BIOPHYSICS AND BIOCHEMISTRY

Effect of Complexes of Apolipoprotein A-I with Tetrahydrocortisol and Pregnenolone on Protein Biosynthesis in Rat Hepatocytes Culture

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Complexes of apolipoprotein A-I with tetrahydrocortisol and pregnenolone exhibit high biological activity and increase the rate of protein biosynthesis in the culture of rat hepatocytes. An important role in this process is played by reduced Δ^4-3-keto group in the A-ring of steroid hormones. A complex of apolipoprotein A-I and pregnenolone modulated the rate of protein biosynthesis in liver cells. Hence, the observed changes are not organ-specific for this steroid. Our results suggest that this mechanism of regulation play an important role in intracellular regeneration and proliferation.

Key Words: *hepatocytes; apolipoprotein A-I; tetrahydrocortisol; pregnenolone; protein biosynthesis*

Our previous studies showed that reduced steroid hormones, *e.g.*, tetrahydrocortisol (THC), in complexes with apolipoprotein A-I (apoA-I) increase the rate of protein biosynthesis in the primary culture of rat hepatocytes [3,9]. Reduced Δ^4-3-keto group in the A-ring of steroid hormones is required for activation of gene expression. Our results are consistent with the role of apoA-I in hormone transport in transcriptionally active chromatin, nuclear matrix, and fraction of acid nonhistone proteins [2].

Reduced Δ^4-3-keto group is present in some steroids, *e.g.* pregnenolone, a precursor of the female sex hormone progesterone. Progesterone binds to cell membrane receptors in target organs (uterus and placenta), enters the cell, interacts with cytosolic receptors, and is transported into the nucleus. In the nucleus, progesterone activates a set of genes, which involves RNA polymerase 2 [7]. Progesterone modulates activity of isocitrate dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, and other enzymes in target organs.

Here we compared the effects of two pairs of steroid hormones (hydrocortisone—THC and progesterone—pregnenolone) in the complex with apoA-I on the rate of protein biosynthesis in the primary hepatocyte culture.

MATERIALS AND METHODS

Experiments were performed on isolated hepatocytes from male Wistar rats weighing 180-200 g. Hepatocytes were isolated by recirculatory perfusion with 0.03% collagenase (ICN Biomedicals, Inc.) and separated from nonparenchymal cells by differential centrifugation. Cell viability assessed by the exclusion of trypan blue (Serva) was ≥90%. The cells were resuspended in RPMI-1640 medium

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(Biolot, pH 7.4) containing 20 mM HEPES (ICN Biomedicals, Inc.), 10% fetal bovine serum (Serva), 2 mM L-glutamine (Vektor), 100 U/ml penicillin, 50 μg/ml gentamicin, 5.6 mM glucose, and 10 nM insulin (Serva). The cells in 6-well plates (Orange Scientific) coated with collagen were incubated in a CO_2 incubator (Cole-Parmer) in atmosphere containing 5% CO_2 and 95% O_2 at 37°C. Cell density in the primary monolayer culture was 800 cells/mm^2.

Blood plasma lipoproteins were isolated by density ultracentrifugation in KBr solutions in the presence of 3 mM EDTA an Optima L-90K ultracentrifuge (Beckman Coulter). High-density lipoprotein (HDL) fractions were delipidated by treatment with a cold mixture of ethanol and acetate (1:1) and repeated washing with ether. ApoA-I was isolated by the method of gel filtration. ApoA-I purity was estimated by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate (Serva).

Complexes of apoA-I and hormones were formed with hydrocortisone, progesterone, and pregnenolone (Serva). The reduced form of steroid hormone THC was presented by Yu. A. Pankov (Academician of the Russian Academy of Medical Sciences, Institute of Experimental Endocrinology). Complexes of apoA-I with hormones were obtained by incubation of their mixture (1:2 molar ratio) in 0.05 M potassium-phosphate buffer (pH 7.4) containing 0.15 mM NaCl at room temperature for 5 min. The concentrations of apoA-I and hormone in the incubation medium were 60 μg/ml and 5×10^{-6} M, respectively.

The rate of protein biosynthesis in cultured hepatocytes was evaluated by ^{14}C-leucine (Amersham) incorporation. The label (2 μCi/ml medium) was added 3 h before the end of incubation. Radioactivity of samples was measured on a Mark-III scintillation counter and expressed in cpm/mg protein. The results were analyzed by Student's *t* test. The significance level was $p<0.05$.

RESULTS

Two independent pairs of steroid hormones were used to study the regulation of gene expression associated with an increase in the protein biosynthesis rate. The 1st pair included adrenal cortex hormones, hydrocortisone, and its reduced form (THC) containing oxidized and reduced Δ^4-3-keto group in the A-ring, respectively. Hydroxyl in the 3-position of THC is in the trans-position. The 2nd pair of hormones was progesterone and pregnenolone. By the structure of the A-ring these hormones correspond to hydrocortisone and THC, respectively. However, the hydroxy group in the 3-position of pregnenolone is in the cis-position.

