Effect of Dexamethasone on the Composition of Skin Phospholipids in Laboratory Animals

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Glucocorticoids are often used in dermatology as antiallergic and anti-inflammatory drugs [6,9]. However, their application in dermatotherapy frequently leads to the development of complications and, in turn, withdrawal of the drugs can be accompanied by complicated relapses [9].

Phospholipids localized mainly in the plasma and intracellular membranes have recently been found to play an important role in both cell function and hormonal transmembrane "signaling" [1,7,8,10,11]. At the same time, little is known about the composition of phospholipids in the skin. For the reasons given above it is clear that a study of glucocorticoid action on the skin phospholipids may be of value for hormonal dermatotherapy and for goal-directed pharmacological correction of the side effects.

The present study explores the effect of dexamethasone on the phospholipid composition in rabbit and rat skin and the activity of phospholipase A_2 (PL A_2), one of the enzymes of phospholipid metabolism.

MATERIALS AND METHODS

The study was carried out on 30 male rats weighing 150-180 g and 30 male gray rabbits weighing 2-2.5 kg. Dexamethasone (Dex) (Sigma, USA) was injected intraperitoneally in doses of 10 and 50 mg/kg. The animals were killed under light narcosis 1 h after hormone administration.

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Lipids were extracted after Folch [14]. Lipid composition was assayed by thin-layer chromatography on Silufol-254 plates in hexane-ethyl ester-acetic acid (80:20:2) and chloroform-methanol-water (65:25:4) for determination of the total lipids and phospholipid fractions, respectively. The plates were scanned at 560 nm in reflected light by an EGR-65 densitometer (Karl Zeiss, Germany).

A homogenate was prepared for the study of PL A_2 activity [5]. Enzyme activity was measured according to Saiton *et al.* [17]. Statistical processing of the results was performed using the Student *t* test [2].

RESULTS

First, the content of total lipids, total phospholipids (PL), and their fractions in the skin of intact rats and rabbits was measured. The content of PL (%) in rat skin was shown to be 1.5 times higher than that in rabbit skin, namely, 35.24 and 22.7%. The quantitative ratio of the different PL fractions was also extremely nonuniform: the amount of phosphatidylcholine (PC) in rat skin was about 10 times higher than in rabbits, whereas the phosphatidylglycine (PG) level was much lower in rat skin (Table 1). Phosphatidylserine (PS) and sphingomyelin, both essential for the function of the membrane-bound enzymes 5-nucleotidase and Na^+,K^+-ATPase [12,15], were detected in negligible amounts in the skin of intact animals. The differences of PC content in rat and rabbit skin appear to be connected with the variations in PL A_2 activity (Table 2).

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TABLE 1. Effect of Dex on Phospholipid Composition in Rat and Rabbit Skin.

Phospholipid	Rabbits			Rats		
	control	Dex		control	Dex	
		10 mg/kg	50 mg/kg		10 mg/kg	50 mg/kg
% of total lipids	22.7±0.98	15.5±3.1*	17.1±2.3*	35.24±2.3	23.23±1.9*	20.41±0.8*
PC	3.75±0.27	5.2±0.4*	4.8±0.45*	31.57±2.13*	50.5±1.3*	54.7±0.91*
PE	17.2±0.92	7.2±1.4*	10.4±1.16*	17.4±1.25	16.0±2.0	14.2±1.35*
PA	4.8±1.03	14.3±0.8*	10.4±1.2*	2.95±0.4	6.2±1.25*	7.5±1.2*
PG	35.17±2.4	20.8±2.4*	23.2±2.5*	1.75±0.1	1.20±0.89	1.41±1.4
PI	12.0±2.0	17.1±0.85*	17.8±3.1*	14.8±3.5	8.6±2.3	7.7±2.8

Note: here and in Table 2 data are presented as means from 6–8 experiments. Asterisks: data are statistically different from control (*: $p<0.05$, **: $p<0.01$).

