Effects of 25-Hydroxycholesterol and Progesterone on Viscosity, Cholesterol Esterification, and Protein-Lipid Interactions in Macrophage Membranes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 2, pp. 145-148, February, 2000
Original article submitted January 20, 2000

25-Hydroxycholesterol stimulates and progesterone inhibits cholesterol esterification in mouse peritoneal macrophages in a dose-dependent manner. Fluorescence assay showed nonlinear changes in lipid viscosity and protein-lipid interactions in macrophage membranes under the effect of various doses of 25-hydroxycholesterol and progesterone, therefore opposite effects of studied steroids on acyl-CoA:cholesterol acyltransferase activity were not necessarily related to these factors.

Key Words: *oxysterols; cholesterol esterification; macrophage membranes; viscosity; protein-lipid interactions*

Biological activity of steroids is primarily realized through modulation of gene expression and interaction with cell membranes. Steroid-induced changes in membrane structure affect the functioning of membrane-bound enzymes. Acyl-CoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is a typical membrane-bound enzyme involved in cholesterol (CH) esterification in cells [10]. Its activity in macrophages sharply increases during internalization of modified low-density lipoproteins (LDL) and excessive release of CH into the cytoplasm [6,12], underlying the formation of foam cells enriched with cholesteryl esters (CE) in atherosclerotic vessels. Regulation of ACAT is poorly understood. It was shown that the CH-induced activation of this enzyme does not depend on the expression of the corresponding gene [11,13]. Our previous experiments on cultured macrophages showed that endogenous oxidized CH derivatives (oxysterols) act as mediators in CH-induced stimulation of CE formation [3]. Some oxysterols and steroid hormones markedly affect the rate of CH esterification in various cells. Progesterone inhibits ACAT [2], while some oxysterols and especially 25-hydroxycholesterol (25-OH-CH) increase ACAT activity in macrophages independently on the cell content of CH [4]. It was shown that progesterone [8] and 25-OH-CH [9] regulate activity of membrane-bound enzymes, in particular, Na^+/K^+-ATPase, by modulating physicochemical properties of membranes.

Here we studied the interrelation between progesterone- and oxysterol-induced changes in lipid viscosity, protein-lipid interactions, and the rate of CE formation in mouse peritoneal macrophages by using a fluorescence assay.

MATERIALS AND METHODS

Macrophages were obtained from peritoneal exudate of C57Bl/6 mice on day 5 after intraperitoneal injection of 5% glycogen. The cells (2×10^6/dish) were incubated in plastic Petri dishes (Falcone) in RPMI-1640 medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin at 37°C (95% air and 5% CO_2) for 12 h [3]. After 12-h incubation, cell monolayer was washed with Hanks' solution and incubated with (or without) various concentrations (1-10 μg/ml) of 25-OH-CH, 7-ketocholesterol (7-ketoCH), 7β-OH-

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0007-4888/00/0002-124$25.00

CH, 22(S)-OH-CH, and progesterone (Sigma) in RPMI-1640 medium containing 50 μg/ml gentamicin and 0.2% bovine serum albumin (BSA) at 37°C for 2 h. The synthesis of CE and triglycerides was estimated by incorporation of 1-^{14}C-oleate (Amersham) into cell lipids. Macrophages were cultured in RPMI-1640 medium containing 0.2 mmol labeled oleate adsorbed on BSA, and the cell monolayer was washed with Hanks' solution. Cell lipids were extracted with isopropanol-hexane (3:2, intravenous) and separated by thin-layer chromatography on Silica G. The radioactivity of CE and triglycerides was measured in a Mark-III scintillation counter (Tracor Analytical) using a toluene scintillator [3,4]. The rate of CH esterification was expressed in nmol esterified oleate formed over a 4-h incubation.

Viscosity of macrophage membrane lipids was estimated using fluorescence probes pyrene (Serva) and DSP-12 (Zonde) and then calculated by comparing the ratio between the efficiency of pyrene excimer formation at 334 nm and the efficiency of resonance energy transfer from pyrene (donor) to DSP-12 (acceptor) in studied membranes and yolk phosphatidylcholine liposomes with known viscosity [1]. Protein-lipid interactions were determined by the efficiency of resonance energy transfer from tryptophan residues in membrane proteins to pyrene localized in the lipid bilayer [1]. Fluorescence was measured in phenol red-free Hanks' solution at 22°C and cell concentration of 2×10^6/ml on an MPF-4 spectrophotometer (Hitachi) in a round cuvette (0.5 cm in diameter).

