PHARMACOLOGY AND TOXICOLOGY

Steroid Hormones Modulate Lipid Spectrum in Lysosomal Membranes of Skin Fibroblasts

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It is shown that estradiol and hydrocortisone modulate lipid composition and affect lipid-protein interaction in fibroblast lysosome membranes, which can promote enzyme release from lysosomes. These effects are particular mechanisms of hormone regulation of functional lysosome activity in the skin.

Key Words: *fibroblasts; steroid hormones; lysosomes; skin; lipids*

Steroid hormones modulate in a dose-dependent manner functional activity of lysosomes [3,7,10], whose lipids participate in transduction of hormone signal into the cell, and simultaneously regulate membrane permeability for various agents and interaction between membrane-bound enzymes with cell substrates [9,13].

Compared with other tissues of the organism, hormone regulation of cell functions in the skin is the least studied. Skin fibroblasts (FB) are not classic target for steroid hormones. However, it has been shown that hydrocortisone and estradiol have a considerable effect of synthetic and proliferative processes in these cells [4,8,14].

In light of this, our objective was to evaluate the effect of hydrocortisone and estradiol on lipid composition of lysosomes in skin FB.

MATERIALS AND METHODS

Experiments were performed on skin FB from L929 rats. The cells were cultured in Carrel glass flasks. Medium 199 supplemented with 5% bovine serum, 5% embryonal serum, 100 U/ml penicillin, and 100 μg/ml streptomycin was used. The cells were detached from glass (for experiments or subculturing) with 0.25% trypsin; enzyme was then inactivated with 5-10 ml complete medium. Cell concentration and viability were evaluated in a Goryaev chamber in the presence of 0.1% Trypan Blue. Estradiol and hydrocortisone (Merck) were added to FB suspension to final concentrations of 10^{-7} and 10^{-5} M. Lysosomes were isolated as described previously [15]. Lipids were extracted by the method of Folch [12] 2 h after addition of steroids and analyzed by thin-layer chromatography on Silufol 254 plates in systems containing normal hexan:ethanol:glacial acetic acid (80:20:2) and chloroform:methanol:water (65:25:4) for total lipids and phospholipids, respectively. Densitometry of chromatograms was performed in a reflected light (560 nm) in an EGR-65 densitometer (Karl Zeiss). The data were processed statistically using the Student *t* test [1].

RESULTS

Lisosomes from intact FB contain 40% phospholipids (Table 1), primarily phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The contents of other phospholipids decrease in the following order: phosphatidylinositol>phosphatidic acid and cardiolipin>sphingomyelin> phosphatidylglycerol> phosphatidylserine. It should be noted that the contents of

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TABLE 1. Effect of Hormones on Phospholipid Spectrum of Lysosome Membranes in Skin FB ($M \pm m$)

Phospholipids	Control	Hydrocortisone	Estradiol
Total phospholipids	38.3±2.2	22.4±2.0*	43.1±1.5
PC	33.6±2.1	50.1±1.8*	46.8±2.0*
PE	19.4±1.3	15.5±2.3	21.9±0.5
Phosphatidic acid+cardiolipin	2.95±0.4	6.0±2.5	2.0±0.5
Phosphatidylglycerol	1.75±0.9	1.6±0.3	1.2±0.1
Phosphatidylinositol	15.1±1.2	9.2±0.8*	6.3±1.1*
Sphingomyelin	2.0±0.5	2.9±0.6	2.3±0.5
PC/PE	1.74±0.071	3.29±0.23*	2.14±0.05*

Note. Data are the means of 6-8 experiments; the content of lipids is presented in % (total phospholipids as percentage of total lipids; individual phospholipids as percentage of total phospholipids). *$p<0.05$ compared with the control.

sphingomyelin and especially phosphatidylserine in lysosome membranes from the skin were considerably lower in comparison with lisosomes from other tissues [5,7].

The duration of incubation with steroids was chosen on the basis of the following data: the most pronounced changes in the lipid spectrum of hepatocyte, myocyte, and some other membranes were observed 2 h after single intraperitoneal injection of a steroid [8,9], whereas maximum shift of lysosome enzymatic activity was observed 3 h after it [11]. Taking this into account, we analyzed lipid spectrum of lysosome membranes from skin FB 2 h after addition of the hormones to cell suspension.

In a concentration of 10^{-7} M none of the hormones induced significant changes in lipid composition of lysosome membranes. Hydrocortisone in a concentration of 10^{-5} M reduced the content of total phospholipids, cholesterol and free fatty acids, whereas the content of triglycerides rose. The content of total phospholipids decreased mainly due to PE, phosphatidylglycerol, and phosphatidylinositol, the content of PC, phosphatidic acid, and cardiolipin being relatively increased.

Unlike hydrocortisone, estradiol in a concentration of 10^{-5} M increased the content of total phospholipids and cholesterol and reduced the content of triglycerides. Total phospholipids rose due primarily to PC, lysoPC, and PE, while the content of phosphatidylglycerol, phosphatidylinositol, phosphatidic acid, and cardiolipin decreased (Table 1).

These data suggest that estradiol and hydrocortisone have different effects on lipid composition in lysosomes of skin FB, which may account for the above-mentioned difference in their effects on functions of FB [4,8,14]. It should be noted that phosphatidylinositol, phosphatidylserine, phosphatidic acid, and cardiolipin are negatively charged phospholipids. When comparing changes in the contents of these phospholipids and, consequently, charges in lysosome membranes, we found that estradiol increases negative charge of the membrane, while hydrocortisone has no effect on this parameter. This, in turn, can modulate electrostatic interactions between lipids and membrane proteins and, consequently, affect their functions. In contrast, hydrocortisone elevates the PC/PE ratio, an indirect index of membrane fluidity, which is consistent with its surfactant properties [2]. These changes indicate that shifts of the membrane phospholipid content in lysosomes of skin FB depend on the type of the steroid.

The effects of hydrocortisone and estradiol on lipid composition in FB lysosomes precede and, presumably, determine changes in activity of some membrane-bound enzymes; the release of a lysosomal enzyme probably results from disturbances in lipid environment and local fluidity of the membrane. This assumption is based on our experimental findings and is confirmed by published data that the ability of an enzyme to be released from lysosome membrane is described by the formula: $y=nX^n$, where n is a coefficient specific for each enzyme and $X=PC/PE$ [6]. Individual coefficients for each enzyme probably reflect the specificity of their membrane lipid environment.

Thus, estradiol and hydrocortisone have dose- and hormone-dependent effects on the phospholipid spectrum of lysosome membrane in skin FB. This may modulate the charge and fluidity of lysosome membrane and disturb the interaction between lipids and proteins in particular, enzymes in the membrane, and consequently, affect functional activity of lysosomes.

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