Opposite Effects of Insulin and Catecholamines on LDL-Receptor Activity in Human Mononuclear Leukocytes

WILHELM KRONE, HERBERT NAEGELE, BERT BEHNKE, AND HEINER GRETEN

The mechanisms by which insulin and catecholamines affect low-density lipoprotein (LDL)-receptor activity were studied in freshly isolated human mononuclear leukocytes. Incubation of cells for up to 24 h in a lipid-free medium resulted in an increase in the specific binding, accumulation, and degradation of ^{125}I-labeled LDL. Insulin stimulated the ability of the cells to bind, accumulate, and degrade the lipoprotein with high affinity, which may be caused by an increase in the LDL-receptor number without altering binding affinity. (−)-Epinephrine inhibited the specific binding, accumulation, and degradation of ^{125}I-LDL. This effect appears to be mediated by a decrease in the number of LDL receptors and not by a change in the binding affinity. (−)-Norepinephrine, the unspecific β-adrenergic agonist (−)-isoproterenol, and the β_2-specific agonist terbutaline mimicked the effect of epinephrine on LDL-receptor activity. Catecholamines and β-adrenergic agonists yielded sigmoidal log-concentration effect curves. The action of epinephrine was attenuated by the β-antagonist (*dl*)-propranolol. These results demonstrate that insulin stimulates and catecholamines suppress the specific binding, accumulation, and degradation of ^{125}I-LDL in human mononuclear leukocytes. The catecholamine action appears to be mediated by β_2-adrenergic receptors. A suppression of LDL-receptor activity resulting from deficiency of insulin and elevated plasma catecholamine concentrations in uncontrolled insulin-dependent diabetic patients may contribute to the increased levels of LDL cholesterol observed in these patients. *Diabetes* 37:1386–91, 1988

Premature development of atherosclerosis is the major cause of morbidity and mortality in insulin-dependent diabetic individuals (1). Abnormalities in lipoprotein levels and metabolism have been demonstrated in these patients (2,3). Epidemiologic studies in the general population have shown that the incidence of coronary heart disease is directly correlated to the levels of plasma cholesterol, more specifically to the plasma concentration of low-density lipoprotein cholesterol (LDL-chol; 4,5). Insulin-dependent diabetic patients are particularly prone to have elevated LDL-chol levels, the prevalence depending on several factors including glycemic control (6). Accordingly, initiation of intensive insulin therapy results in a marked decrease of LDL-chol levels (7–9). This may be caused by a decreased cholesterol synthesis or an increased degradation of LDL due to a rise in LDL-receptor activity.

Poor control of insulin-dependent diabetic patients is associated with insulin deficiency and elevated plasma catecholamine levels (10). These hormonal changes may contribute to the increase in plasma LDL-chol levels by affecting the LDL-receptor–mediated pathway. Accordingly, previous studies from our laboratory (11) and from Mazière et al. (12) have shown that epinephrine suppressed LDL-receptor activity in human mononuclear leukocytes and fibroblasts, respectively, whereas Chait et al. (13,14) reported a stimulation of the receptor-mediated LDL processing by insulin in cultured human skin fibroblasts. This study was undertaken to elucidate the mechanisms by which insulin and catecholamines affect the high-affinity binding, accumulation, and degradation of LDL. Freshly isolated mononuclear leukocytes were used because they reflect the in vivo milieu and possess insulin (15) and LDL (16,17) receptors, as well as β_2-adrenergic receptors, which are well defined by radioligand-binding studies (18), and pharmacological characterization (19).

MATERIALS AND METHODS

Materials. Bovine serum albumin (BSA; essentially fatty acid free), theophylline, dibutryl cyclic AMP (dbcAMP), crystalline

From the Medizinische Kernklinik und Poliklinik, Universitäts-Krankenhaus Eppendorf, Hamburg, Federal Republic of Germany.

Address correspondence and reprint requests to Wilhelm Krone, MD, Medizinische Kernklinik und Poliklinik, Universitäts-Krankenhaus Eppendorf, Martinistr. 52, 2000 Hamburg 52, FRG.

