BIOPHYSICS AND BIOCHEMISTRY

Role of Apolipoprotein A-I in Steroid-Induced Activation of DNA and Protein Synthesis in Hepatocytes

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It is demonstrated that anabolic effect of steroid hormones is produced by steroid-apolipoprotein A-I complexes. These complexes accelerate not only protein, but also DNA synthesis and produce both cell hypertrophy and hyperplasia. For realization of their anabolic effect steroid hormones are modified by α- and β-reductases in resident macrophages.

Key Words: *protein biosynthesis; DNA biosynthesis; steroid hormones*

Protein and DNA syntheses in various cells are regulated by hormones, first of all, steroid hormones with pronounced anabolic activity [2,7], including adrenal hormones (dehydroepiandrosterone) and sex hormones (androgens and estrogens). The mechanisms of their effects on DNA and protein metabolism remain unclear. DNA replication in target cells is preceded by activation of protein synthesis [1].

We have previously demonstrated that tetrahydroderivatives of glucocorticoid hormones in a complex with apolipoprotein A-I (apoA-I) accelerate protein synthesis in hepatocytes [4]. Reduction of Δ^4,3-keto group of glucocorticoid A-ring is performed by resident macrophages [12]. These cells bind HDL and release apoA-I [5]. It was shown that HDL specifically bind various steroid hormones [3]. This binding is mediated by apoA-I.

Experiments with tetrahydrocortisol showed that Δ^4,3-keto-group plays an important role in activation of gene expression. Reduction of this group in macrophages by α- and β-reductases yields OH-group in the C3 position of the A-ring. This group can form hydrogen bonds with DNA bases. This determined the choice of test hormones in the present study.

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Androsterone, a testosterone metabolite possessing 10% testosterone activity, contains OH-group in the cis-configuration. Dehydroepiandrosterone (hormone of the reticular zone of the adrenal gland) contains OH-group in the trans-configulation, while its androgenic activity is about 10% of testosterone activity. This hormone is released in a sulfated form. Sulfate residue is bound to the C3 position instead of OH-group. This form actively forms hydrogen bonds. Tetrahydrocortisol, the main cortisol metabolite, contains OH-group in thje cis-configuration and C5 hydrogen atom in the trans-configuration.

Our aim was to study the effect of steroid hormones alone or in a complex with apoA-I on DNA and protein biosynthesis in hepatocytes.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180-200 g. Serum lipoproteins were isolated by ultracentrifugation in a KBr [9] gradient on an L5-75 centrifuge equipped with 75 Ti rotor (Beckman L5-75). The isolated lipoproteins were delipidated with cold (-16°C) ethanol-diethyl ester mixture. ApoA-I was isolated by gel filtration on Sepharose 4B (Pharmacia) in 0.01 Tris-HCl buffer (pH 8.6) containing 6 M urea. Peak 2 was purified by ion-exchange chromatography on DEAE-Toyopearl 650 M (TSK) in 0.01 Tris-HCl

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TABLE 1. Effect of Test Hormones and ApoA-I on the Rate of Protein and DNA Synthesis in Cultured Hepatocytes (*M*±*m*)

Test substances	^{14}C-Leucine incorporation, cpm/well		^{3}H-Thymidine incorporation, cpm/well	
	without apoA-I	with apoA-I	without apoA-I	with apoA-I
Control	760.10±34.72	385.5±21.1^{+}	4469.7±157.9	3319.7±511.1^{+}
Androsterone	676.6±32.0	481.5±31.1^{*+}	3731.9±190.2*	4258.7±430.7
Dehydroepiandrosterone	996.9±42.4*	643.1±58.5^{*+}	4310.5±129.8	3877.9±107.0^{+}
Dehydroepiandrosterone sulfate	852.2±51.1	1881.6±149.1^{*+}	3333.3±297.7*	4921.0±104.4^{*+}
Tetrahydrocortisol	692.1±34.7	1098.8±107.4^{*+}	3353.8±333.9*	4928.0±115.3^{*+}

Note. $p<0.05$: *compared to the corresponding control, $^{+}$compared to wells without apoA-I.

buffer (pH 8.6) containing 6 M urea. The protein was eluted with the initial buffer using a 0.01-0.5 M NaCl gradient. The purity of isolated apoA-I was verified by PAAG-electrophoresis [10] using Pharmacia weight marker kit. Protein concentration was measured after Lowry [11]. Dehydroepiandrosterone (Amersham) and Dehydroepiandrosterone sulfate (Sigma) were used. Androsterone and tetrahydrocortisol were kindly provided by Yu. A. Pankov, Academician of the Russian Academy of Medical Sciences.

