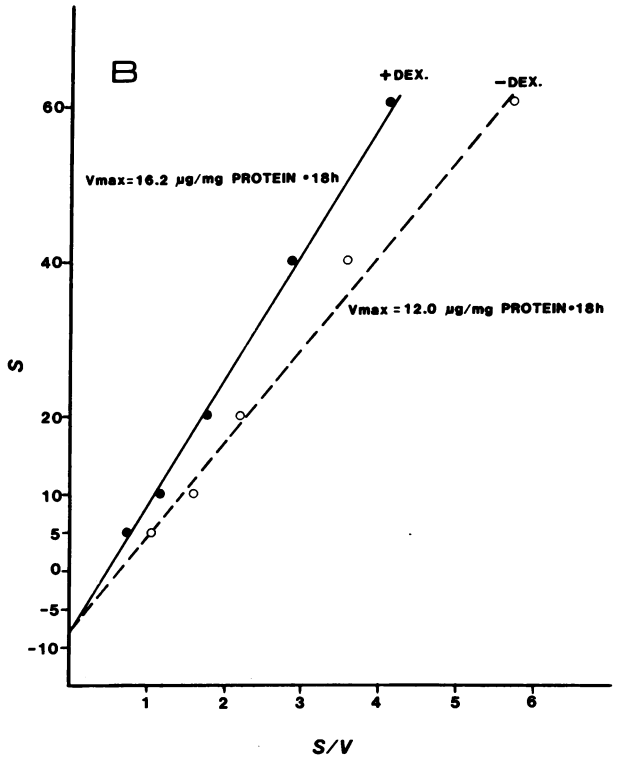


Figure 2. Kinetics of human macrophage ^{125}I-ALDL degradation: effect of dexamethasone. Cells were obtained and treated as in Fig. 1. ^{125}I-ALDL was added in increasing amounts as shown, and degradation products measured. ○, control cells, no dexamethasone. ●, cells with 2.5×10^{-8} M dexamethasone. Mean±SEM. (*A*) ^{125}I-ALDL saturation curves. (*B*) Linearization plot of the data in *A* (31). Nonspecific ALDL degradation has been subtracted. The slope of the line = apparent V_{max}, and the *y*-intercept = −apparent K_m. R values for each line >0.99.

Table I. Human Macrophage Cholesterol Esterification and Cholesterol Content in the Presence of ALDL: Stimulation by Dexamethasone

	Free cholesterol	CE	[^{14}C]oleic acid CE
	μg/mg cell protein		*cpm/mg cell protein*
Control	26.2±0.8	14.8±0.4	943±91
Dexamethasone (2.5×10^{-8} M)	34.6±1.9*	32.8±2.3‡	4,015±586§

Cells were isolated and cultured as in Fig. 1. ALDL (50 μg/ml)±dexamethasone were added to wells used for determination of free cholesterol and CE mass for 66 h before cell extraction. Wells used for the measurement of [^{14}C]oleic acid incorporation into CE were incubated with or without dexamethasone for 63 h. ALDL (25 μg/ml) was then added 3 h before the addition of [^{14}C]oleic acid (final concentration: 0.2 μCi/ml). Cells were harvested after an additional 2 h.

* $P < 0.02$ vs. control cells.
‡ $P < 0.002$ vs. control cells.
§ $P < 0.01$ vs. control cells.

poprotein metabolism were mediated via glucocorticoid receptor pathways, HMM were incubated in 2.5×10^{-8} M dexamethasone, progesterone, or 11α-epicortisol, a non–receptor-binding glucocorticoid analogue (19, 22). As shown in Fig. 3, only dexamethasone increased ALDL degradation. As a further confirmation, cells were incubated in dexamethasone alone or dexamethasone plus a 100-fold excess of 17α-OH progesterone, a competitive antagonist for the glucocorticoid receptor without intrinsic glucocorticoid activity (32). As shown in Fig. 4, 17α-OH progesterone at 2.5×10^{-6} M had no effect on ALDL degradation. However, it completely abolished the dexamethasone-mediated stimulation of ALDL receptor activity, as would be expected if dexamethasone's actions occurred through glucocorticoid-receptor pathways.

