Two Mechanisms of Progesterone Action on the Skin (Results of Biochemical Analysis)

T. V. Ukhina, M. M. Shegai, and G. Zh. Kalmagambetova

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Effects of progesterone on functional activity of lysosomes and lipid peroxidation are measured in the skin of rats in relation to its dose, duration of exposure to it, and skin tissue type (epidermis and dermis). This hormone is shown to regulate both lipid peroxidation intensity and lysosomal activity. It is concluded that these two affects represent two mechanisms through which progesterone exerts its anti-inflammatory effect on the skin.

Key Words: *lysosomes; progesterone; skin; lipid peroxidation*

Impaired lysosomal function and intensified free-radical lipid peroxidation in the skin are important factors in the pathogenesis of some skin diseases, particularly those of inflammatory nature [6,11].

In health, lipid peroxidation (LPO) is implicated in the regulation of membrane permeability and the activity of membrane-bound enzymes, including lysosomal enzymes, while lysosomes regulate both the formation of lipid hydroperoxides and the hydrolysis of LPO products.

There is evidence that progesterone (PG) can exhibit anti-inflammatory activity [8], but the influence exerted by this hormone on lysosomes and free-radical lipid peroxidation in the skin remains unexplored and is the subject of the present study.

MATERIALS AND METHODS

A total of 130 random-bred rats of both sexes (body weight 100-130 g) were used. Female rats were in the diestrus phase as determined by the conventional method [5]. PG was injected subcutaneously in a single dose of 1, 5, or 15 mg/kg body weight. Intact rats served as controls. Rats were killed at different times postinjection (10 and 30 min and 1, 3, 6, 12, 18, and 24 h), and homogenates of their skin (dermis and epidermis) were prepared as previously described [5]. Functional activity of lysosomes was evaluated by the ratio of total to free activities of the enzymes β-glucosidase, cathepsin D, and phospholipase A_2 [10,13, 14]. The LPO rate was assessed by the level of 2-thiobarbituric acid-reactive products [7] and by intensity of the "slow burst" of Fe^{2+}-induced chemiluminescence [3]. Protein was estimated by a microbiuret method [2]. The results were statistically analyzed by Student's *t* test [1].

RESULTS

In the control (intact) groups, acid mucopolysaccharides were broken down by lysosomal β-glucosidase more intensely in females and in the dermis than in males and in the epidermis, whereas no such differences were noted for the interaction of cathepsin D with proteoglycans or of phospholipase A_2 with phospholipids. No significant sex differences were found in dermal or epidermal levels of the LPO parameters, although the levels were somewhat higher in males.

At 5 mg/kg, PG altered lysosomal activity starting at minute 30 postinjection. Although β-glucosidase and cathepsin D activities after this dose were lowered in absolute terms, the strength of bonds formed by these enzymes with the lysosomal mem-

Chair of Molecular Pharmacology and Radiobiology, Biomedical Department, Russian State Medical University, Moscow

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TABLE 1. Effect of Progesterone (PG) on Lysosomal Enzyme Activity in Dermis and Epidermis (mmol/min×mg protein; *M*±2*m*; *n*=8-10)

Group	Enzyme	Activity	Females		Males	
			dermis	epidermis	dermis	epidermis
Control	β-Glucosidase	free	1.31±0.13	1.81±0.23	0.35±0.03	0.42±0.08
		total	2.89±0.19	4.82±0.41	0.84±0.09	0.97±0.09
		% of free	44.6±2.70	37.5±1.20	40.6±1.90	43.2±1.80
	Cathepsin D	free	0.25±0.02	0.31±0.03	0.15±0.02	0.36±0.03
		total	0.38±0.03	0.49±0.04	0.26±0.03	0.42±0.03
		% of free	61.2±3.70	60.8±3.70	55.2±2.90	60.2±2.30
PG, 5 mg/kg	β-Glucosidase	free	0.50±0.03*	0.70±0.01*	0.20±0.03	0.40±0.05
		total	1.07±0.10*	1.80±0.13*	0.37±0.01*	0.90±0.05
		% of free	50.1±3.80	39.7±2.90	55.1±2.07*	44.3±2.50
	Cathepsin D	free	0.15±0.03	0.20±0.02*	0.12±0.07	0.25±0.03
		total	0.26±0.03	0.35±0.03*	0.23±0.01	0.52±0.04*
		% of free	59.4±2.23	56.7±2.50	52.2±2.80	48.7±1.40*

Note. Here and in Table 2: *$p<0.05$ relative to the control.

brane remained virtually unchanged (Table 1). No statistically significant differences of phospholipase A_2 activity or LPO intensity from their control values were noted (Figs. 1 and 2).