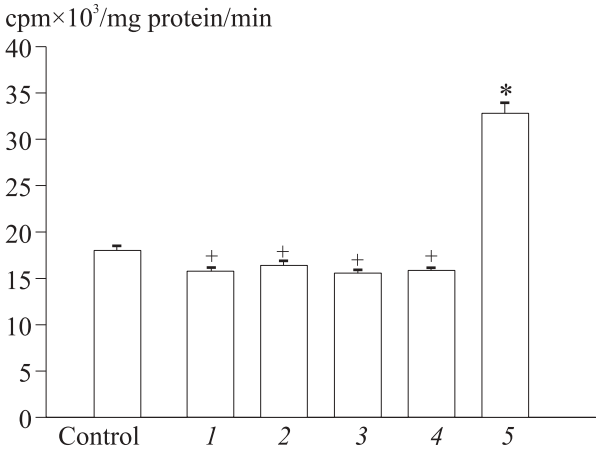

Fig. 1. Rate of protein biosynthesis in rat hepatocytes after incubation with complexes of apoA-I with hydrocortisone and THC: apoA-I (*1*), hydrocortisone (*2*), THC (*3*), apoA-I-hydrocortisone (*4*), and apoA-I-THC (*5*). **p*<0.001 compared to the control; +*p*<0.05 compared to an apoA-I-THC complex.

In our previous experiments we used apoA-I as a carrier of steroid hormones and showed that reduced Δ^4-3-keto group played an important role in activation of gene expression in the liver under the influence of steroid hormones. The molecular mechanisms of this process were evaluated [4,8]. We also showed that only apoA-I—THC complex significantly increases the rate of protein biosynthesis in hepatocytes (Fig. 1). Hydrocortisone, apoA-I—hydrocortisone complex, and THC were little effective.

Experiments with the pregnenolone-progesterone pair showed that only the complex of apoA-I

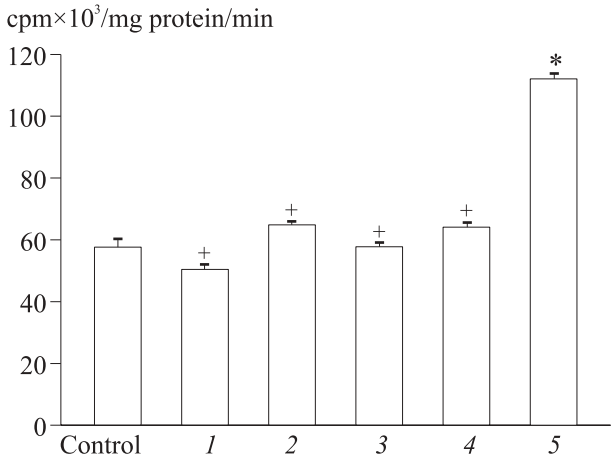

Fig. 2. Rate of protein biosynthesis in rat hepatocytes after incubation with complexes of apoA-I with progesterone and pregnenolone: apoA-I (*1*), progesterone (*2*), pregnenolone (*3*), apoA-I-progesterone (*4*), and apoA-I—pregnenolone (*5*). **p*<0.001 compared to the control; +*p*<0.05 compared to apoA-I—pregnenolone complex.

and pregnenolone significantly increases the rate of protein biosynthesis in cultured hepatocytes (Fig. 2), while apoA-I—progesterone complex, progesterone, and pregnenolone induced only minor changes. These results are consistent with data on the hydrocortisone-THC pair. It should be emphasized that the hydroxy group in the 3-position of the hormone molecule (THC and pregnenolone) does not play an important role. When the effect is expressed in percent of the control, the cis-position has some advantages over the trans-position (increase in the protein biosynthesis rate by 95 and 82%, respectively).

Our results indicate that not only reduced hydrocortisone (THC), but also pregnenolone with similar structure of the A-ring exhibited biological activity in the complex with apoA-I. Previous studies showed that plasma HDL bind and transport a variety of steroid hormones, including corticosterone, deoxycorticosterone, hydrocortisone, and pregnenolone [1]. Progesterone also interacts with HDL. Kupffer cells play an important role in HDL metabolism in the liver [6]. They bind HDL carrying progesterone, disintegrate HDL with the formation of apoA-I in secondary lysosomes of Kupffer cells, and reduce steroid hormones present in HDL with the formation of tetrahydro compounds, since activity of α-reductase and β-reductase is very high in liver macrophages. ApoA-I and hormone then form a bioactive complex. Reduction of progesterone leads to the formation of the bioactive complex of apoA-I and pregnenolone, which accelerates protein biosynthesis. This mechanism of the formation of the apoA-I—THC complex was discussed previously [4]. It can be hypothesized that this mechanism mediates progesterone-induced increase in proliferative processes in the uterus and mammary glands in pregnant women.

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