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bovine insulin, (−)-epinephrine, (−)-norepinephrine, (−)-isoproterenol, and (*dl*)-propranolol were purchased from Sigma (Munich, FRG). Terbutaline was a gift from Astra (Wedel, FRG). RPMI-1640 culture medium and penicillin/streptomycin mixture were obtained from Gibco (Glasgow, UK). Ficoll-Hypaque (Lymphoprep) was purchased from Nyegaard (Oslo). ^{125}I-labeled sodium in dilute NaOH solution was purchased from Amersham (Amersham, UK). Conical tubes of 50 ml were obtained from Falcon (Oxnard, CA) and 22-mm 12-well tissue-culture clusters were purchased from Costar (Cambridge, MA).

Isolation of LDL and preparation of lipoproteins. Human LDL (density 1.019–1.063 g/ml) was isolated from plasma of normolipemic healthy subjects by sequential ultracentrifugation as described by Havel et al. (20). ^{125}I-labeled LDL was prepared by the iodine monochloride method of McFarlane (21) as modified by Bilheimer et al. (22). After iodination, free iodine was removed by passage through a QAE-Sephadex A-50 anion-exchange column and sterilized by 0.45-μm Millipore filtration. Analysis of iodinated lipoprotein substrates showed that <1% of the radioactivity resulted from free iodine and <0.02% represents trichloroacetic acid–soluble noniodine material. Specific activity of the preparations was expressed as counts per minute per nanogram protein and was usually between 80 and 150.

Isolation and incubation of mononuclear leukocytes. Peripheral blood mononuclear leukocytes were isolated from heparinized blood obtained from healthy subjects after an overnight fast by the method of Böyum (23). Blood (10 ml) was diluted with an equal volume of 0.15 M NaCl. Lymphoprep (10 ml) was layered under the mixture of blood and saline and centrifuged at 400 × *g* for 40 min at room temperature. The resultant band of mononuclear leukocytes was washed twice in RPMI-1640 and then suspended in RPMI-1640 with 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were then plated at a density of 2 × 10^6/500 μl in 22-mm dishes and incubated in a humidified atmosphere with 5% CO_2 at 37°C.

Cell viability, as assessed by trypan blue exclusion, was >95% before and after incubation up to 30 h in cells untreated or treated with insulin or adrenergic agents. Purity of cell preparations was routinely assessed by staining smears with Wright-Giemsa stain. Differential counts before and after incubations showed that 85–95% of the cells were lymphocytes; the rest were monocytes as identified by their ability to ingest latex particles.

Measurement of LDL-receptor activity. After an incubation period of 24 h, the cells were chilled to 4°C for 30 min. Then 50 μl of 20% BSA in RPMI-1640, native LDL, and ^{125}I-LDL were added in various concentrations. After 2 h of incubation on a rotary shaker (100 oscillations/min at 4°C), the medium with the nonadherent cells was overlayered on a solution of 80 mg/ml BSA in 0.15 M NaCl/20 mM Tris-HCl buffer, pH 7.4 in 5-ml tubes. The dishes were then rinsed once with buffer without BSA, and the adherent cells were carefully removed with a rubber policeman. This cell suspension was also overlayered on the same solution followed by centrifugation (1800 rpm for 6 min, 4°C). The medium and BSA buffer were then sucked off, and the remaining cell pellet was resuspended in 0.5% BSA buffer followed by a short centrifugation (1800 rpm for 3 min, 4°C). This washing procedure was repeated three times. Radioactivity was then determined in a Packard γ-counter to calculate the binding of ^{125}I-LDL. Specific binding was defined as the difference of values with and without addition of a 25-fold excess of unlabeled LDL after subtracting blank values of incubations without cells. Surface binding of ^{125}I-LDL to cells was expressed as nanograms of ^{125}I-LDL protein associated with the cell pellet per 2 million cells.