After a PG dose of 15 mg/kg, the release of β-glucosidase from dermal and epidermal lysosomes of female rats was found to decrease throughout the 24-hour observation period. In male dermal lysosomes, it decreased until hour 6 postinjection and then rose by hour 12. In contrast, no differences in cathepsin D release under hormonal action were observed either between males and females or between dermis and epidermis. Free phospholipase A_2 activity was lowered only 1 h after PG injection (Fig. 1), possibly as a result of the genome-mediated inhibition of this activity [11].

The impact of PG on LPO also depended on the type of skin and on sex. In females at 1 h postinjection, the slow burst amplitudes (Table 2) and 2-thiobarbituric acid-reactive products (Fig. 2) were higher in the epidermis, but had returned to control values by 24 h. In males, these LPO parameters in the dermis had higher values than in the epidermis; the decreases in slow burst amplitudes recorded 1 h postinjection were followed by their elevation later (at 3 h in the epidermis and at 6 h in the dermis).

The observed effects of PG on the activity of dermal and epidermal lysosomal enzymes are similar to those of hydrocortisone, which is widely used in dermatology [9], while the antioxidative effect of PG on the skin even exceeds that of glucocorticoids.

Our results suggest that PG is most likely to exert its anti-inflammatory effect on the skin through two interrelated mechanisms, namely, by reducing LPO intensity and by regulating lysosomal functional activity.

TABLE 2. Slow Burst Amplitudes of Fe^{2+}-Induced Chemiluminescence in Dermis and Epidermis at Different Times after Progesterone (PG) Injection at 15 mg/kg (rel.units; *M*±2*m*; *n*=8-10)

Time postinjection, h	Slow burst amplitude			
	males		females	
	dermis	epidermis	dermis	epidermis
0	2.0±0.1	1.5±0.1	1.8±0.2	1.4±0.2
1	1.4±0.2*	0.9±0.1*	1.2±0.1*	0.8±0.1*
3	2.4±0.1	1.8±0.1*	2.2±0.1	1.8±0.1
6	2.5±0.2*	2.0±0.2*	2.2±0.1	1.8±0.1
12	2.2±0.2	1.5±0.1	1.8±0.2	1.6±0.2
18	1.8±0.2	1.5±0.1	1.4±0.1	1.6±0.1
24	2.0±0.2	1.4±0.1	1.6±0.2	1.4±0.2