The preincubations for the above experiments were performed for 48 h in serum-free medium in order to eliminate the possibility that dexamethasone's effects might be obscured by

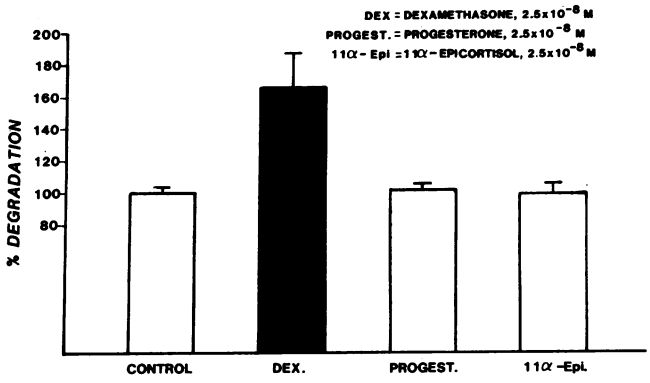


Figure 3. Effects of various steroids on human macrophage ^{125}I-ALDL degradation. Cells were obtained and treated as in Fig. 1. 2.5×10^{-8} M dexamethasone, progesterone, or 11α-epicortisol were added to cells for 48 h, then ^{125}I-ALDL degradation was determined. Mean±SEM. Values for control cell ALDL degradation = 10.5±0.4 μg/mg protein · 18 h.

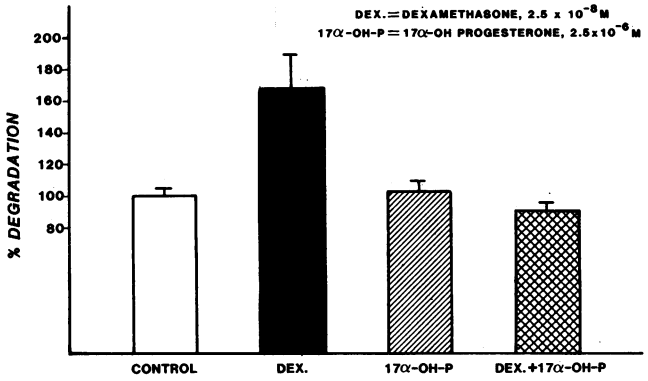


Figure 4. Antagonism of dexamethasone stimulation of human macrophage ^{125}I-ALDL degradation by 17α-OH progesterone. Cells were obtained and treated as in Fig. 1, and steroids were added as shown. Mean±SEM. Values for control cell ALDL degradation = 19.5±0.6 μg/mg protein · 18 h.

the small amount of glucocorticoid hormones present in 20% autologous serum. Placing HMM in serum-free medium for this length of time has, however, been reported to depress scavenger receptor activity (13). Fig. 5 shows that while ALDL degradation was lower in cells preincubated under serum-free conditions, dexamethasone stimulation of scavenger receptor activity was similar whether preincubations were done in serum-containing or serum-free media. In additional experiments (not shown) we determined that the stimulatory effect of dexamethasone was maximal by 24 h.

Aside from macrophages, only endothelial cells are known to express the modified LDL binding site (33). In order to determine whether or not dexamethasone regulation of the scavenger receptor was unique to macrophages, we incubated confluent human umbilical vein endothelial cells with or without dexamethasone and measured native and acetylated-LDL receptor activity. As shown in Fig. 6, dexamethasone decreased native LDL degradation by 31% ($P < 0.001$) but had no effect on ALDL degradation by endothelial cells.

Discussion

Macrophages are established target cells for glucocorticoid hormones and possess a high affinity glucocorticoid binding site with a dissociation constant of 10^{-9}–10^{-8} M for dexamethasone (19). We now report that dexamethasone modulates human monocyte-derived macrophage lipoprotein receptor activity in a dose-dependent manner with maximal effects at 2.5×10^{-8} M and by 24 h. While dexamethasone inhibits native LDL degradation by HMM, it simultaneously promotes scavenger receptor activity via glucocorticoid receptor pathways, as indicated