After various incubation periods, the dishes received 50 μl of 20% BSA in RPMI-1640, native LDL, and ^{125}I-LDL. After an incubation period of 6 h (37°C, 5% CO_2), cells were chilled down on ice. Cells were separated from the medium and washed as described. Specific accumulation was defined as the difference of values with and without addition of a 25-

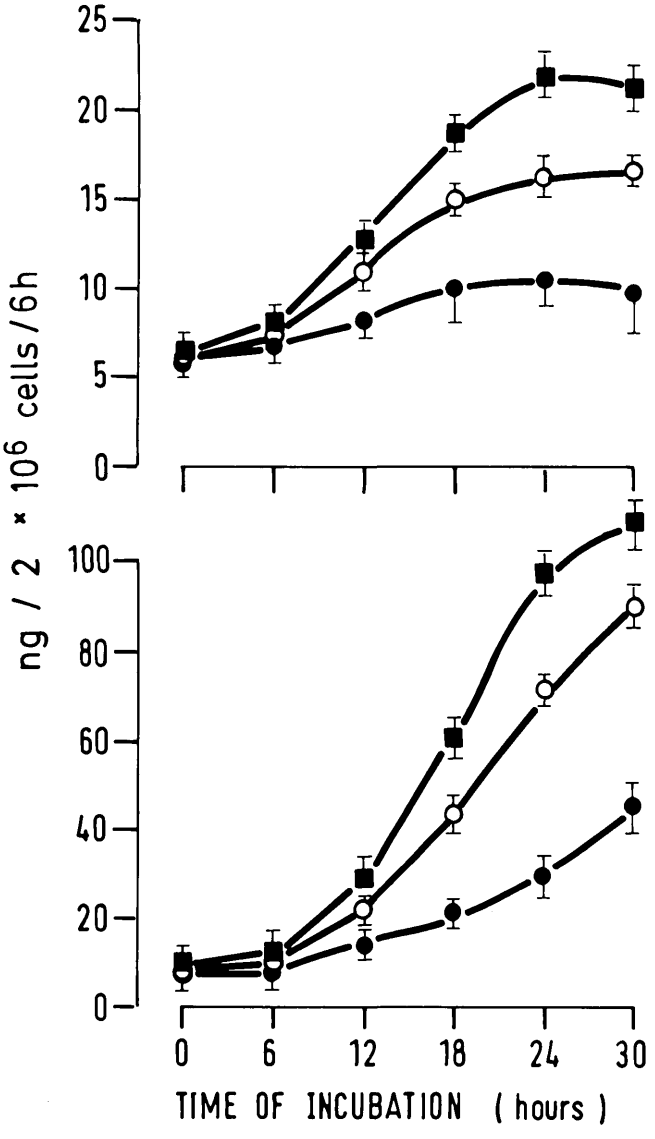

FIG. 1. Effects of insulin and epinephrine on ability of freshly isolated human mononuclear leukocytes to accumulate (*top*) and degrade (*bottom*) ^{125}I-LDL with high affinity as function of incubation time. Cells were incubated at 37°C in RPMI-1640 without lipoproteins with insulin (10 mU/ml, ■), epinephrine (0.1 mM, ●), or neither (○). At each indicated time interval, cells were incubated for 6 h at 37°C with ^{125}I-LDL (10 μg/ml). Accumulation and degradation assays were performed as described in MATERIALS AND METHODS. Values are means ± SE of 3 experiments performed in triplicate.

