Effect of Tetrahydrocortisol on Protein Biosynthesis in Hepatocytes

L. E. Panin, I. F. Usynin, and A. V. Khar'kovskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 2, pp. 171-173, February, 2000
Original article submitted July 26, 1999

Cooperative effect of high-density lipoproteins and glucocorticoids on protein biosynthesis in hepatocytes was associated with the formation of biologically active hormone in Kupffer cells. Apolipoprotein A-I, a constituent of high-density lipoproteins, transported the active hormone from liver macrophages to hepatocytes. Apolipoprotein A-I-induced stimulation of protein biosynthesis was observed only in Kupffer cells. Apolipoprotein A-I alone or in combination with hormones did not change the rate of protein biosynthesis in liver endotheliocytes.

Key Words: *glucocorticoids; tetrahydrocortisol; high-density lipoproteins; apolipoprotein A-I; hepatocytes; Kupffer cells*

Kupffer cells (KC) are involved in the regulation of various processes. Synthesis of interleukins, prostaglandins, and other cytokines in KC attracts considerable attention. Inteleukin-1, and inteleukin-6 and tumor necrosis factor-α regulate the biosynthesis and production of acute-phase proteins in the liver [8]. However, these cytokines did not affect protein biosynthesis in hepatocyte culture [14], which attests to specificity of their effects in the liver.

KC are involved in the cooperative effect of high-density lipoproteins (HDL) and glucocorticoids on gene expression and protein biosynthesis in hepatocytes [3]. Stimulation of KC with lipopolysaccharides (LPS) potentiates HDL_3 uptake and stimulates protein biosynthesis in the liver [5]. These changes specifically involve hepatocytes, but not nonparenchymal cells [4].

Here we evaluated the role of KC in these processes.

MATERIALS AND METHODS

Experiments were performed on female outbred albino rats weighing 180-200 g. Liver cells were isolated as described elsewhere [13] with some modifications [6]. The liver was reperfused with 0.03% collagenase (Boehringer Mannheim). After dissociation, hepatocytes were purified from nonparenchymal cells by differential centrifugation. Nonparenchymal cells were fractionated in a J2-21 centrifuge (Beckman) equipped with a JE-6 a counterflow elutriation rotor at 2500 rpm. KC and endotheliocytes were washed at flow rates of 22 and 42 ml/min, respectively [11]. The cells were counted in a Goryaev chamber. The viability and purity of cell fractions were estimated by trypan blue exclusion under light or electron microscopes.

The cells resuspended in DMEM medium containing 15 mM HEPES, 10% fetal serum, and 50 μg/ml gentamicin were incubated in a CO_2 incubator in 24-well plates (Linbro) coated with type I collagen. The primary culture contained 700 hepatocytes and 7000 endotheliocytes and KC per 1 mm^2 (final densities). HDL, apolipoprotein A-I (apoA-I, 100 μg/ml medium), cortisol, tetrahydrocortisol (final concentration 10^{-6} M), and *Serratia marcescens* LPS (prodigiozan, Moscow Plant of Chemical and Pharmaceutical Reagents, 7.5 μg/ml medium) were added to the incubation medium.

^{14}C-Leucine (3.7×10^4 Bq/ml) was added to wells 2 h before the end of incubation to estimate the rate

Institute of Biochemistry, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

0007-4888/00/0002-146$25.00 ©2000 Kluwer Academic/Plenum Publishers

of protein biosynthesis in cultured cells. The reaction was stopped with 0.2 N NaOH, and the content of each well was placed on membrane filters to measure radioactivity.

Plasma HDL were isolated by centrifugation in a KBr density gradient [10] using a L5-75 centrifuge (Beckman) equipped with a 75Ti rotor. The results were analyzed by Student's *t* test.

RESULTS

Previous experiments on cultured liver slices retaining peculiar cell-cell interactions showed that HDL and glucocorticoids considerably increased the rates of ^{3}H-uridine and ^{14}C-leucine incorporation into RNA and total protein, respectively [1]. Our studies on isolated liver cells demonstrated that HDL, cortisol, and LPS did not accelerate protein biosynthesis in primary hepatocyte culture. Their simultaneous addition into the incubation medium produced no effects (Table 1). *In vivo* stimulation of resident macrophages with LPS only slightly increased the rate of protein biosynthesis in nonparenchymal liver cells. In the present study, conditioned medium after incubation of these cells with cortisol, HDL, or LPS transferred to primary hepatocyte culture had no effect on the rate of protein biosynthesis in hepatocytes. At the same time, incubation of cells in the presence cortisol, HDL, and LPS markedly increased the rate of protein biosynthesis in hepatocytes.

