PRELIMINARY REPORT

Corticotropin Increases the Receptor-Specific Uptake of Native Low-Density Lipoprotein (LDL)—But Not of Oxidized LDL and Native or Oxidized Lipoprotein(a) [Lp(a)]—in HEPG2 Cells: No Evidence for Lp(a) Catabolism Via the LDL-Receptor

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To understand the interaction of corticotropin (ACTH) and lipid catabolism, we analyzed the influence of ACTH on receptor-mediated lipoprotein uptake and compared the uptake and degradation of human native (N-LDL) and oxidized (Ox-LDL) low-density lipoprotein and native (N-Lp(a)) and oxidized (Ox-Lp(a)) lipoprotein(a) by human hepatoma (HepG2) cells. The receptor affinity of N-LDL, Ox-LDL, N-Lp(a), and Ox-Lp(a) was comparable (K_d, 33, 13, 24, and 13 μg/mL medium), whereas the maximum degradative capacity was 10.5-fold higher in N-LDL ($\dot{V}_{max}$, 1,978 ng/mg cell protein) compared with Ox-LDL (189 ng/mg). In N-LDL, it was 4.5-fold higher than in N-Lp(a) (442 ng/mg) and eightfold higher than in Ox-Lp(a) (246 ng/mg) ($P < .05$). Addition of ACTH to the cell cultures increased receptor-specific degradation of N-LDL by 44% (2,866 *v* 1,978 ng/mg, $P < .05$), whereas changes in Ox-LDL, N-Lp(a), and Ox-Lp(a) showed no significant increase. No differences in uptake specificity were observed with or without ACTH. In addition, a 12-hour preincubation of liver cells with LDL increased Lp(a) uptake by 40% to 50% with (411 *v* 620 ng/mg) and without (393 *v* 558 ng/mg) ACTH administration. These data indicate that ACTH elevates receptor-specific uptake of N-LDL, but only to a low extent versus Ox-LDL, N-Lp(a), or Ox-Lp(a). These results support the hypothesis that catabolism of oxidized lipoproteins and Lp(a) through the LDL receptor pathway is only a minor route of lipid metabolism, whereas LDL receptor activity itself can be stimulated by ACTH.

ELEVATED PLASMA CONCENTRATIONS of low-density lipoprotein (LDL) and lipoprotein(a) [Lp(a)] have been associated with an increased risk of premature coronary artery disease.[1,2] The in vivo mechanism of cellular interaction and uptake of Lp(a) and the organ responsible for its catabolism are unknown. Lipoprotein metabolism is known to be regulated by corticotropin (ACTH).[3] Cholesterol uptake from both LDL and high-density lipoprotein into the adrenal gland is promoted by ACTH for the synthesis of steroid hormones.[4] Recently, it was demonstrated that ACTH administration resulted in a rapid decrease of LDL, Lp(a), and apolipoprotein B in humans in vivo.[5] These data suggested an upregulation of LDL receptor activity after administration of ACTH and the receptor-specific uptake of both LDL and Lp(a). To understand the influence of ACTH on lipoprotein uptake kinetics, we investigated LDL receptor uptake and degradation of native (N) and oxidized (Ox) LDL and Lp(a) by human hepatoma cells. Previous in vitro studies have reported binding of Lp(a) to the LDL receptor of human fibroblasts,[6] whereas the interaction of Lp(a) with the LDL receptor of human liver cells was of low affinity and nonsaturable.[7]

MATERIALS AND METHODS

ACTH was purchased from Sigma (Deisenhofen, Germany). [^{125}I]-sodium iodide and 2-[^{14}C]-sodium acetate were from Amersham (Braunschweig, Germany). Dulbecco's modified Eagle's medium/F $_{12}$, fetal calf serum, glutamine, sodium pyruvate, HEPES buffer, and nonessential amino acids were from Seromed (Berlin, Germany). HepG2 cells were from the American Type Culture Collection (Rockville, MD). Human LDL was isolated by sequential ultracentrifugation.[8] A Lp(a)-enriched regeneration fluid was obtained from an LDL-apheresis system (Braun, Melsungen, Germany). Lp(a) was purified using Sephadex G-25M gel chromatography and lysine Sepharose 4B chromatography and dialyzed against 150 mmol/L NaCl/1 mmol/L EDTA (pH 7.4). Lp(a) purity was determined by agarose gel electrophoresis and 4% sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Apolipoprotein(a) isoform determination was made by immunoblotting.[9] A monoclonal antibody against apolipoprotein(a) (Cappel, Durham, NC) was used as first antibody. Detection was made using the Vectastain ABC test kit (Vector, Burlingame, CA).

