Effect of Apolipoprotein A-I Complex with Tetrahydrocortisone on Protein Biosynthesis and Glucose Absorption by Rat Hepatocytes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 8, pp. 173-175, August, 2009
Original article submitted February 11, 2009

We studied the effect of apolipoprotein A-I—tetrahydrocortisone complex on ^{14}C glucose absorption and lactate accumulation and on the rate of protein biosynthesis in isolated rat hepatocytes. The presence of apolipoprotein A-I—tetrahydrocortisone complex in the incubation medium increased absorption of labeled glucose by hepatocytes by 52%, while lactate content in the conditioning medium increased 4-fold. The rate of protein biosynthesis increased by 80% in comparison with control cells. It is hypothesized that the increase in protein biosynthesis rate in hepatocytes under the effect of apolipoprotein A-I—tetrahydrocortisone complex is due to stimulation of energy metabolism, specifically, of its glycolytic component.

Key Words: *hepatocytes; apolipoprotein A-I; tetrahydrocortisone; protein biosynthesis; glycolysis*

We previously showed that reduced forms of steroid hormones (tetrahydrocompounds) are characterized by high biological activity and in complex with apolipoprotein A-I (apoA-I) increase the rate of protein and nucleic acid biosynthesis in normal and tumor cells [1-4]. These complexes are formed in resident macrophages during cooperative capture of HDL and steroid hormones [14]. The molecular mechanisms underlying the effects of apoA-I—tetrahydrocompound complexes were studied [13]. Acceleration of biosynthetic processes is associated with stimulation of energy metabolism.

We studied the glycolytic component of energy metabolism under the effect of apoA-I—tetrahydrocortisone (ApoA-I—THC) complex.

MATERIALS AND METHODS

The study was carried out on isolated hepatocytes of male Wistar rats (180-200 g). Hepatocytes were isolated by recirculatory enzymatic perfusion with 0.03% collagenase solution (ICN Biomedicals Inc.) and separated from nonparenchymatous cells by differential centrifugation. Cell viability was evaluated by trypan blue (Serva) exclusion method and was at least 90%. The resultant cells were resuspended in RPMI-1640 (Biolot), pH 7.4, containing 20 mM HEPES (ICN Biomedicals Inc.), 10% FCS (Serva), 2 mM L-glutamine (Vector), 100 U/ml penicillin, 50 μg/ml gentamicin, 5.6 mM glucose, and 10 nM insulin (Serva). The cells were incubated in a CO_2 incubator (Cole-Parmer) at 5% CO_2 and 95% air and 37°C in 6-well plates (Orange Scientific) coated with collagen-1 (Serva). Cell density in primary monolayer culture was 800 cell/mm^2.

Plasma HDLP were isolated by isodense centrifugation in KBr solutions with 3 mM EDTA-Na_2 on an Optima L-90K centrifuge (Beckman Coulter). Delipidation of HDLP was carried out by cold ethanol:acetate (1:1) mixture with subsequent repeated washing in ether. ApoA-I was isolated by gel filtration (column: 1.6×100 cm, CL-6B Sepharose (Amersham Biosciences); eluent: 0.01 M Tris-HCl buffer (pH 6.8) with

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6 M urea, 0.01% sodium azid, 1 mM phenylmethane sulfonylfluoride). The elution profile was recorded using an UV detector 2151 (LKB) at λ=280 nm. Analysis of apoA-I purity was carried out by PAAG disc electrophoresis with sodium dodecylsulfate (Serva). A set of low-molecular reference proteins served as the markers. Protein bands were visualized with 0.1% Coumassie G-250 in methanol and 10% acetic acid mixture (1:1). ApoA-I was separated from urea by gel filtration (column: 0.8×30 cm, Sephadex G-25 (Amersham Biosciences); eluent: 0.05 M potassium phosphate buffer (pH 7.4) with 0.15 M NaCl).

