Glucocorticoid-Dependent Regulation of the Expression of High-Density Lipoprotein Binding Sites in Rat Hepatocytes

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UDC 616.36-018.1-008.939.
15-02:577.175.53-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 1, pp. 47-50, January, 1994
Original article submitted July 8, 1993

It is shown that dexamethasone increases the number of HDL binding sites in cultured hepatocytes both *in vivo* and *in vitro*. The glucocorticoid acts in a dose-dependent and reversible manner within a wide range of concentrations, including physiological and subphysiological doses.

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

Binding sites for high-density lipoproteins (HDL) have been found on the surface of cells from various animal and human tissues. The binding of HDL by cells meets the criteria of receptor binding and is characterized by specificity, saturability, and high affinity, and is accompanied by biological effects [7,8,11]. Regulation of receptor interaction of apolipoprotein E-free HDL has been well studied in peripheral tissues, where cholesterol (CH) and its 25-hydroxy derivatives has been shown to positively regulate the expression of HDL-binding sites on the cell surface [17]. At the same time, glucocorticoids possessing a similar sterol structure mediate a number of receptor-dependent changes in the liver that are related to CH metabolism both *in vivo* and in cultured hepatocytes. Thus, a short-term administration of small doses of cortisol leads to a rise of the level of HDL CH in volunteers, and up-regulates the synthesis of lipoproteins A1 and A4 and the expression of the corresponding mRNA, while on the other hand it does not change the synthesis of apolipoprotein E [5,6]. Glucocorticoid hormones elevate the expression of acyl coenzyme A: cholesterol acyltransferase (ACAT) and cholesterol 7-O-hydroxylase. Chronic excess of glucocorticoids leads to a drastic increase of CH in low-density lipoproteins (LDL), which seems to be the result of a reduced number of LDL binding sites in the liver [15].

However, at present there are no reliable data on the effect of glucocorticoids on the number of HDL binding sites in hepatocytes, although the above findings, which illustrate numerous effects of these hormones on CH metabolism, suggest their

TABLE 1. Effect of Dex on Parameters of Specific Binding of HDL by Cultured Hepatocytes

Culture conditions	Binding paraneters	
	K_d, M	N, ng/mg proteim
Without Dex	2×10^{-7} (5)	48
Dex (10^{-5} M)	2×10^{-7} (5)	128*

Note. Number of experiments presented in parentheses, K_d – dissociation constant, N – number of binding sites, *: $p<0.05$ in comparison with the control.

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0007-4888/94/0001-0048$12.50

TABLE 2. Effect of Adrenalectomy on Binding of $^{125}I-HDL$ by Parenchymal Liver Cells *in Vivo* ($M \pm m$)

Specific Binding of ^{125}I-HDL_3, ng/mg protein		
intact rats	adrenalectomized rats	adrenalectomized rats + Dex
28.1±2.6(19)	8.7±0.6(5)*	26.4.1(5)

Note. Number of experiments shown in parentheses. *: $p<0.05$ in comparison with the initial level of HDL_3 binding sites.

involvement in the regulation of the uptake of apolipoprotein A-containing lipoproteins (LP) in the liver.

MATERIALS AND METHODS

Mature male Wistar rats weighing 250-300 g were used in the experiments. Bilateral adrenalectomy was carried out as described elsewhere [2] and 14 days later the animals were taken in the experiment. The completeness of adrenalectomy was verified upon dissection.

In the course of glucocorticoid therapy dexamethasone (Dex, 50 μg/100 g body weight) was injected intramuscularly for 3 days. The level of HDL binding sites was determined one day after the last injection of the hormone.