Liver cells were isolated after recirculation perfusion with 0.3% collagenase-I (Sigma). Hepatocytes and nonparenchimatous cells (NPC) were fractionated by differential centrifugation [11]. The yields were (38±3)×10^{6} hepatocytes and (40±3)×10^{6} NPC per 1 g tissue, respectively. According to the trypan blue exclusion test, 90% hepatocytes and 95% NPC were viable. Hepatocytes were resuspended in 5 ml Krebs—Ringer phosphate buffer (2-3 mg cell protein per 1 ml buffer) and incubated on 24-well plates (Linbro) coated with type I collagen for 1 day. Test hormones were added to the incubation medium in a concentration of 10^{-6} M, apoA-I in a concentration of 70 μg/ml. The rate of protein and DNA synthesis was measured with ^{14}C-leucine and ^{3}H-thymidine added to the incubation medium in a dose of 37 kBq/ml) Radioactivity was measured on a Mark-III counter. Specific activity was calculated in cpm/well. The data were processed statistically using Student's *t* test.

RESULTS

Test hormones had practically no effect on the rate of protein synthesis in hepatocytes (Table 1). The only exception was dehydroepiandrosterone, which considerably accelerated protein synthesis. ApoA-I alone reduced the rate of protein synthesis in hepatocytes by binding with the corresponding sites on DNA. The complex consisting of apoA-I and hormone significantly accelerated protein synthesis, dehydroepiandrosterone, tetrahydrocortisol, and dehydroepiandrosterone sulfate being most effective. The maximum rate was attained with the apoA-I–dehydroepiandrosterone sulfate complex (Table 1). These findings suggest that all test hormone in complexes with apoA-I exhibit anabolic effect and accelerate protein synthesis in the liver by the previously described mechanism [4,6].

All test hormones either inhibited or had no effect on DNA synthesis. ApoA-I alone reduced the rate of DNA synthesis (Table 1). ApoA-I–androsterone and apoA-I–dehydroepiandrosterone complexes did not changes the rate of DNA synthesis in hepatocytes, while apoA-I–dehydroepiandrosterone sulfate and apoA-I- tetrahydrocortisol complexes considerable accelerated this process. Since the rate of ^{3}H-thymidine incorporation reflects mitotic activity, apoA-I–dehydroepiandrosterone sulfate and apoA-I–tetrahydrocortisol complexes not only accelerated protein synthesis, but also promoted cell proliferation.

Thus, anabolic steroids act in the complex with apoA-I. These complexes increase the rate of not only protein, but also DNA synthesis, *i. e*., induce both cell hyperplasia and hypertrophy. Realization of the anabolic effect of many steroids depends on chemical modifications by α- and β-reductases in resident macrophages.

REFERENCES

1. O. I. Epifanova, *Hormones and Cell Proliferation* [in Russian], Moscow (1965).
2. N. P. Mertvetsov, *Regulation of Gene Expression by Steroid Hormones* [in Russian], Novosibirsk (1990).
3. L. E. Panin, L. M. Polyakov, A. A. Rozumenko, and N. G. Biushkina, *Vopr. Med. Khimii*, No. 5, 56-58 (1988).
4. L. E. Panin, F. V. Tuzikov, N. A. Tuzikova, *et al.*, *Mol. Biol.*, **33**, No. 4, 1-6 (1999).
5. L. E. Panin, I. F. Usynin, and O. N. Poteryaeva, *Byull. Eksp. Biol. Med.*, **126**, No. 7, 43-45 (1998).
6. L. E. Panin, I. F. Usynin, O. M. Trubitsina, *et al.*, *Biokhimiya*, **59**, No. 3, 353-359 (1994).
7. P. V. Sergeev, P. A. Galenko-Yaroshevskii, N. L. Shimanovskii, *Essay on Biochemical Pharmacology* [in Russian], Moscow (1996).

8. I. F. Usynin, *New Methods in Clinical and Experimental Medicine* [in Russian], Moscow (1980), pp. 96-98.
9. F. Hatch and R. Lees, *Adv. Lipid Res.*, **6**, 2-68 (1968).
10. U. K. Laemmli, *Nature*, **227**, No. 5259, 680-685 (1970).
11. O. H. Lowry, N. G. Rosenbrough, A. L. Farr, and R. J. Kandall, *J. Biol. Chem.*, **193**, 256-275 (1951).
12. N. Y. Sawyer, J. T. Oliver, and R. S. Troop, *Steroids*, **2**, 213-227 (1963).