Tetrahydrocortisone (kind gift from Academician Yu. A. Pankov, Institute of Experimental Endocrinology) served as the reduced form of steroid hormones. ApoA-I—THC was obtained by 5-min exposure of a mixture of apoA-I and THC in 1:2 molar proportion in 0.05 M potassium phosphate buffer (pH 7.4) with 0.15 M NaCl at ambient temperature. The concentrations of ApoA-I and THC in incubation medium were 60 μg/ml, 5×10^{-6} M, respectively.

The rate of protein biosynthesis in hepatocyte culture was evaluated by incorporation of ^{14}C leucin (Amersham) in a concentration of 2 μCi/ml medium. The reaction was stopped by addition of 0.2 n NaOH. The contents of the well was transferred onto cellulose filters (Whatman 3 MM) and radioactivity was measured. Glucose absorption by hepatocytes was evaluated by the level of ^{14}C glucose (Amersham) in cell lysate. ^{14}C-glucose was added to incubation medium in a concentration of 2 μCi/ml. Binding of ^{14}C glucose to apoA-I and ApoA-I—THC was studied by gel filtration (column: 0.8×30 cm, Sephadex G-25 (Amersham Biosciences); eluent: 0.15 M NaCl, 0.05 Tris-HCl buffer, pH 8.0). Radioactivity was measured on a Mark-III scintillation counter.

Lactate was measured by the enzymatic method [9] using standard reagents (Boehringer Mannheim) and Hitachi spectrophotometer at λ=340 nm.

The results were statistically processed using Student's *t* test at $p<0.05$ level of significance.

RESULTS

The ApoA-I—THC complex accelerated protein biosynthesis in rat hepatocyte culture by 80% in comparison with the control (Fig. 1). It is noteworthy that ApoA-I or THC alone did not modify the protein biosynthesis. The rate of protein biosynthesis is an energy-consuming process associated with activation of energy metabolism. The presence of ApoA-I—THC in the incubation medium increased absorption of labeled glucose by 52% in comparison with the control cells (Fig. 2). This was paralleled by significant accumulation of lactate in conditioning medium: 0.77±0.05 mg/dl *vs.* 0.18±0.02 mg/dl in the control ($p<0.001$). These results indicated stimulation of glycolytic component of the energy metabolism and increased hepatocyte membrane permeability for lactate.

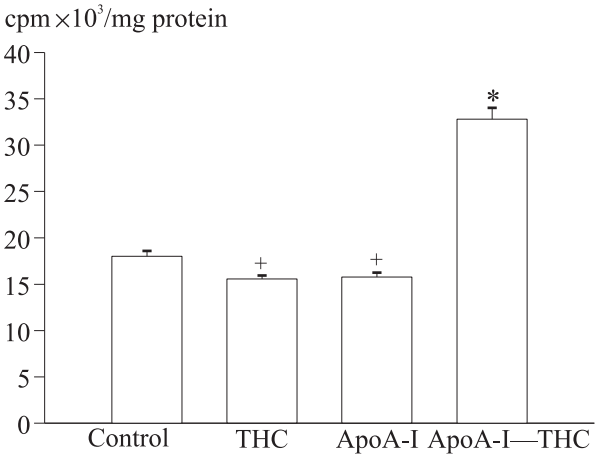

Fig. 1. Effect of ApoA-I—THC on the rate of protein biosynthesis in rat hepatocyte culture. $p<0.001$ compared to: *control, +ApoA-I—THC.

Presumably, ApoA-I serves as an extra carrier of glucose into cells under conditions of our experiment. The capacity of HDLP and their main structural component apoA-I to bind and transport compounds of different nature, *e.g.* steroids [5,10,12], thyroid hormones [8], tocopherols [7], oligonucleotides [11], polysaccharides [6], xenobiotics [5], *etc.* was demonstrated not once. We studied the possibility of binding of labeled glucose to apoA-I and to ApoA-I—THC by column chromatography on Sephadex G-25. In the former case, ^{14}C glucose was released in the same volume as apoA-I, which fact indicated that they formed a complex, while in the latter case the output of labeled glucose and ApoA-I—THC did not coincide (Fig. 3). This means that apoA-I in complex with THC did not bind glucose and hence, could not carry it.

