BIOPHYSICS AND BIOCHEMISTRY

Effects of Glucocorticoids and High Density Lipoproteins on Activity of Hepatocyte Nuclei

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Combined exposure to high-density lipoproteins and glucocorticoids caused changes in the nucleus of rat hepatocytes. These changes most rapidly (within 10 min) appeared after stimulation of sinusoid cells and manifested themselves in enlargement of nucleoli, loosening of the fibrillar and accumulation of the granular components, and in an increase in nuclear membrane pore density. The increased binding and acridine orange enhanced red fluorescence attested to chromatin activation and enhanced gene expression. Resident macrophages play a key role in the realization of this mechanism.

Key Words: *lipoproteins; glucocorticoids; protein synthesis; hepatocyte*

All cells in different organs and tissues are continuously renewed (physiological regeneration). This is associated with rapid proteins catabolism. For instance, about 40% proteins are catabolized daily in rat liver [1]. It is generally accepted that structural homeostasis in organs is maintained by a definite level of protein biosynthesis, specific for each functional state [10]. Many extracellular transmitters and intracellular messengers remain unknown. This is especially true for molecular mechanisms of cell-cell interactions in the liver.

We have previously described a cooperative facilitating effect of glucocorticoids and high-density lipoproteins (HDL) on protein biosynthesis in the parenchymal cells of parenchymatous organs [7,8]. We hypothesized that these agents are taken up by resident macrophages together with cell degradation products and enzymatically modified in macrophage secondary lysosomes with the formation of apoA-1—tetrahydrocortysol complex, which enhances gene expression and accelerates protein biosynthesis in parenchymal cells [4,5].

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This article describes the results of fluorescent and electron microscopic investigation supporting this assumption.

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 180-200 g. Chromatin activation was studied in cultured liver slices using acridine orange (AO) DNA fluorescent probe (Sigma). AO incorporates into deproteinized DNA fragments between the base pairs and fluoresces in a green range (λ=530 nM) upon interaction with double-stranded DNA and in a red range (λ=615-640 nM) upon interaction with single-stranded DNA. The application of AO and rat liver slices for the analysis of chromatin activity was described in detail [6]. The slices were incubated for 2.5 h in a medium containing HDL (0.2 mg protein/ml) and hydrocortisone (3×10^{-6} M).

For morphometric analysis, the liver was perfused *in vitro* with Eagle's medium (pH=7.4) containing 20 mM HEPES, 10 μM hydrocortisone, and colloid gold—lipoprotein conjugates (100 μg protein/ml). Home-produced lipopolysaccharide (LPS) prodigiozan (20 ng/ml) was used to stimulate macrophages. Vari-

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ous lipoproteins were isolated from rat serum by ultracentrifugation [12]. Colloid gold (9-10 nm particle size) was prepared as described elsewhere [11] and conjugated with lipoproteins [9]. Hepatocyte morphometry and counting of gold particles were performed using a JEM-100S electron microscope (×5000-30,000).

The material for electron microscopy was fixed in 2.5% glutaraldehyde, postfixed in 1.5% OsO_4, and embedded in Epon—Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

RESULTS

Incubation of liver slices with hydrocortisone and HDL increases AO binding to DNA by 98% (Table 1), which indicates a 2-fold increase in the number of deproteinized DNA fragments in nuclear chromatin. Previous data [3] suggest that this increase is associated with activation of proteolytic enzymes in hepatocyte nuclei. HDL alone produce no such effect, while hydrocortisone caused a 2-fold increase in K_D without changing the number of binding sites [6]. Thus, this experiment revealed a cooperative effect of hydrocortisone and HDL on chromatin activity.

Metachromatic properties of AO provide additional information on changes in chromatin activity. Interaction with single-stranded nucleic acids shifts AO fluorescence spectrum toward a red range. The red to green fluorescence ratio (I_{615}/I_{530}) significantly increased after the incubation with hydrocortisone — HDL mixture (Table 1). This shift attests to not only enhanced expression of active genes, but also to activation of new genes. The latter is supported by the fact that the combined exposure to glucocorticoids and HDL accelerates incorporation of H^3-uridine into RNA and ^{14}C-leucine into proteins [3].

TABLE 1. Molar Concentration of Acridine Orange Binding Sites (*1*) and a Single-Stranded/Double Stranded DNA Ratio (*2*) in Nuclei Isolated from Rat Liver Slices (*M±m*)

Parameter	Control	Experiment
1	7.02±0.54 (35)	13.76±1.68* (25)
2	8.35±0.21 (117)	9.20±0.25* (17)

Note. Number of measurements is indicated in parentheses. Here and in Table 2: *$p<0.05$ in comparison with the control.

Electron microscopy of perfused liver revealed some new facts clarifying this mechanism. Two HDL subtypes (HDL_2 and HDL_3) were separately labelled with colloid gold and added to the perfusion medium together with hydrocortisone. After passing the blood-tissue barrier these compounds were taken up by endotheliocytes and macrophages through receptor-mediated endocytosis. After 1-h perfusion without stimulation of sinusoid cells endotheliocytes accumulated equally slow HDL of both subtypes, while the content of HDL_3 in macrophages 3-fold surpassed that of HDL_2 (Fig. 1, *a*). LPS dramatically increased labeling and after 10-min perfusion the uptake of HDL_3 by macrophages surpassed that of HDL_2 (Fig. 1, *b*). In none of these experiments the count of labeled hepatocytes exceeded 3%.

