Effect of Dexamethasone on the Expression of Binding Sites for High-Density Lipoproteins in Cultured Rat Hepatocytes (Independence of the Hormone Effect on the Intracellular Cholesterol Pool)

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It is shown that glucocorticoids play a key regulatory role directed toward the maintenance of an optimal level of binding and internalization of HDL_3 in hepatocytes. Their stimulatory effect on the expression of HDL receptors proves to be independent of changes in the CH content in parenchymal cells.

Key Words: *high-density lipoproteins; hormone regulation; glucocorticoids; dexamethasone; hepatocyte culture; adrenal-ectomized rats*

A central role in the maintenance of CH homeostasis in mammals is played by the liver, which directs the uptake and metabolism of high-density lipoproteins that transport CH primarily in the form of CH esters [2]. Since, in the first place, specific binding sites for apolipoprotein-A-containing lipoproteins (LP) - HDL "receptors" - have been found both in the liver membrane fraction and in cultured hepatocytes [6,8,11] and, second, the apolipoprotein-E-mediated uptake of LP is not, at least in rats, crucial for the delivery of CH esters to the liver [14], a study of the physiological regulators of the expression of HDL "receptors" in the liver is of indisputable interest. It is well known that a key physiological role in the systemic regulation and self-regulation of various functions of the liver is played by hormones, which, through the corresponding cell receptors, exert a potent effect on various enzymatic processes in the liver cells [3]. For instance, glucocorticoids have been shown to regulate CH synthesis [9] and the activity of 7-O-cholesterol hydroxylase [10] and hydroxy-methylglutaryl coenzyme A reductase (HMG-CoA reductase) [9]. It is also known that through their involvement in CH transport the HDL receptors regulate CH efflux from peripheral cells [2]. Moreover, the content of CH and of its 25-hydroxy derivatives up-regulates the number of HDL binding sites in peripheral cells [17]. However, the data on the CH-dependent regulation of HDL "receptors" in hepatocytes are contradictory [5,7].

In the present study we investigated the regulation by dexamethasone (Dex) of the expression of HDL binding sites in cultured rat hepatocytes and proved that the effect of the hormone is independent of the intracellular CH content.

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MATERIALS AND METHODS

Mature male Wistar rats weighing 250-300 g were used for the experiments.

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HDL_3 (1.125-1.216 g/cm^3) were isolated from the plasma of healthy volunteers by preparative ultracentrifugation in a NaBr solution [13]. The HDL_3 preparation contained no more then 0.1% apolipoprotein E, as shown by electrophoresis in 10% polyacrylamide gel (Bio-Rad, USA), with subsequent Coomassie blue staining and laser scanning of the gel. The preparations were dialyzed against two changes (1:1000 v/v) of 0.145 M NaCl, and 0.3 mM EDTA (Sigma, USA). The HDL were labeled with ^{125}I as described earlier [12]. The labeled HDL contained 2% of radioactivity in the trichloroacetic acid-soluble fraction, the specific radioactivity being 400-600 cpm/ng.

A suspension of hepatocytes was obtained by *in situ* enzymatic perfusion of the liver with a collagenase solution, as described elsewhere [1]. Hepatocytes were seeded on collagen-precoated plastic dishes and cultured in William's E medium (Flow Laboratories, UK), supplemented with 10 μg/ml insulin (Sigma), 100 μg/ml kanamycin (Sigma), and 20 mM HEPES (Flow), and containing various concentrations of Dex (Sigma). The medium was replaced every day. The cells retained 98% viability during 5 days of culturing as determined by trypan blue exclusion.

The binding and internalization were evaluated by the single point binding assay. For binding experiments the hepatocytes were incubated with 10 μg/ml ^{125}I-HDL at 37°C for 2 hours in the presence or absence of a 20-fold excess of unlabeled ligand. The cells were then washed three times with ice-cold Hanks solution and incubated on ice for 30 min in Hanks solution additionally containing dispase and protease (100 μg/ml each, Sigma). The specific binding was determined as the difference between the radioactivity of the obtained solution after incubation in the presence or absence of HDL_3. For measurement of the internalization of HDL the attached cells were scraped off, transferred to counting vials, and assessed for radioactivity. The specific internalization was also calculated as the difference of radioactivity in the presence or absence of a 20-fold excess of unlabeled ligand.

In order to prevent internalization, Scatchard binding analysis was carried out at 4°C. The dose dependence of binding was studied by varying the ^{125}I-HDL concentrations from 2.5 to 40 μg/ml. Nonspecific binding was determined in the presence of a 20-fold excess of unlabeled ligand [15].

CH synthesis was measured as described elsewhere [17]. Briefly, the hepatocytes were incubated for 2 hours with 30 μCi/ml ^{14}C sodium acetate (Amersham) and washed twice with ice-cold solution, after which CH was precipitated with digitonin after Sperry (Sigma) [16]. The protein content in the samples was measured after Bradford [4].