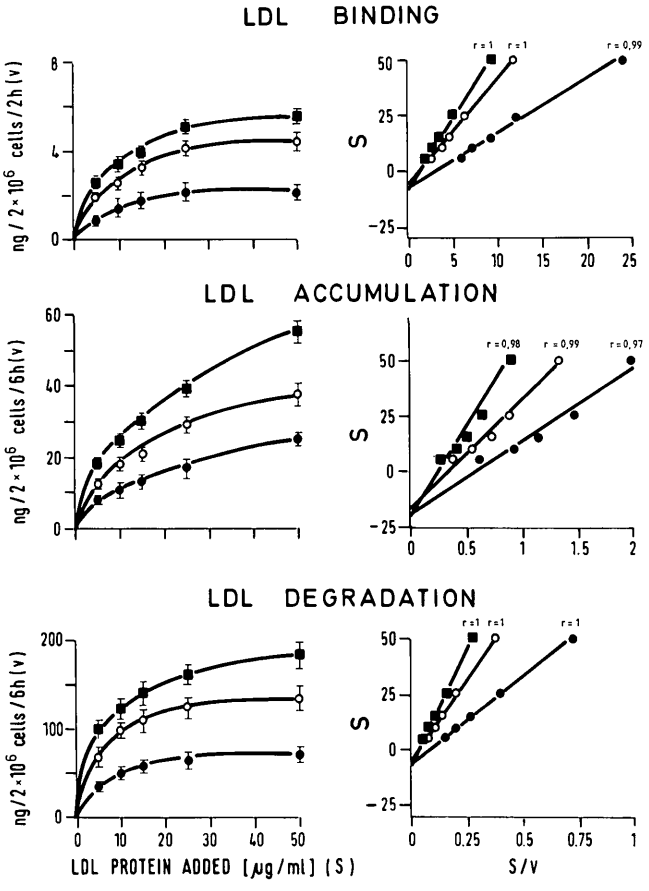

FIG. 2. Effects of insulin and epinephrine on high-affinity binding, accumulation, and degradation of ^{125}I-LDL in freshly isolated human mononuclear leukocytes as function of ^{125}I-LDL concentration. Cells were incubated at 37°C in RPMI-1640 with insulin (10 mU/ml, ■) epinephrine (0.1 mM, ●) or neither (○) for 24 h. ^{125}I-LDL was then added at indicated concentrations for 2 h at 4°C (binding) or for 6 h at 37°C (accumulation and degradation). Assays were performed as described in MATERIALS AND METHODS. Values represent means ± SE of 3–5 experiments performed in triplicate. Data on *right* represent reciprocal plots of data on *left*. Slope equals B_{max} for binding or apparent V_{max} for accumulation and degradation. Points of intersection with *y*-axis equal $-K_d$ or apparent $-K_m$, respectively.

fold excess of unlabeled LDL after subtracting blank values of incubations without cells. Cellular accumulation (i.e., the ^{125}I-LDL on the cell surface and within the cell) was expressed as nanogram of ^{125}I-LDL protein associated with the cell pellet per 2 million cells.

Cellular degradation was determined by carefully removing the medium from the supernatant after the 6-min centrifugation period as described above. The medium was then treated with trichloroacetic acid to a final concentration of 10% (vol/vol). After 30 min of incubation at 4°C, the precipitate was pelleted by centrifugation (3500 rpm for 10 min, 4°C), and the supernatant was treated with hydrogen peroxide and extracted with chloroform to remove free iodine. An aliquot of the aqueous phase was then counted. Specific degradation was defined as the difference of values with and without addition of a 25-fold excess of unlabeled LDL after subtracting blank values of incubations without cells. Degradation activity is expressed as nanogram of ^{125}I-LDL protein degraded to acid-soluble noniodine material per 2 million cells.

Other assays. The content of protein was determined by the method of Lowry et al. (24) with bovine serum albumin as a standard.

Data analysis. Values were expressed as means (of *n* experiments) ± SE. The significance of differences between means was established by Student's paired *t* test.

RESULTS

Freshly isolated mononuclear leukocytes exhibit a low number of high-affinity binding sites (16). These cells also exhibit a relatively low ability to degrade ^{125}I-LDL (Fig. 1). However, incubation of cells at 37°C in a medium devoid of lipoproteins led to a progressive rise in their ability to bind and accordingly to accumulate and degrade ^{125}I-LDL with high affinity. The specific accumulation and degradation was 3- and 12-fold, respectively, after an incubation of 30 h. Insulin (10 mU/ml) added to the incubation medium at time 0 led to a further increase in the specific high-affinity accumulation and degradation of ^{125}I-LDL (10 µg/ml), which was 135 and 136%, respectively, compared with controls without hormone at 24 h. At insulin concentrations of 1 and 100 mU/ml, the specific accumulation was 115 and 141%, and the specific degradation was 122 and 147%, respectively (data not shown).