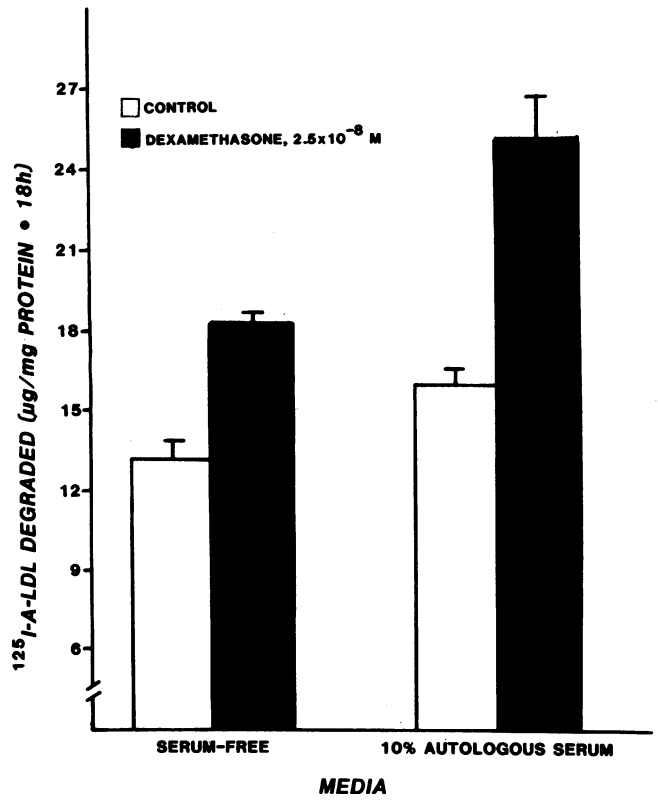


Figure 5. Dexamethasone stimulation of human macrophage ^{125}I-ALDL degradation in serum-free or serum-containing medium. Cells were obtained and treated as in Fig. 1, except for the serum-containing wells, which were washed and incubated in DME with 10% autologous serum instead of DME-0.2% BSA. Mean±SEM.

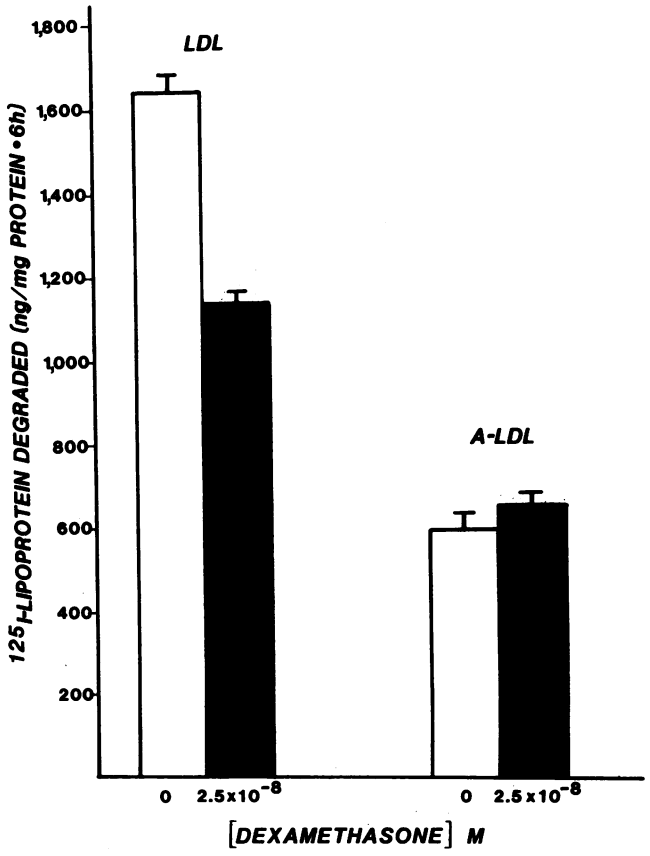


Figure 6. Dexamethasone effects on ^{125}I-lipoprotein degradation by human umbilical vein endothelial cells. Human umbilical vein endothelial cells were grown to confluence in 10% fetal bovine serum in DME. Preincubations were begun by washing twice with DME and adding 0.2% BSA in DME ± dexamethasone for 24 h. ^{125}I-LDL (*left*) or ^{125}I-ALDL (*right*) were added and degradation products measured after 6 h. Mean±SEM.

by loss of the effect in the presence of competitive antagonists for that receptor (19, 32). Dexamethasone also increases cellular cholesterol esterification rates and CE content in the presence of ALDL. These effects are most likely accounted for by the dexamethasone stimulation of scavenger receptor activity because glucocorticoids have been shown to be without direct effect on macrophage acyl-coenzyme A:cholesterol acyltransferase activity (29). In confluent cultured human umbilical vein endothelial cells, on the other hand, dexamethasone has no effect on ALDL degradation but depresses LDL receptor activity.