Liver perfusion with HDL_2 and hydrocortisone did not change the morphometric parameters of hepatocyte nuclei (Table 2). HDL_2 in combination with hydrocortisone and LPS or HDL_3 with hydrocortisone increased the relative volume of the granular component in nucleoli, which attests to enhanced synthesis of ribosomal subunit precursors [2]. Since no significant changes were observed in other relevant parameters we considered these changes as spontaneous and nonspecific.

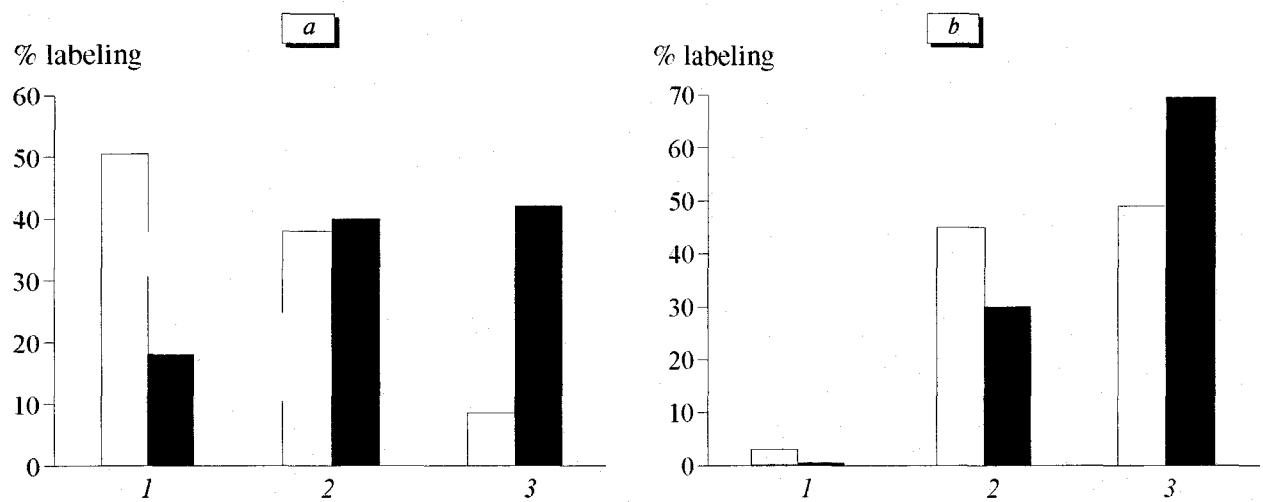

Fig. 1. Distribution of labeled HDL_2 (open bars) and HDL_3 (filled bars) in extracellular space (*1*), endotheliocytes (*2*), and macrophages (*3*) of the liver *in vitro* perfused with hydrocortisone. The data are averaged over 1 h before (*a*) and 10 min after (*b*) addition of lipopolysaccharide.

TABLE 2. Morphometric Indices of Hepatocyte Nuclei after Liver Perfusion with Eagle's Medium with Test Compounds ($M \pm m$)

Experiments	Nucleus				Relative volume of nucleolus granular component, %
	diameter, μ	relative volume, %		pore density per 10 μ membrane	
		euchromatin	nucleolus		
Control	7.3±0.15	71.4±0.68	8.0±0.52	2.7±0.13	34.6±2.11
HDL_2+HC	6.8±0.17	73.1±0.97	9.2±0.56	2.5±0.18	37.2±1.71
HDL_2+HC+LPS	7.4±0.17	74.6±0.94	8.4±0.56	2.2±0.21	50.0±1.80*
HDL_3+HC	7.1±0.15	73.7±0.68	8.4±0.59	3.0±0.15	46.6±1.93*
HDL_3+HC+LPS	6.6±0.16*	68.4±0.85	11.1±0.58*	5.1±0.34*	69.9±3.52*

Note. Mean values for 10-60-min perfusion are presented. HC, hydrocortisone, HDL_2, HDL_3, high density lipoproteins of the second and third subtypes, LPS, lipopolysaccharide.

HDL_3 in combination with hydrocortisone and LPS slightly reduced the volume of nuclei and induced minor condensation of euchromatin as soon as after 10-min incubation. Nucleoli and their granular components reached their maximum relative volumes (11.6± 1.12 and 84.3±1.06%, respectively) and acquired nucleonemic structure. The fibrillar component appeared loosened and finely dispersed with multiple fibrillar centers. These changes persisted during 30-min perfusion, while the density of nuclear membrane pores increased more than 2-fold compared to the control (7.3±0.46 per 10 μ), which indicated active transport of nucleolar synthesis products into the cytoplasm. The signs of activation of nuclear apparatus persisted after 60-min perfusion.

Thus, two independent methods showed that the combined action of HDL and glucocorticoids (cooperative effect) induces functional changes in the nuclear apparatus of rat hepatocytes. These changes more rapidly appeared (within 10 min) after stimulation of sinusoid cells with LPS and manifested themselves in nucleolar activation and enhanced synthesis of ribosomal subunits. The increased binding and red fluorescence of AO attest to chromatin activation and enhancement of gene expression. Resident macrophages play a key role in the realization of this mechanism.

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