To determine whether the rise in LDL-receptor activity caused by insulin results from an increased affinity of LDL to its receptor or from an increased number of LDL receptors, LDL concentration curves were performed in the presence or absence of insulin (10 mU/ml). Increasing concentrations of ^{125}I-LDL in the incubation medium led to a saturation of binding, accumulation, and degradation of ^{125}I-LDL (Fig. 2). Half-maximal saturation as achieved at 7.8, 17.9, and 5.5 µg/ml, respectively. In the presence of insulin (10 mU/ml), the rate of specific binding, accumulation, and degradation was greater at all concentrations used. Double reciprocal plots (25) of the data indicate that the major effect of insulin was to produce an increase in maximum binding (B_{max}) of LDL from 5.0 to 6.4 ng · 2 × 10^{-6} cells · 2 h^{-1} and a rise in apparent V_{max} of LDL accumulation and degradation from 50 to 81 ng · 2 × 10^{-6} cells · 6 h^{-1} and from 150 to 207 ng · 2 × 10^{-6} cells · 6 h^{-1}, respectively. In the presence of insulin, no significant change was observed in K_d of binding and in apparent K_m of accumulation and degradation.

To evaluate whether catecholamines and β-agonists influence LDL-receptor activity, the effects of (−)-epinephrine, (−)-norepinephrine, (−)-isoproterenol, and terbutaline on the high-affinity binding, accumulation, and degradation of ^{125}I-LDL were studied. Epinephrine (0.1 mM) added to the incubation medium at time 0 inhibited both high-affinity accumulation and degradation of ^{125}I-LDL (10 µg/ml) for up to 30 h (Fig. 1). The log-concentration–effect curves are shown in Fig. 3. Incubation of cells for 24 h with increasing concentrations of epinephrine progressively suppressed specific accumulation and degradation of ^{125}I-LDL (42 and 52% inhibited, respectively, 0.1 mM). Norepinephrine also inhibited LDL-receptor activity but to a lesser extent than epinephrine. To evaluate whether catecholamines act via β-adrenergic receptors, the effects of the unspecific β-agonist isoproterenol and of the β_2-specific agonist terbutaline on LDL-receptor activity were studied. Both adrenergic agonists caused a marked inhibition of the high-affinity accumulation