Experiments were performed in 3 repetitions, and the results were analyzed by Student's *t* test.

RESULTS

Incubation of macrophages in the presence of various steroids in a concentration of 5 μg/ml accelerated CH esterification in cells (Table 1). The ability of oxysterols to stimulate the CE biosynthesis decreased in the following order: 25-OH-CH>22(S)-OH-CH >7-keto-CH>7β-OH-CH. It should be noted that 22(S)-OH-CH, 7-keto-CH, and 7β-OH-CH stimulated the incorporation of labeled oleate into triglycerides, whereas 25-OH-CH had no effect on triglyceride synthesis. Progesterone inhibited CE biosynthesis, but produced no effect on the rate of triglyceride formation in macrophages (similarly to 25-OH-CH, Table 1). The stimulatory effect of 25-OH-CH and inhibitory effect of progesterone on the rate of CE biosynthesis were dose-dependent (Fig. 1, *a*). The maximum stimulatory effect was produced by 5 μg/ml 25-OH-CH, while the maximum inhibitory effect of progesterone was observed at a concentration of 10 μg/ml.

7-Keto-CH and 7β-OH-CH suppress mitochondrial functions of macrophages [7]. Damage to mitochondrial membranes produced by these substances impairs β-oxidation of fatty acids and induces their accumulation in cells and enhanced incorporation in triglycerides and CE. The data suggest that effects of these oxysterols on CE biosynthesis in macrophages are not related to their direct action on ACAT activity. At the same time, our findings and experiments on membrane fractions of various cells [5] indicate that the opposite effects of 25-OH-CH and progesterone on CE biosynthesis are associated with their direct action on ACAT activity. Changes in the activity of ACAT probably depend on structural rearrangements in cell membranes caused by these steroids. Therefore, we studied the effects of various concentrations of 25-OH-CH and progesterone on lipid viscosity (Fig. 1, *b*) and protein-lipid interactions (Fig. 1, *c*) in macrophage membranes. Structural characteristics of macrophage membranes change to a different degree under the effect of various doses of progesterone and 25-OH-CH. Progesterone and 25-OH-CH in concentrations of 8-10 and 2-4 μg/ml, respectively, reduced

TABLE 1. Effects of Oxysterols and Progesterone (5 μg/ml Medium) on the Rate of 1-^{14}C-Oleate Incorporation into CE and Triglycerides in Macrophages (*M±m*)

Steroids	1-^{14}C-Oleate incorporation, nmol/mg cell protein	
	CE	Triglycerides
Control	2.3±0.2	6.4±0.5
7-keto-CH	12.4±2.1*	22.5±2.6*
7β-OH-CH	9.6±1.2*	6.7±1.6*
22(S)-OH-CH	22.9±2.6*	31.2±4.2*
25-OH-CH	34.4±4.1*	3.7±4.1
Progesterone	0.4±0.3*	7.5±0.8

Note. *$p<0.05$ compared to the control.