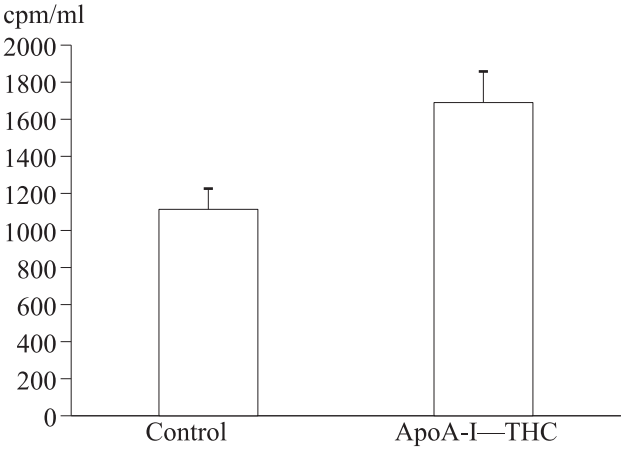

Fig. 2. Effect of ApoA-I—THC on absorption of ^{14}C glucose by isolated rat hepatocytes. *$p<0.05$ compared to control.

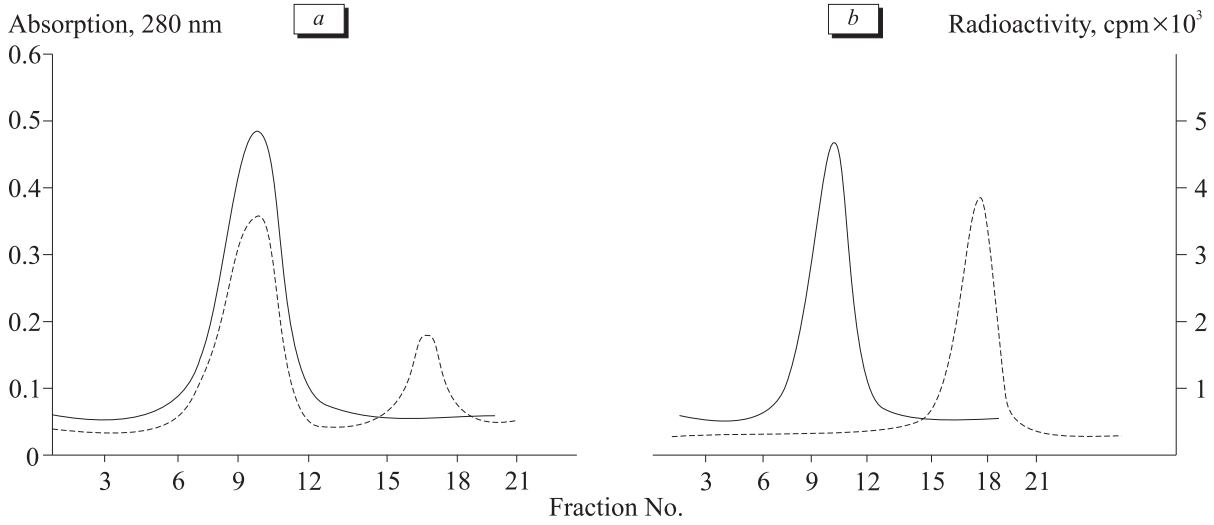

Fig. 3. Chromatographic analysis of ^{14}C glucose binding to apoA-I and ApoA-I—THC. *a*) ^{14}C glucose and apoA-I; *b*) ^{14}C glucose and ApoA-I—THC. Solid line: protein absorption at 280 nm; dotted line: radioactivity.

Hence, acceleration of biosynthetic processes (protein synthesis) in rat hepatocytes under conditions of their incubation with ApoA-I—THC is associated with stimulation of energy metabolism, specifically, of its glycolytic component.

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