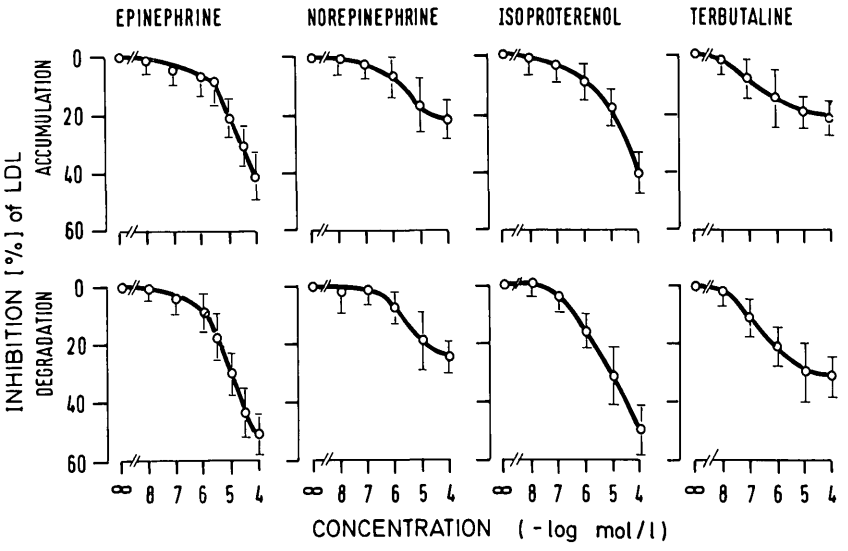

FIG. 3. Log-concentration–effect curves of epinephrine, norepinephrine, isoproterenol, and terbutaline on rate of high-affinity accumulation and degradation of ^{125}I-LDL in freshly isolated human mononuclear leukocytes. Cells were incubated at 37°C in RPMI-1640 with or without agents for 24 h after adrenergic agonists were added at various concentrations. ^{125}I-LDL (10 μg/ml) was then added, and accumulation and degradation assays were performed as described in MATERIALS AND METHODS. Values without agents at 24 h were defined as 0% inhibition. Each *point* represents mean ± SE of 4–6 experiments performed in triplicate.

and degradation of ^{125}I-LDL (10 μg/ml). Epinephrine, norepinephrine, and the β-adrenergic agonists yielded sigmoidal log-concentration–effect curves. The unspecific β-antagonist (*dl*)-propranolol (10 μM) attenuated the action of epinephrine on high-affinity accumulation and degradation when added to the incubation medium (Fig. 4). This β-blocker, which per se had no effect on LDL-receptor activity up to a concentration of 10 μM, caused a shift to the right of the log-concentration–effect curve produced by epinephrine.

As shown in Fig. 2, the LDL-receptor activity measured as specific binding, accumulation, and degradation of ^{125}I-LDL was saturable in the presence of epinephrine and was lower at all tested concentrations of LDL. Double-reciprocal plots of the data suggest that epinephrine suppresses B_{max} of binding from 5.0 to 2.4 ng · 2 × 10^{-6} cells · 2 h^{-1} and inhibits apparent V_{max} of accumulation and degradation from 50 to 33 ng · 2 × 10^{-6} cells · 6 h^{-1} and from 150 to 79 ng · 2 × 10^{-6} cells · 6 h^{-1}, respectively, without significantly altering binding affinity and apparent K_m (25). dbcAMP and theophylline added in increasing concentrations to the incubation medium at time 0 resulted in an inhibition of both high-affinity accumulation and degradation of ^{125}I-LDL (10 μg/ml; Table 1).

DISCUSSION

Freshly isolated human mononuclear leukocytes catabolize LDL from plasma after binding to high-affinity cell-surface receptors (16) that have been visualized by Western blotting and immunoprecipitation techniques (17). The LDL is degraded, and the resulting free cholesterol then regulates the activity of the microsomal enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis (26). We have previously reported that sterol synthesis in human mononuclear leukocytes is under additional hormonal control, with insulin stimulating (27) and catecholamines suppressing (28) the pathway.

This study demonstrates that insulin and catecholamines also have opposite effects on LDL-receptor activity. As to the mechanisms, insulin appears to raise the number rather than the affinity of LDL receptors, because analysis of LDL-concentration curves revealed an increase in B_{max} and apparent V_{max} without any change in K_d and apparent K_m of LDL-receptor activity (Fig. 2). Similar data have been obtained by Chait et al. (13,14) for cultured human skin fibroblasts in which insulin stimulates LDL-receptor activity. Also, HMG-CoA reductase activity (29,30) and sterol synthesis rate (14,29,30) were increased in fibroblasts by the peptide hormone, as we have have demonstrated in human mononuclear leukocytes (27).

Our results and those of Chait et al. (14) contrast with a report by Suresh et al. (31), who showed in freshly isolated human lymphocytes that insulin caused an increase in HMG-CoA reductase activity and cholesterol synthesis rate but a surprising decrease in the binding, internalization, and degradation of ^{125}I-LDL as a consequence of a decrease in binding affinity (Figs. 1 and 2; 27). This apparent contradiction cannot yet be explained. Note, however, that certain hormones that stimulate cell growth, e.g., insulin (14), thyroxine (32), and platelet-derived growth factor (33), cause simultaneous increases in LDL-receptor activity and cholesterol synthesis rate.

A simultaneous suppression of LDL-receptor activity and sterol synthesis rate is followed by an incubation of human mononuclear leukocytes with epinephrine (Figs. 1–3; 28).