Lp(a) was oxidized in a similar fashion as LDL.[8] Briefly, antioxidant-free LDL or Lp(a) (0.3 mg protein/mL) was incubated with $CuSO_4$ (5

Table 1. Uptake and Degradation of Native and Oxidized ^{125}I-LDL and ^{125}I-Lp(a) After Incubation With and Without ACTH

	−ACTH		+ACTH	
Lipoprotein	$\dot{V}_{max}$	k_d	$\dot{V}_{max}$	k_d
N-LDL				
Uptake	4,252	38	4,729	34
Degradation	1,978	33	2,866	40
Ox-LDL				
Uptake	469	28	502	25
Degradation	189	13	216	14
N-Lp(a)				
Uptake	547	31	646	35
Degradation	442	24	440	25
Ox-Lp(a)				
Uptake	724	18	835	19
Degradation	246	13	288	14

NOTE. $\dot{V}_{max}$ is given as ng/mg cell protein; k_d is given as μg/mL medium.

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μmol/L) for 24 hours at 23°C. The degree of oxidation was quantified by (1) the increase in relative mobility on agarose gel and (2) the formation of thiobarbituric acid–reactive substances.[10] Homogeneity of lipoproteins was tested by agarose gel electrophoresis (Immuno, Heidelberg, Germany). Radioiodination of LDL and Lp(a) was performed by the method used by Bilheimer et al.[11] Final preparations of ^{125}I-LDL and ^{125}I-Lp(a) were sterilized by passage through a 0.45-μm Millipore (Molsheim, France) filter, and protein content was estimated by the Lowry method.

To induce apolipoprotein B/E receptor activity, HepG2 cells were incubated in 10% lipoprotein-deficient serum (LDS)-containing medium. After 48 hours, LDS-containing medium was replaced with ^{125}I-labeled lipoproteins with or without a 25-fold excess of unlabeled lipoproteins. The cultures were incubated (5 hours at 37°C) with or