HDL_3 (1.125-1.216 g/cm³) were isolated from plasma of healthy volunteers by preparation ultracentrifugation in NaBr [14]. The HDL_3 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 free of KBr 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 enzymatic *in situ* perfusion of the liver with 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

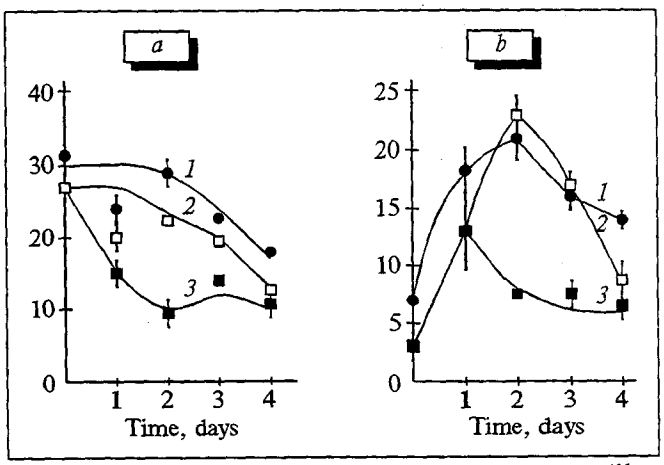

Fig. 1. Level of specific binding and internalization of $^{125}I-HDL$ in hepatocytes as a function of cultivation time. Hepatocytes were cultured over 48 h in the presence of Dex: *1*) 10^{-5} M, *2*) 10^{-7} M, or *3*) in the absence of the hormone. Ordinate: *a*) specific binding; *b*) specific internalization, ng/mg cell protein

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.

In the single-point binding assay the hepatocytes were incubated with 10 μg/ml ^{125}I-HDL at 37°C over 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). 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 internalization of HDL the attached cells were scraped off, transferred to counting vials, and assessed for radioactivity. As in the binding experiments, the specific internalization was 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 binding dose dependence was studied by

TABLE 3. Reversibility of the Effect of Dex on $^{125}I-HDL$ Specific Binding in Cultured Hepatocytes from Adrenalectomized Rats ($M \pm m$, $n=5$)

Experimental conditions	Time in culture, h		
	2	24	48
Control (without Dex)	8.1±0.5	9.1±0.6	8.6±0.5
Dex, 10^{-5} M, (with hormone)	8.1±0.6	29.3±0.4	30±2.5
Dex, 10^{-5} M, (without hormone)	8.2±0.4	29.8±0.7	14.3±2.1

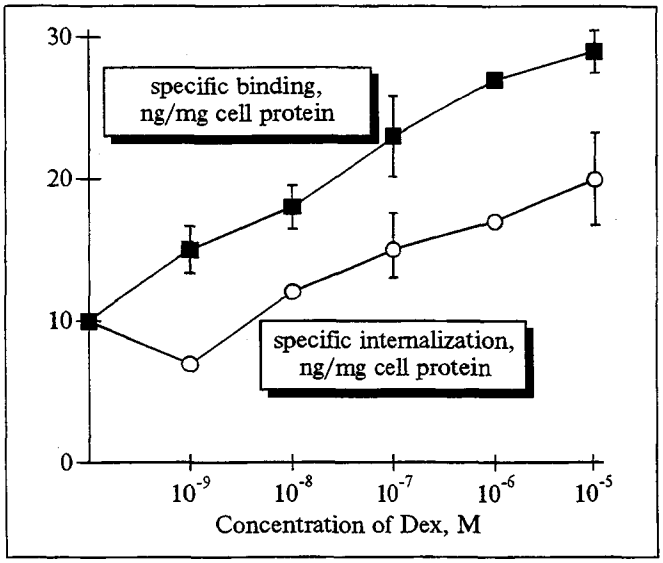

Fig. 2. Effect of different concentrations of Dex on specific binding and internalization of ^{125}I–HDL in cultured hepatocytes. Abscissa: concentration of Dex, M; ordinate: specific binding (*1*) and specific internalization (*2*), ng/mg cell protein.

varying the concentrations of ^{125}I-HDL from 2.5 to 40 μg/ml. The nonspecific binding was determined in the presence of a 20-fold excess of unlabeled ligand [16]. The content of protein in the samples was measured after Bradford [3].