TABLE 1
Effects of dbcAMP and theophylline on rate of accumulation and degradation of high-affinity ^{125}I-LDL in freshly isolated human mononuclear leukocytes

Agents	Inhibition (%)	
	Accumulation	Degradation
Control	0	0
dbcAMP (10^{-6} M)	2 ± 4	4 ± 5
dbcAMP (10^{-5} M)	38 ± 6*	37 ± 5*
dbcAMP (10^{-4} M)	52 ± 8*	58 ± 7*
Theophylline (10^{-5} M)	24 ± 6*	20 ± 6*

Indicated concentrations of agents were added to incubation medium at beginning of incubations (see Fig. 3 legend). Values are means ± SE of 4–6 experiments performed in triplicate.
* $P < .01$ vs. control.

The hormone led to a decrease in B_{max} and apparent V_{max} of LDL-receptor activity without affecting K_d and apparent K_m, suggesting that catecholamines may decrease the number of receptors rather than altering binding affinity (Fig. 2). Radioligand-binding studies have shown that human mononuclear leukocytes possess β-adrenergic receptors that are exclusively of the β_2-subtype (18). We have previously reported that stimulation of β_2-adrenergic receptors suppresses sterol synthesis in these cells (19). As demonstrated in this study, catecholamines may also regulate LDL-receptor activity by stimulating β_2-adrenergic receptors. This can be deduced from experiments in which the β-agonist isoproterenol and the selective β_2-agonist terbutaline caused a decrease in the high-affinity LDL accumulation and degradation, while the β-antagonist propranolol attenuated the action of epinephrine on LDL-receptor activity (Figs. 3 and 4).