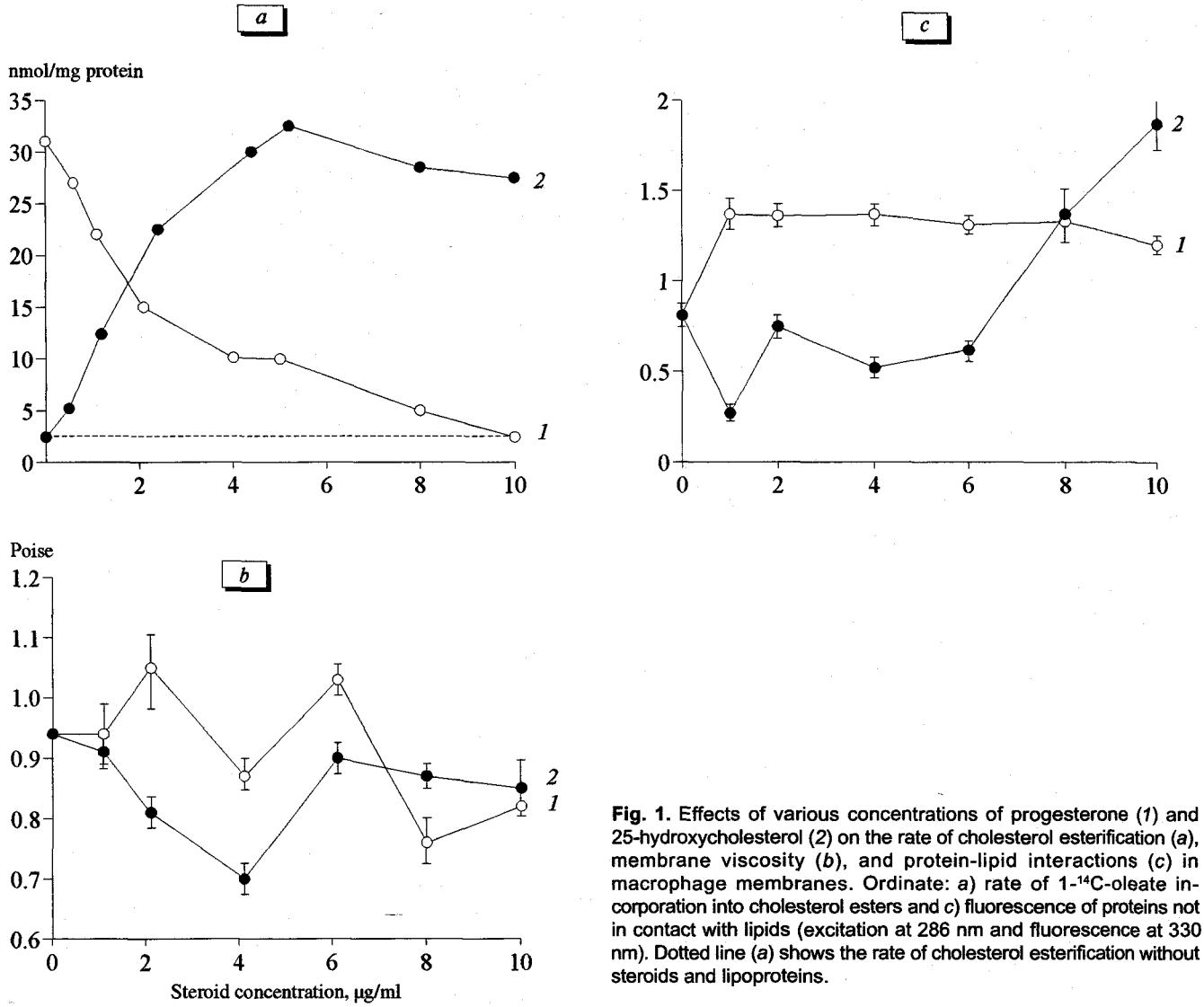

Fig. 1. Effects of various concentrations of progesterone (*1*) and 25-hydroxycholesterol (*2*) on the rate of cholesterol esterification (*a*), membrane viscosity (*b*), and protein-lipid interactions (*c*) in macrophage membranes. Ordinate: *a*) rate of 1-^{14}C-oleate incorporation into cholesterol esters and *c*) fluorescence of proteins not in contact with lipids (excitation at 286 nm and fluorescence at 330 nm). Dotted line (*a*) shows the rate of cholesterol esterification without steroids and lipoproteins.

viscosity of membrane lipids. We observed no correlation between changes in the rate of CH esterification and viscosity of membrane lipids induced by progesterone and 25-OH-CH. Furthermore, changes in the activity of ACAT were not associated with changes in the viscosity of membrane lipids caused by other oxysterols.

Inductive resonance energy transfer from tryptophan residues of membrane proteins to the acceptor localized in the lipid phase (pyrene) depends on the spatial organization of protein-lipid complexes. Progesterone and 25-OH-CH in various concentrations induced opposite changes in protein-lipid interactions (Fig. 1, *c*). Progesterone in concentrations of 0.5-1.0 and 6-10 μg/ml and 25-OH-CH in concentrations of 8-10 μg/ml decreased the efficiency of resonance energy transfer from tryptophan residues to pyrene. Therefore, the content of proteins not in contact with lipids increased, which indicated a higher proportion of protein associates and/or more surface localization of protein complexes in macrophage membranes [1]. On the contrary, in a concentration of 0.5-6.0 μg/ml 25-OH-CH decreased this parameter and increased the efficiency of energy transfer. The linear increase in ACAT activity was also observed in the presence of the same concentration of 25-OH-CH. Surprisingly, high efficiency of protein fluorescence quenching induced by 2-4 μg progesterone did not affect the dose-dependent inhibition of ACAT.

These data suggest that studied steroids modulate lipid viscosity and protein-lipid interactions in macrophage membranes. Nonlinear changes in the studied parameters indicate that the opposite effects of steroids on ACAT activity in macrophages are not necessarily related to these membrane characteristics.

This study was supported by the Russian Foundation for Basic Research (grants No. 99-04-49943 and 99-04-49744) and the Program on Atherosclerosis.

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