These data indicate that the cooperative effect of HDL and glucocorticoids on protein biosynthesis in the liver is associated with biotransformation of glucocorticoids in nonparenchymal cells, in particular, in macrophages, leading to the formation of bi- and tetrahydrohormones. This process depends on the presence of 5α- and 5β-reductases restoring double bonds between positions 3 and 4 in ring A of the hormone with the formation of tetrahydrocompounds [12]. After stimulation with LPS, macrophages, but not endotheliocytes, internalize HDL_3 [2], whose protein components consist mainly of apoA-I [9].

TABLE 1. Effect of Incubation Medium of Nonparenchymal Liver Cells Cultured with HDL, Cortisol, and LPS for 24 h on Protein Biosynthesis in Hepatocytes (*M*±*m*, *n*=9)

Culture conditions	^{14}C-leucine incorporation into total protein, cpm/well	
	control (DMEM medium)	test (incubation medium)
Without additives	19,790±1190	18,610±1790
LPS	18,490±1190	17,740±1530
Cortisol	17,750±1460	14,780±980
HDL	17,890±1410	16,470±680
HDL+cortisol+LPS	18,810±1950	33,940±5210*

Note. *Significant differences compared with the control.

Taking this fact into account, in further experiments we incubated hepatocytes, KC, and endotheliocytes with various bioactive substances. Cortisol and tetrahydrocortisol did not stimulate protein biosynthesis in liver cells (Table 2). ApoA-I significantly increased the rate of ^{14}C-leucine incorporation into proteins only in KC. Our previous experiments showed that HDL and apoA-I display high affinity for steroid hormones [2]. The complex consisting of apoA-I and cortisol significantly inhibited protein biosynthesis in hepatocytes, while the apoA-I-tetrahydrocortisol complex considerably stimulated this process.

It was believed that biological inactivation of steroid hormones begins with reduction of double bond in ring A of glucocorticoids and the formation of tetrahydrocompounds [7] and leads to the production of water-soluble glucuronides excreted with urine. Our experiments showed for the first time that glucocorticoid tetrahydroderivatives represent biologically active

Table 2. Effects of ApoA-I, Cortisol, and Tetrahydrocortisol on Protein Biosynthesis in Primary Cultures of Hepatocytes, KC, and Liver Endotheliocytes (*M*±*m*, *n*=9)

Incubation conditions	Protein biosynthesis, cpm/well		
	hepatocytes	KC	endotheliocytes
Control	3490±70	1420±100	1580±190
Cortisol	3190±60	1380±140	1470±190
Tetrahydrocortisol	3630±120*	1540±310	1220±190
ApoA-I	3840±90*	2790±420*	1640±180
Cortisol+ApoA-I	3080±60*	1832±190	1550±80
Tetrahydrocortisol+ApoA-I	4860±500*+	1890±390	1350±300

Note. Significant differences: *compared with the control and +compared with apoA-I.

hormones involved in the regulation of protein biosynthesis in hepatocytes, and that apoA-I transports these hormones from KC to parenchymal liver cells.

Author is grateful to Yu. A. Pankov (Academician of the Russian Academy of Medical Sciences) for gifted tetrahydrocortisol.

REFERENCES

1. L. E. Panin, *Vopr. Med. Khim.*, **33**, No. 5, 96-102 (1987).
2. L. E. Panin, *Byull. Sib. Otd. Rus. Acad. Med. Sci.*, No. 2, 62-69 (1996).
3. L. E. Panin, M. V. Sokolova, and I. F. Usynin, *Ibid.*, No. 4, 98-100 (1989).
4. L. E. Panin, M. V. Sokolova, and I. F. Usynin, *Tsitologiya*, No. 5, 528-532 (1990).
5. L. E. Panin, I. F. Usynin, O. M. Trubitsyna, *et al.*, *Biokhimiya*, **59**, No. 5, 353-359 (1994).
6. I. F. Usynin, *New Methods of Scientific Studies in Clinical and Experimental Medicine* [in Russian], Novosibirsk (1980), pp. 96-98.
7. N. A. Yudaev, S. A. Afinogenova, and M. A. Krekhova, *Biochemistry of Hormones and Endocrine Regulation* [in Russian], Moscow (1976).
8. K. Decker, *Eur. J. Biochem.*, **192**, 245-261 (1990).
9. A. Eklund and L. Sjoblom, *Biochem. Biophys. Acta*, **877**, 135-140 (1986).
10. F. T. Hatch and R. S. Lees, *Adv. Lipid Res.*, **6**, 2-68 (1968).
11. D. L. Knook, N. Blansjaar, and E. C. Slyster, *Exp. Cell Res.*, **109**, 317-329 (1977).
12. N. J. Sawyer, J. T. Oliver, and R. S. Troop, *Steroids*, **2**, 213 (1963).
13. P. Seglen, *Methods Cell Biol.*, **13**, 30-83 (1976).
14. M. A. West, T. R. Billiar, J. E. Mazuski, *et al.*, *Arch. Surg.*, **123**, 1400-1405 (1988).