In the second set of experiments the effect of Dex on the above parameters was studied. Dex changed both the PL content and PL fraction ratio in a dose-dependent and an animal-specific manner. For example, Dex (10 mg/kg) reduced the relative content of total PL in both species, while the administration of 50 mg/kg Dex led to a further reduction of PL content in rat skin and to normalization of that in rabbit skin (Table 1). A nonuniformity of rat and rabbit skin responses to different Dex doses was also noted in the PL fractions. The PC/PE ratio, which characterizes membrane "fluidity" [3] was elevated in the skin of both animals at 10 mg/kg, but decreased in the rabbit skin with the 5-fold higher dose of Dex.

In untreated animals PL A_2 activity correlated directly with the PC/PE ratio in the skin (Table 2). Since PL A_2 activity is lowered by Dex (Table 1), the gradual reduction of PL content under hormone treatment is not connected with PL degradation. The PL reduction observed could be caused by an inhibition of its biosynthesis [13]. Moreover, the drop of the relative PE content together with the simultaneous increase of PC under the hormone treatment, correlating with the Dex dose, appears to be the result of PE-enhanced methylation and its transformation into PC [16]. This correlation may be of significance in the regulation of the activity of the membrane-bound enzymes in the skin.

On the other hand, the increase of PC, which is the main cellular substrate for PL A_2, can reflect a decrease of PL A_2 activity. The elevation of PC and drop of PE are far less pronounced in the rabbit than in the rat skin. It is possible that in rabbits the amount of PC changes solely due to a decrease of enzyme activity.

The increase of phosphatidic acid (PA) in the rat skin concurrently with the reduction of phosphatidylinositol (PI) suggests a connection between the cellular "recognition systems" for glucocorticoids and the phosphoinositol cycle [4,10]. However, Dex induces a simultaneous elevation of both PA and PI in rabbit skin. Consequently, this assumption needs experimental verification. Another explanation of the revealed PA level rise may be the stimulation, reported by some authors [18], of its synthesis as a result of glucocorticoid action.

Thus, the comparison study of the phospholipid composition in the skin of intact rabbits and rats showed that the percent of PL in the content of total lipids was higher in rats than in rabbits, with PC and PE predominating among the PL. This has been also noted for human skin [16]. Dex is able to change both the PL content and PL fractions ratio in a dose-dependent and animal-specific manner. The hormone-induced alteration of the normal PL composition and PL fractions ratio promotes an increased membrane "fluidity" and, consequently, may modulate membrane function, in turn leading to a change in cell metabolism as well as cell regulation.

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TABLE 2. Effect of Dex on PL A_2 Activity and Index of Membrane "Fluidity" in Rat and Rabbit Skin ($M\pm m$); Enzyme Activity Expressed in nmol/min/g Protein.

Phospholipid	Rabbits			Rats		
	control	Dex		control	Dex	
		10 mg/kg	50 mg/kg		10 mg/kg	50 mg/kg
PL A_2	12.3±1.2	10.1±0.7	73±0.35*	8.5±0.73	6.1±0.51*	4.5±0.25*
PC/PE	0.2±0.06	0.7±0.05*	0.46±0.05*	1.81±0.2	3.15±0.35*	3.85±0.28*

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Effect of Cimetidine on Experimental Atherogenesis in Rabbits

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Cimetidine is an antiulcer drug that blocks the H_2-receptors and inhibits cytochrome P-450 in the liver. It is known that a low activity of liver monooxidases promotes damage to the intima in experimental atherosclerosis in rabbits, whereas P-450 induction has an antiatherogenic effect [2,10]. On the other hand, antioxidative properties of cimetidine have recently been discovered [14], which may be important for atherogenesis inhibition.

In the present study the effect of cimetidine on experimental atherogenesis in rabbits was investigated.

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MATERIALS AND METHODS

The experiments were carried out on 27 male chinchilla gray rabbits weighing 2.1-2.9 kg. Experimental atherosclerosis was modeled by daily (6 times a week) feeding of a 10% cholesterol solution in sunflower oil (200 mg/kg) through gastric intubation during 12 weeks. All rabbits were divided into 3 groups, with 9 animals in each. The first group consisted of control animals which received sunflower oil (2 ml/kg) through a gastric tube in addition to standard laboratory chow. The second group received the cholesterol solution in sunflower oil. The third group received 10 mg/kg cimetidine (histodyl, Gedeon Richter, Hungary) in a 1% starch suspension through a gastric tube together with cholesterol. Blood for

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