Glucocorticoid hormone suppression of HMM and endothelial cell LDL receptor activity is in agreement with data previously reported by Henze et al. (34) on human skin fibroblasts and arterial smooth muscle cells. In the latter studies, cortisol-mediated inhibition of LDL degradation was due to specific depression of LDL receptor internalization, with no change in lipoprotein binding. Whether this mechanism is responsible for the inhibition of LDL receptor function in HMM cannot be determined with certainty in the absence of binding studies. Such studies were not performed because HMM are nondividing cells and did not form a continuous monolayer in our series of experiments, leading to significant levels of background lipoprotein binding to the exposed surface of the tissue culture well (35). In addition, even though dexamethasone did not substantially alter cell protein per dish, we could not systematically rule out an effect of dexamethasone on cell shape which might lead to confounding differences in background binding. Finally, HMM are resistant to release from tissue culture surfaces by enzymes or calcium chelators, thereby precluding binding assays in cell suspension. The inhibition of HMM-LDL degradation by dexamethasone was, however, specific, and not a steroid-induced cell membrane effect or generalized suppression of receptor-mediated endocytosis given the opposite effects on ALDL degradation. Our kinetic data and analysis indicate that dexamethasone-mediated stimulation of ALDL degradation derives from an increase in the V_{max} of the process without a change in K_m. Because ligand-receptor internalization and intracellular processing are very rapid, making ligand-receptor association rate-limiting, such increases in V_{max} for lipoprotein degradation are thought to reflect increased cell surface receptor number (17, 18, 28, 36). For the LDL binding site, it has already been demonstrated that there is no large pool of intracellular receptors (37). If the same is true for the scavenger receptor, dexamethasone stimulation of receptor number may require new receptor synthesis.

Macrophage scavenger receptor activity is influenced by a number of factors; serum-free medium, bacterial endotoxin, and lymphocyte-conditioned medium all inhibit scavenger receptor function (13–15). Maturation of monocytes to macrophages, increased cell density, macrophage-conditioned medium, and heat labile serum factors all enhance receptor activity (13, 16). We found ALDL degradation to be somewhat decreased after preincubation in serum-free compared with serum-containing medium, in agreement with Fogelman et al. (13), but the stimulatory effect of dexamethasone was similar in both conditions. Regulation of scavenger receptor function by dexamethasone represents the first example of hormonal regulation of macrophage lipoprotein receptor activity. In addition, dexamethasone is the first well-characterized factor shown to augment scavenger receptor activity. The modulation of scavenger function by lymphocyte products and bacterial endotoxin has led to the suggestion that the activity of this receptor may be a marker for macrophage activation (15). The data reported here that scavenger receptor activity is enhanced by dexamethasone, an agent known to depress a number of macrophage inflammatory and immune functions (20, 22), support this proposal.

Regulation of cell surface hormone receptors by dexamethasone in other cell types has been previously demonstrated. Baker et al. found that dexamethasone treatment increases epidermal growth factor binding to human fibroblasts (38). Dexamethasone has also been shown to enhance insulin binding to hepatocytes (39) and 3T3 cells (40). Glucocorticoid hormone enhancement of macrophage cellular endocytic receptor activity, however, has only recently been described. Shepard et al. (21) found that macrophage mannose receptor activity is stimulated in a dose-dependent manner by dexamethasone; the stimulation is maximal by 24 h and is due to increased numbers of receptors, as in our studies. Increased mannose receptor activity promotes the endocytosis and removal of lysomal enzymes (previously secreted by activated macrophages) from the extracellular space. In an analogous manner, dexamethasone stimulation of scavenger receptor activity may enhance the removal of LDL previously modified by interaction with platelets (6), endothelial (8), or arterial smooth muscle cells (9).

In summary, results from the present study indicate that dexamethasone promotes HMM intracellular CE accumulation in the presence of modified lipoproteins, and that this is due to a stimulation of modified lipoprotein receptor activity. The potential relevance of glucocorticoid hormone regulation of HMM lipoprotein receptor activity to human atherosclerosis derives from the central role played by the monocyte-macrophage in atherogenesis (10–12) and will require further clinical study.

Acknowledgments

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