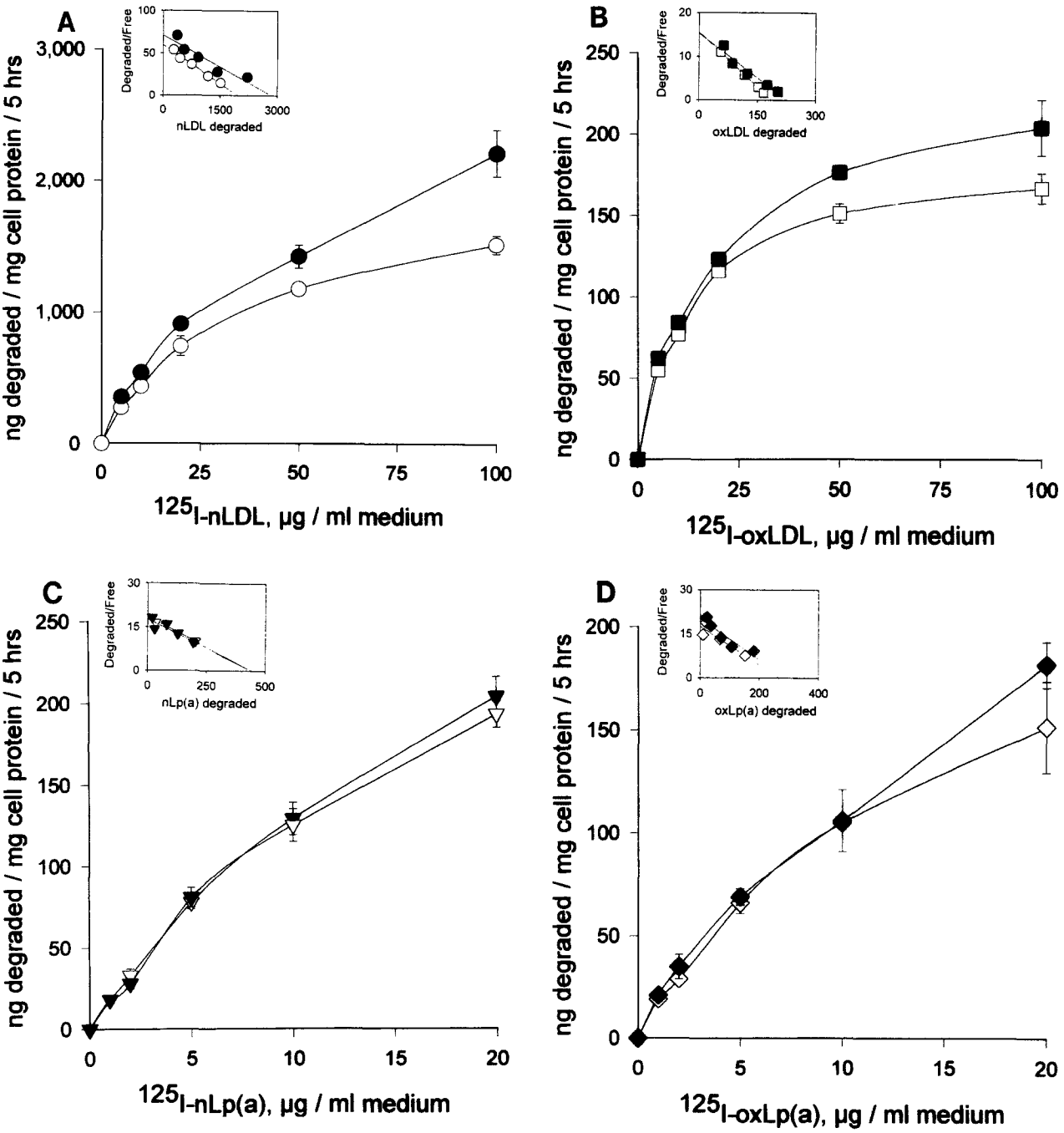

Fig 1. Concentration-dependent degradation of [^{125}I] N-LDL (a), Ox-LDL (b), N-Lp(a) (c), and Ox-Lp(a) (d) without (open symbols) and with (closed symbols) ACTH in cultures of HepG2 cells. After 48 hours in 10% LDS medium, various (n = 5) concentrations of labeled lipoproteins were added to HepG2 cells for 5 hours with or without ACTH (10^{-6} mol/L). Regression coefficients were as follows: N-LDL, r = −.9931 (−ACTH) and −.9322 (+ACTH); Ox-LDL, −.9874/−9642; N-Lp(a), −.9837/−.9685; Ox-Lp(a), −.9611/−.9256. Data are the mean ± SE of triplicate experiments. For symbols without error bars, the SE was less than the symbol size.

without ACTH at a concentration of 10^{-6} mol/L. Assays for uptake and degradation were performed by standard methods.[12] Nonspecific uptake and degradation were defined as the amount of lipoprotein taken up in the presence of a 25-fold excess of unlabeled ligand.

To investigate the effect of LDL on the degradation of Lp(a), HepG2 cells were incubated with 100 μg LDL 12 hours before addition of Lp(a). After this pretreatment, Lp(a) (2 and 10 μg) was added and uptake kinetics were analyzed with and without ACTH as already described.

Results are given as the mean ± SEM. The mean of the triplicates from each experiment served as a single experiment. Comparison was performed by paired or unpaired Student's *t* test as appropriate.

RESULTS

HepG2 cells bound, internalized, and degraded native and oxidized LDL and Lp(a) in a concentration- and receptor-dependent manner, and the process was saturable for LDL but nonsaturable for Lp(a). Receptor binding specificity was high for LDL (87% to 97%) and lower for Lp(a) (50% to 58%). Scatchard plot analysis of uptake and degradation showed comparable k_d values for native and oxidized LDL and Lp(a) with and without preincubation with ACTH (Table 1). The maximum degradative capacity ($\dot{V}_{max}$) was 10.5-fold higher in N-LDL (1,978 ng/mg) compared with Ox-LDL (189 ng/mg), 4.5-fold higher than in N-Lp(a) (442 ng/mg), and eightfold higher compared with Ox-Lp(a) (246 ng/mg) ($P < .01$). The concentration-dependent degradation of N-LDL, Ox-LDL, N-Lp(a), and Ox-Lp(a) and the corresponding Scatchard plots (ie, regression analysis) are shown in Fig 1.

After administration of ACTH, an increase in concentration-dependent lipoprotein uptake (4,252 *v* 4,729 ng/mg) and degradation (1,978 *v* 2,866 ng/mg) was observed for N-LDL ($P < .01$), whereas the effect of ACTH on the uptake and degradation of Ox-LDL and N- or Ox-Lp(a) was not significant.