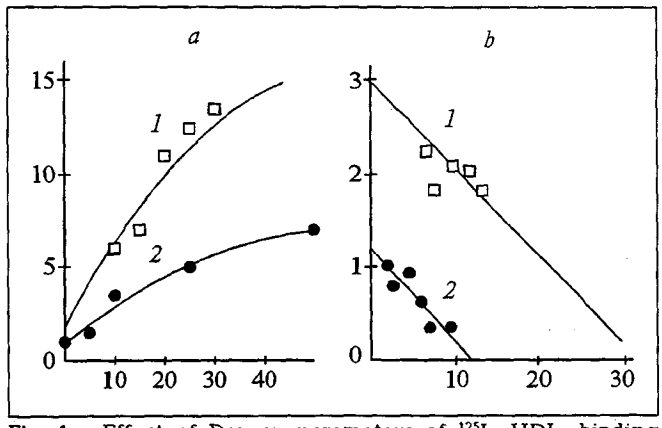

Fig. 1. Effect of Dex on parameters of $^{125}I-HDL_3$ binding by cultured rat hepatocytes. Hepatocytes were cultured for 48 h in the presence (*1*) or absence (*2*) of Dex (10^{-5} M). *a*) dose dependence of $^{125}I-HDL_3$ binding; abscissa: concentration of $^{125}I-HDL_3$, μg/ml; ordinate: binding, ng/mg cell protein. *b*) Scatchard plot of $^{125}I-HDL_3$ binding; abscissa: bound $^{125}I-HDL_3$, ng/ml, ordinate: bound/free ($\times 10^{-3}$). The data are representative of four experiments.

RESULTS

Figure 1, *a* shows dose-dependence curves of ^{125}I-HDL_3 binding. The experiments were conducted at 4°C to prevent internalization of bound HDL_3. Both curves reach a plateau at an LP concentration of 25-30 μg/ml, but Dex increases the number HDL binding sites 1.5-fold. Scatchard analysis (Fig. 1, *b*) revealed that the ligand-receptor interaction curves are typical for monomolecular reactions, and although Dex increased the number

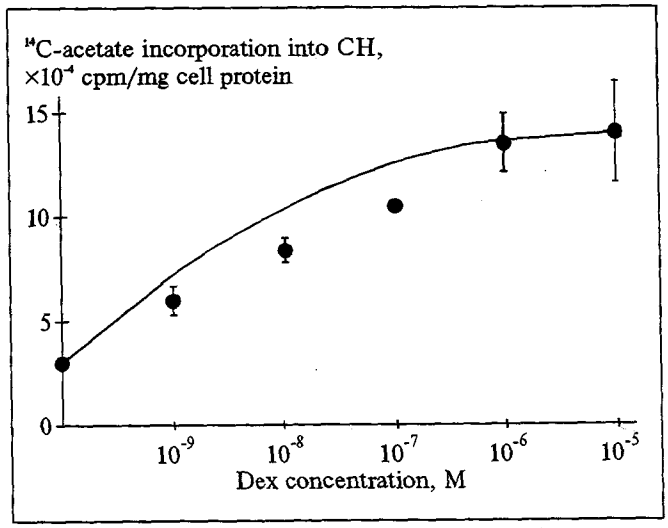

Fig. 2. Effect of Dex on synthesis of CH from $^{14}C-$acetate in cultured rat hepatocytes. Hepatocytes were cultured in the presence of various concentrations of Dex over 48 h, and $^{14}C-$acetate (30 μCi/ml) was added for 2 h. The data are averaged for three independent experiments.

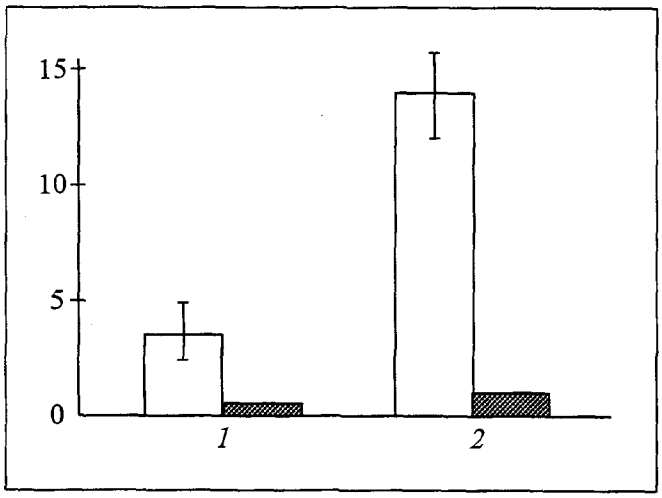

Fig. 3. Effect of lovastatin on synthesis of CH from ^{14}C-acetate in cultured rat hepatocytes. Hepatocytes were cultured over 48 h in the presence (*1*) or absence (*2*) of Dex (10^{-5} M) after 24 h the media were replaced with either lovastatin-containing (100 nM) (dark bars) or lovastatin-free (light bars) media. Synthesis of CH was measured 48 h after seeding. Ordinate: ^{14}C-acetate incorporated into CH, $\times 10^{-4}$ cpm/mg cell protein. The data are averaged for three independent experiments.

of binding sites (from 48 to 128 ng/mg protein), it did not affect their affinity to HDL_3, the dissociation constant in both cases being $2 \cdot 10^{-7}$ M.