RESULTS

The single-point assay revealed an accelerated drop of specific binding and internalization of HDL in hepatocytes cultured without Dex (Fig. 1, *a, b*). During the first 48 hours the specific binding dropped from 30 to 12 ng/mg cell protein. Internalization of the ligand also gradually decreased during the incubation and reached 90% of the maximal value. In both physiological (10^{-7}) and pharmacological (10^{-5}) doses Dex increased the binding measured after 48 and 72 hours in culture to the level observed soon after isolation of the cells. It should be noted that internalization measured 2 h after isolation of the cells did not exceed 4 ng/mg cell protein (which is evidently a result of changes in the intracellular transport systems during the isolation procedure), but it recovered to 15 ng/mg cell protein after 24 h in culture, when the hepatocytes were completely plated. The incubation in the absence of the hormone was accompanied by a gradual decrease (to 30% of the initial level) in the internalization of the ligand.

Scatchard analysis of HDL binding to the cells cultured in the presence or absence of Dex revealed (Table 1) that the elevated binding is due to the increased number of HDL binding sites from 48 to 128 ng/mg cell protein (i.e., from 300,000 to 850,000 binding sites per cell). The dissociation constant of the ligand-receptor interaction remains unchanged, being 2×10^{-7} M (with respect to a ligand molecular weight of 100 kD).

When hepatocytes were cultured in the presence of various concentrations of Dex, a dose-dependent increase of specific binding and internalization of HDL was observed, which seems to be due to the increased number of HDL binding sites (Fig. 2). The reversibility of the effect of Dex on the number of HDL-specific binding sites was tested both *in vivo* and *in vitro*. For assessment of the *in vivo* HDL_3 uptake by the liver parenchymal compartment the specific binding was assayed 2 hours after seeding. The HDL binding by cultured cells from adrenalectomized animals was lowered by 60% in comparison with that from normal rats. Glucocorticoid therapy restored the HDL binding in the adrenalectomized animals to a normal value (Table 2).

In cultured hepatocytes isolated from the adrenalectomized animals the level of HDL binding and internalization in the absence of Dex remained unchanged over 72 hours followed by a drop (as much as 50%) at 96 h. Dex added to the culture medium in either a physiological or

TABLE 4. Effect of Cycloheximide on Binding and Internalization of ^{125}I–HDL in Cultured Hepatocytes Obtained from Intact and Adrenalectomized Rats ($M\pm m$, $n=5$)

Rats	Cycloheximide	Dex	Binding	Internalization
			HDL, ng/mg protein	
Intact	No	No	9.5±1.1	6.8±0.5
	Yes	No	2.6±0.1	2.4±0.1
	No	Yes	28.9±2.7	25.3±2.1
	Yes	Yes	4.7±0.5	6.4±1.3
Adrenalectomized	No	No	9.2±0.83	5.1±0.4
	Yes	No	2.5±0.3	1.7±0.2
	No	Yes	28.3±0.4	20.8±1.7
	Yes	Yes	5.1±0.3	3.8±0.4

Note. All values are reliably different ($p<0.01$) from the corresponding controls.

pharmacological concentration restored both the binding and internalization of HDL to a normal level, while washing of the cells and their subsequent incubation without Dex led to reduced binding and internalization parameters for hepatocytes obtained from adrenalectomized animals in the absence of Dex (Table 3). Cycloheximide, an inhibitor of protein synthesis, was shown to reduce the HDL binding in normal and adrenalectomized animals 2-5-fold (Table 4). This effect was observed in the presence or absence of Dex, however, Dex-pretreated cells were more sensitive to the inhibitor, which completely blocked the effect of the hormone.

Thus, the data presented here show that Dex up-regulates the number of HDL binding sites in cultured hepatocytes. The effect of the hormone is expressed both *in vivo* and *in vitro*. The effect of the glucocorticoid is dose-dependent and reversible, and it manifests itself in a wide range of concentrations, including both phisiological and subphysiological doses. The inhibitor of protein synthesis not only prevents the expression of HDL binding sites in response to Dex, but also, it seems, reduces their synthesis in hepatocytes not stimulated with the hormone. In line with published data on the presence of glucocorticoid receptors in cultured hepatocytes [4,13] the obtained results suggest that the effect of glucocorticoids on the expression of HDL binding sites is mediated through the corresponding cell receptors.

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