Maximal capacities for the uptake of native Lp(a) were significantly higher after LDL preincubation (without ACTH, 393 *v* 558 ng/mg; with ACTH, 411 *v* 620 ng/mg; $P < .01$) compared with HepG2 cells without LDL preincubation (Fig 2). The effect of ACTH was observed only to a nonsignificant extent.

DISCUSSION

Recent in vivo studies have reported a marked reduction of LDL and Lp(a) after ACTH administration,[5] suggesting a major role for the LDL receptor in Lp(a) clearance. Studies by the same group have shown that patients with Cushing's disease exhibit dyslipidemia that is not reversed after adrenalectomy.[13] These findings suggest that ACTH influences lipid metabolism independently of corticosteroids.

Data concerning the role of the LDL receptor in Lp(a) metabolism are conflicting. Studies with familial hypercholesterolemic (FH) patients reported elevated Lp(a) levels,[14] suggesting that the LDL receptor mediates Lp(a) catabolism. In vitro studies demonstrated that Lp(a) can bind to the LDL receptor in fibroblasts[6] and HepG2 cells.[7] But in most of these studies, the affinity of Lp(a) for the LDL receptor was markedly lower than that of LDL.

In contrast, there is considerable evidence against a role for the LDL receptor in Lp(a) metabolism. Heterozygous subjects within FH kindreds were not found to have elevated Lp(a) when compared with unaffected family members.[15] Monkeys in a FH animal model do not exhibit elevated Lp(a) concentrations,[16] and drugs that upregulate the LDL receptor do not decrease Lp(a).[17] Recent kinetic studies established that the absence of a functional LDL receptor does not result in delayed Lp(a) catabolism.[18]

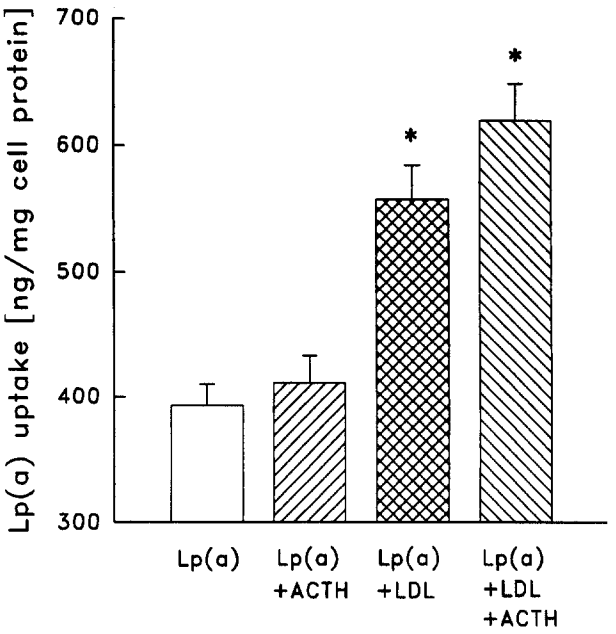

Fig 2. Uptake of [^{125}I] N-Lp(a) after a 12-hour preincubation with 100 μg LDL in cultures of HepG2 cells. Data are the mean ± SE of triplicate experiments. *$P < .01$ for Lp(a) *v* Lp(a) + LDL and for Lp(a) + ACTH *v* Lp(a) + ACTH + LDL.

The results of our study emphasize that the LDL receptor is not required as a physiologically important route in Lp(a) catabolism for N- or Ox-Lp(a). We further demonstrated in vitro that ACTH stimulates receptor-specific uptake and degradation of N-LDL, whereas no significant induction of uptake or degradation could be observed for Lp(a). In addition, we confirmed the recent finding by Kostner[7] that preincubation of HepG2 cells with LDL increases Lp(a) uptake. Whereas preincubation with LDL caused the expected reduction in LDL binding (data not shown), Lp(a) had an opposite effect. Unexpectedly, when cells were preincubated with LDL, we observed a marked increase in Lp(a) binding. The lack of sufficient competitive inhibition of LDL binding suggests an alternative but unknown mechanism for Lp(a) uptake.

In conclusion, the present investigation suggests that ACTH affects LDL receptor–dependent lipoprotein uptake in man, indicating that it modulates rate-limiting, saturable mechanisms in lipoprotein metabolism directly, not only via the pathways of endocrine glands. This LDL receptor–dependent lipoprotein uptake is assumed to be dominant for N-LDL, whereas it represents a minor route of catabolism for Ox-LDL, N-Lp(a), and Ox-Lp(a).

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