Thus, it may be surmised that Dex stimulates the expression of HDL_3-binding sites in hepatocytes.

For a study of the effect of Dex simultaneously on binding and internalization, experiments were conducted at 37°C. Dex (10^{-9}-10^{-5} M) up-regulates both the binding and internalization of ^{125}I-HDL_3 in a dose-dependent manner, the maximum being attained at a hormone concentration of 10^{-5} M. The number of internalized HDL was twice as high under the influence of Dex.

As already mentioned, there are published data on the regulation by glucocorticoids of the activity of 7-O-cholesterol hydroxylase and HMG-CoA reductase, key enzymes in the synthesis of cholesterol and bile acids [9]. Moreover, some workers have demonstrated the stimulating effect of CH and its 25-hydroxy derivatives on HDL binding in various cells [17]. Therefore, it cannot be ruled out that the effect of Dex on HDL_3 binding and internalization is due to enhanced CH synthesis in hepatocytes. For elucidation of the role of the Dex-dependent *de novo* synthesized CH pool in the regulation of binding and internalization of HDL_3 in hepatocytes, we studied the effect of the hormone on the above processes in the presence of lovastatin, an inhibitor of HMG-CoA reductase and, subsequently, of CH synthesis.

Figure 2 shows that Dex up-regulated in a dose-dependent manner CH synthesis in hepatocytes preincubated with the hormone for 48 h. The curve reached a plateau at a Dex concentration of 10^{-7} M, and CH synthesis was elevated 2-4 times. It is important to note that Dex acted in a broad range of concentrations, including both physiological and subphysiological doses.

Lovastatin in a concentration of 100 nM inhibited the synthesis of CH by 95% (Fig. 3). The effect of lovastatin was observed both in the presence and absence of the hormone. However, unlike in peripheral cells, where lovastatin was shown to down-regulate both the CH synthesis and the number of HDL binding sites, in hepatocytes it neither abolished the stimulating effect of Dex on HDL_3 binding and internalization (Table 2) nor lowered these parameters in hepatocytes cultured in the absence of the hormone.

On the basis of the above observations, it may be concluded that Dex affects the expression of the HDL "receptor" via the glucocorticoid receptor-mediated pathway.

TABLE 1. Dose Dependence of the Effect of Dex on Specific Binding and Internalization of ^{125}I-HDL

Parameter, ng/mg cell protein	Concentration of Dex, M			
	0	10^{-9}	10^{-7}	10^{-5}
Binding	10.0±1.5	16.2±1.2*	23.1±1.4*	29.2±3*
Internalization	10.1±0.9	9.2±1.1	13.1±1.5*	18.2±2.1*

Note. *: $p<0.05$ in comparison with the corresponding control.

TABLE 2. Effect of Lovastatin and Dex on Binding and Internalization of HDL_3 (ng/mg Cell Protein) by Cultured Hepatocytes ($M \pm m$, $n=4$)

Lovastatin, 10^{-7} M	Dex, 10^{-5} M	Binding	Internalization
No	No	9.5±1.1	6.8±0.5
Yes	No	8.3±0.95	5.9±1.1
No	Yes	28.9±2.7*	25.3±2.1*
Yes	Yes	22.5±2.5*	23.3±3.1*

Note. *: $p<0.05$ in comparison with the control (without either Dex or lovastatin).

The findings suggest that glucocorticoids have an important regulatory role directed toward the maintenance of the optimal level of binding and internalization of HDL_3 by hepatocytes. Their stimulatory effect on the expression of HDL "receptors" is independent of the CH content in parenchymal liver cells.

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Interaction between Multiply Modified (Desialylated) Low-Density Lipoproteins Isolated from Blood of Atherosclerotic Patients and Cell Receptors

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It is shown that binding of native LDL to fibroblasts expressing the B,E-receptors is twice as high as that of desialylated LDL. An excess of acetylated LDL inhibits binding, uptake, and degradation of ^{125}I-desialylated LDL by macrophages, while an excess of desialylated LDL inhibits binding, uptake, and degradation of acetylated LDL. Desialylated LDL may interact with both B,E and scavenger receptors.

Key Words: *low-density lipoproteins; sialic acid; cell culture; metabolism of low-density lipoproteins*

Previously we found that blood serum of patients with coronary atherosclerosis possesses an atherogenic potential, i.e., it is capable of stimulating lipid accumulation by cultured cells from intact aorta intima [3,6]. The atherogenicity of the serum was shown to be due to the presence of a subfraction of low-density lipoproteins (LDL) with a reduced content of sialic acid - desialylated LDL, which, unlike native (sialylated) LDL, induce lipid

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