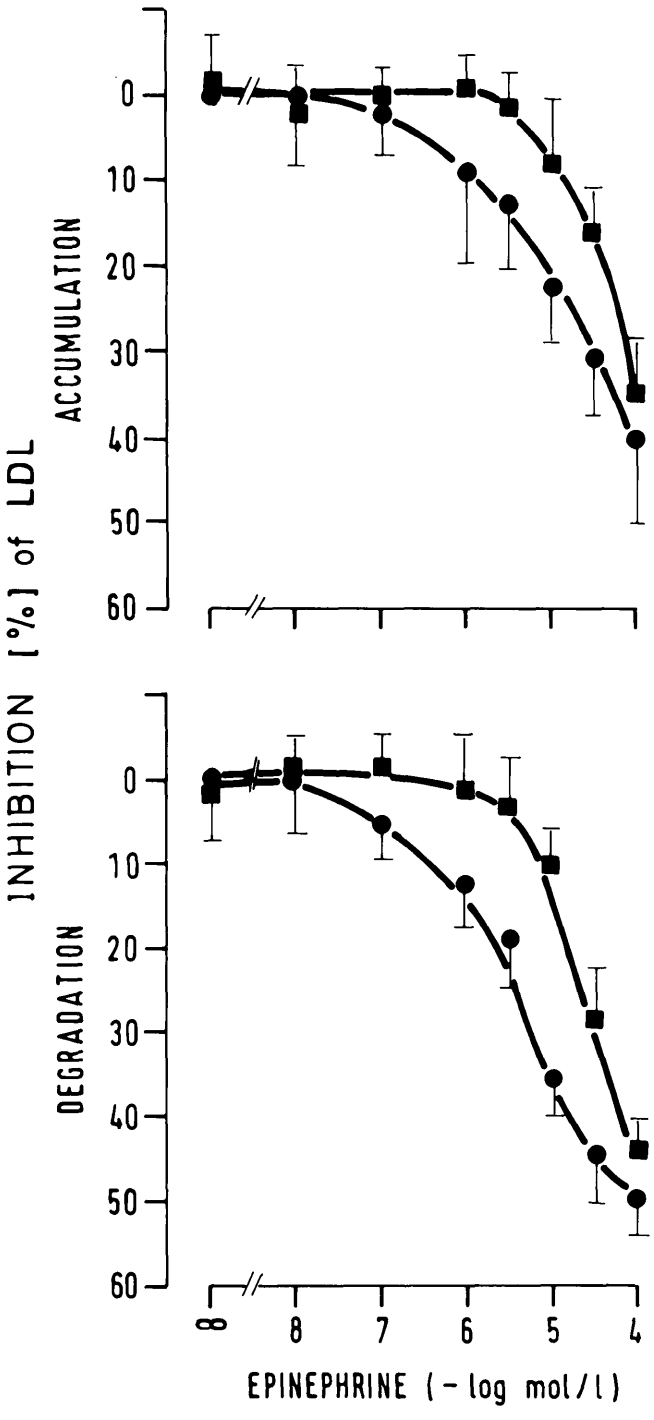

FIG. 4. Effect of β-antagonist propranolol on epinephrine-induced inhibition of high-affinity accumulation and degradation of ^{125}I-LDL in freshly isolated mononuclear leukocytes. Cells were incubated in RPMI-1640 at 37°C for 24 h with various concentrations of epinephrine in absence (●) or presence (■) of propranolol (10 μM) added 5 min before agonist. After 24 h, ^{125}I-LDL (10 μg/ml) was added, and accumulation and degradation assays were performed as described in MATERIALS AND METHODS. Values without agents at 24 h were defined as 0% inhibition. Each *point* represents mean ± SE of 4 experiments performed in triplicate.

Stimulation of β-adrenergic receptors in mononuclear leukocytes leads to a raised level of cAMP (34). Accordingly, the catecholamine-induced inhibition of LDL-receptor activity in these cells could be mimicked by dbcAMP (Table 1), as has been shown in fibroblasts (35,36) and arterial smooth muscle cells (35). Theophylline, which inhibits phosphodiesterase, has similar effects (Table 1). As to the clinical significance of these results, Brown and Goldstein (37) have presented strong evidence that LDL-chol levels in plasma are inversely associated with the number of LDL receptors. Thus, the increased LDL-chol concentrations observed in poorly controlled diabetic patients could be, at least partly, explained by a decreased removal of LDL from the circulation (38), which may be due to suppression of LDL receptors caused by insulin deficiency and high plasma catecholamine levels in these patients (10). The therapeutic implications of the LDL-receptor studies center on strategies for increasing the LDL-receptor activity, thereby lowering LDL-chol levels. In poorly controlled diabetic patients, a marked decrease of plasma LDL-chol can be achieved by intensive insulin therapy, which corrects the insulin deficiency and plasma catecholamine levels (39,40). If freshly isolated mononuclear leukocytes are representative of cells in vivo (37), this study suggests that the increased insulin concentrations and decreased catecholamine levels in well-controlled diabetic patients may be at least partly responsible for the fall in LDL-chol levels due to a stimulation of LDL-receptor activity. This hypothesis is supported by the demonstration by Mazzone et al. (41) that insulin infusion in human subjects accelerates the degradation of LDL.

The biochemical properties of the extrahepatic and hepatic LDL receptors are remarkably similar (42). Both receptors are subject to regulation by hormones (42). However, the liver was found to produce by far the largest number of LDL receptors (37) and accordingly to take up ~70% of LDL in the total body (43,44). Therefore, it is of interest that the adrenergic receptors in human liver may be predominately of the β_2- and α_1-subtypes (45). Whereas the control of the LDL receptors by α-adrenergic receptors remains to be established, our findings clearly demonstrate that β-adrenergic receptors regulate its activity. These studies may also have therapeutic implications when adrenergic drugs (e.g., for treatment of hypertension) have to be given.

Taken together, our results indicate that high-affinity binding, accumulation, and degradation of LDL is stimulated by insulin and suppressed by catecholamines that may act via β_2-adrenergic receptors. A suppression of LDL-receptor activity due to insulin deficiency and elevated plasma catecholamine levels in uncontrolled insulin-dependent diabetic

patients may contribute to the increased level of LDL-chol observed in these patients.

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