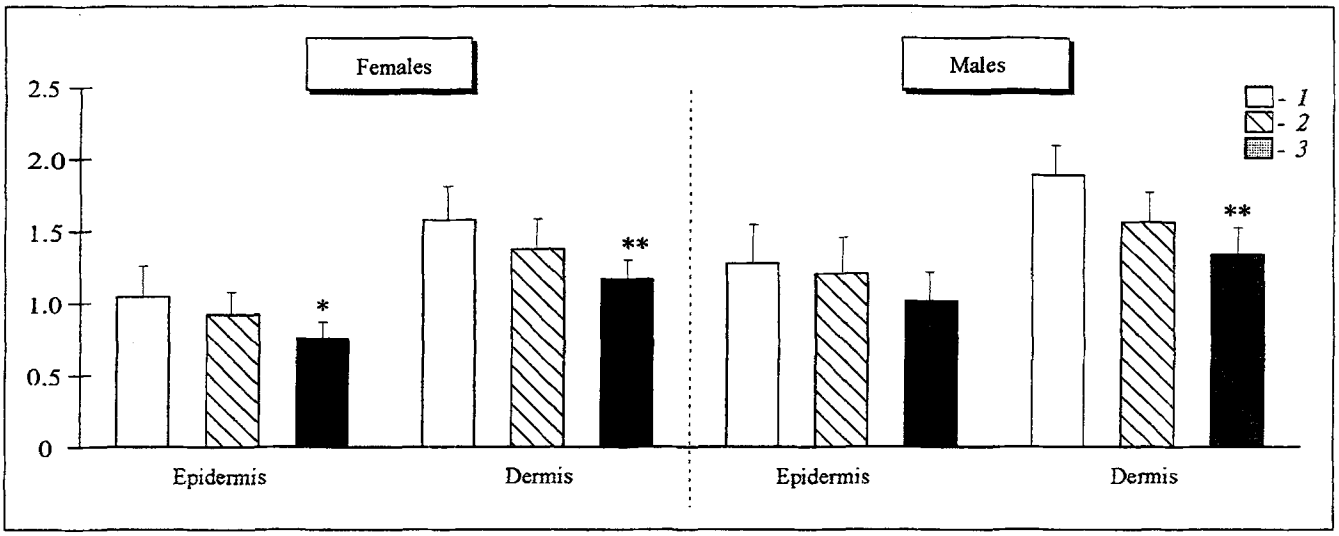

Fig. 2. Effect of progesterone on the level of 2-thiobarbituric acid-reactive products (nmol/mg protein). *$p<0.1$, **$p<0.05$ relative to control. *1*) control; *2*) progesterone 5 mg/kg; *3*) progesterone 15 mg/kg.

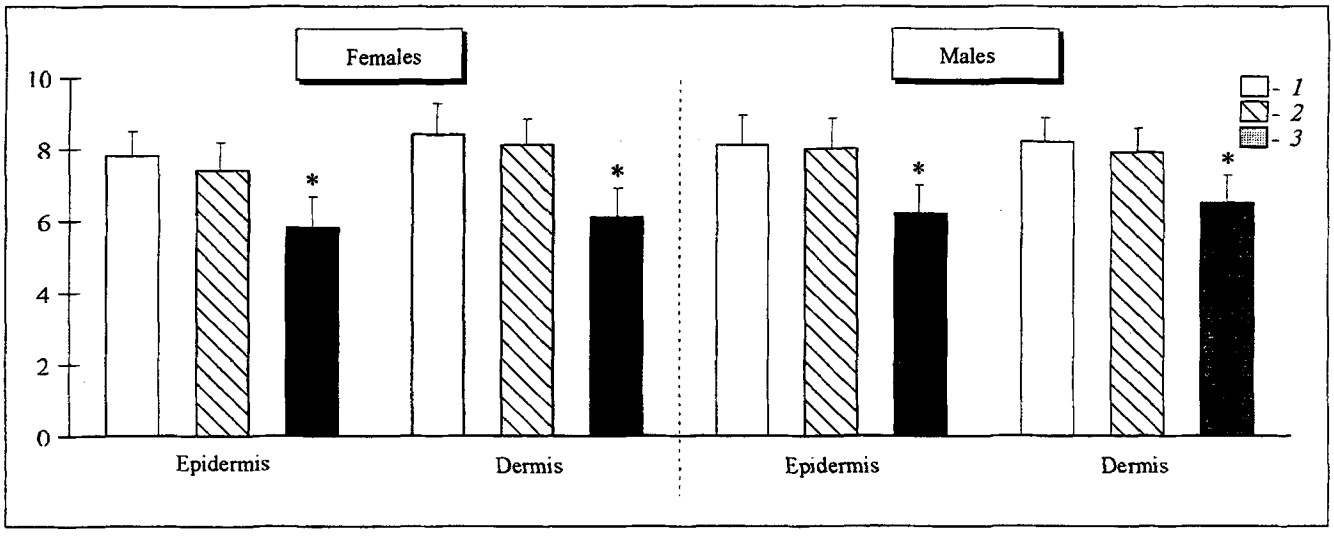

Fig. 1. Effect of progesterone on phospholipase A_2 activity (nmol/min*g protein). *$p<0.05$ relative to control. *1*) control; *2*) progesterone 5 mg/kg; *3*) progesterone 15 mg/kg. The values shown here and in Fig. 2 are means of 8-10 assays.

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