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Effect of High-Density Lipoproteins and Hydrocortisone on Apolipoprotein E Production in Kupffer Cells

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High-density lipoproteins *in vitro* captured by resident liver macrophages (Kupffer cells) are rapidly degraded in secondary lysosomes, but their protein components are not hydrolyzed completely. Immunological properties of apolipoprotein A-I secreted back to the incubation medium are completely preserved. This process is accompanied by enhanced production of apolipoprotein E by Kupffer cells and is markedly promoted by hydrocortisone.

Key Words: *Kupffer cells; high density lipoproteins, apolipoproteins A-I and E; hydrocortisone*

Eighty percent of apolipoprotein E (apoE) is secreted by the liver, while the reminder 20% is produced by the kidneys, spleen, adrenals, ovaries, brain, etc. [12,13]. In the liver, apoE is synthesized by both hepatocytes and resident macrophages (Kupffer cells, KC). Of particular interest is apoE produced by macrophages, since this apoprotein is believed to participate in the development of diseases allergic encephalomyelitis [10] and Alzheimer disease [11]. In Alzheimer disease excessive amounts of apoE are produced by astrocytes.

Production of apoE is regulated by a number of factors: accumulation of cholesterol and its oxidized metabolites (25 hydoxycholesterol) in macrophages promotes [7,8], while endotoxin, colony-stimulating factor [14], and interferon-γ [5] inhibit the biosynthesis and secretion of this protein. We have previously demonstrated a cooperative stimulation of protein biosynthesis in parenchymal cells of various organs by high-density lipoproteins (HDL) and glucocorticoids [2]. Resident macrophages play an important role in this effect, protein biosynthesis in macrophages being only slightly increased under these conditions. In the present study we attempted to correlate this increase with production of apoE by macrophages.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180-200 g. The animals were decapitated under light ether narcosis, the liver was removed and perfused in a recirculatory regime with 0.03% collagenase solution (Boehringer Mannheim) as described early [9] with some modifications [4]. This method provided $40\pm3\times10^6$ nonparenchymal cells per gram tissue, of them more than 95% excluded Trypan Blue. Resident macrophages (KC) were purified by a counterflow elutriation at 2500 rpm in a JE-6 rotor and a J2-21 centrifuge (Beckman). Freshly isolated KC were incubated at 37°C for 30 min in Hanks' saline without supplements containing 100 μg/ml HDL alone or in combination with 10^{-6} M hydrocortisone. The cells were then centrifuged, washed 2 times with cold Hanks' saline to remove HDL and hydrocortisone, and incubated for additional 30 or 90 min. The cell suspension contained

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3.5×10^6 macrophages/ml medium. The content of apoA-I and apoE was determined by enzyme-linked immunosorbent assay [3].

RESULTS

Previously, we evaluated by electron microscopy the rate of binding and internalization of HDL by hepatic sinusoidal cells. Experiments with liver perfusion showed that colloid gold-labeled HDL are rapidly (within 10 min), bound by KC, internalized by receptor-mediated endocytosis, and delivered to secondary lysosomes [1]. Taking these data into account, in the present study KC were preincubated with HDL for 30 min, the time sufficient for internalization of HDL and their initial disintegration catalyzed by lysosomal hydrolases. It is accepted that secondary lysosomes completely hydrolyze proteins. However, apolipoproteins are an exception. Our experiments showed that apoA-I is resecreted into the incubation medium (Table 1). This rapid process takes about 30 min; apoA-I concentration in the medium does not increase and even slightly decreases during subsequent incubation, probably due to a simultaneous release of lysosomal proteases into the incubation medium. Some apoA-I can be released into cytoplasm and interact with cytoplasmic structures. This phenomenon probably underlies cooperative regulatory effect of HDL and glucocorticoids on protein biosynthesis [2] observed in hepatocytes and, to a lesser extent, in macrophages. In the present study hydrocortisone markedly potentiated resecretion of apoA-I by KC, i.e., we observed a cooperative interaction of apoA-I and glucocorticoids.

TABLE 1. Resecretion of ApoA-I by KC Preincubated with HDL and Hydrocortisone ($M\pm m$, $n=7$)

Incubation conditions	Resecretion of apoA-I, ng/mg cell protein	
	30 min	90 min
Without additives (control)	1.6±0.5	1.4±0.3
HDL	82.5±15.4*	60.9±3.3*
HDL+hydrocortisone	154.1±141*	89.8±22.4*

Note. Here and in Table 2: *$p<0.05$ compared with the control.

TABLE 2. Production of ApoE by KC Preincubated with HDL and Hydrocortisone ($M\pm m$, $n=7$)

Incubation conditions	Secretion of apoE, ng/mg cell protein	
	30 min	90 min
Without additives (control)	37.8±6.7	39.3±6.1
HDL	34.1±6.6	52.9±11.6
HDL+hydrocortisone	91.1±8.5*	108.9±8.9*

High-density lipoproteins alone and in the combination with hydrocortisone considerably stimulated the apoE production in liver macrophages (Table 2). The effect of HDL was noted after a 90-min incubation, while the effect of the HDL+hydrocortisone combination developed within 30 min and increased up to the 90th min of incubation, suggesting a progressive increase of apoE secretion due to only not mobilization of preexisting protein but also to its enhanced biosynthesis.

Hyperproduction of apoE in KC induced by HDL and glucocorticoids may underlie other regulatory effects in the organism. For instance, lymphocytes, monocytes, and macrophages interact with apoE via surface immunomodulatory receptors [6]. The binding of apoE inhibits Ca^{2+} entry and cyclic nucleotide metabolism in lymphocytes rendering these cells resistant to mitogen stimulation. ApoE plays an important role in cholesterol homeostasis in various tissues and other regulatory processes.

Thus, HDL uptake by KC is accompanied by resecretion (rather than complete degradation) of their protein components into the incubation medium. Uptake of HDL particles by macrophages stimulates apoE secretion in these cells. Both processes are considerably potentiated by hydrocortisone.

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