Effect of Hydrocortisone on the Activity of Skin Lysosomal Enzymes

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It is well established that glucocorticoids can influence the stability of lysosomal membranes [7,11], increasing or decreasing it in different cases. The peculiarities of the hormonal effects depend on the dose of exogenously administered hormones, their concentration in the blood, and anatomical and physiological parameters of the responding organs and tissues. The influence of steroid hormones, including glucocorticoids, on the skin lysosomes, especially those of different skin layers, has not been studied up till now.

The aim of this study was to examine the mechanism of glucocorticoid action on the functional activity of dermal and epidermal lysosomes of the rat.

MATERIALS AND METHODS

The experiments were performed on 300 rats of both sexes weighing 100-130 g. Female rats in the diestrus phase, determined by the usual method [5], were used. In the *in vivo* experiments hormone was injected intraperitoneally in doses of 0.01, 0.1, and 1 mg per 100 g body weight; *in vitro* hormone was added to the respective tissue homogenates up to a final concentration of 10^{-8}-10^{-4} M. The animals were sacrificed under ether 1 hour after hydrocortisone injection. Homogenates were prepared as described earlier [3]. Total and free activity of enzymes (β-glucosidase, β-galactosidase and cathepsin D) was determined by spectrophotometry [8,10]. Triton X-100 served as the detergent in the determination of total activity. The functional activity of lysosomes was evaluated by the ratio of free to total enzyme activity. Adrenalectomy was performed according to the routine method [4]. Animals were included in the experiments 7 days after the operation. The protein content was measured after Lowry [9]. Statistical calculations were performed using Student's *t* test [1].

RESULTS

As shown in Table 1, in the control group (intact rats) enzyme activity as well as the level of enzyme-substrate interaction (especially regarding cathepsin D) in both skin layers studied is higher in females than in males. The excess of lysosomal functional activity in females as compared with males is easily traced in the derma and to a lesser degree in the epidermis. Of the enzymes studied, β-glucosidase is most strongly and cathepsin D most weakly bound to the lysosomal membrane, irrespective of the sex of the animal.

Analysis of enzyme activity in the derma and epidermis revealed that in males the epidermal lysosomal enzymes surpass the dermal enzymes in the capacity for enzyme-substrate interaction, whereas in females the layers do not differ significantly regarding the functional activity of the lysosomes.

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TABLE 1. Effect of Hydrocortisone on the Lysosomal Enzyme Activity in the Derma and Epidermis (M±m, n=6–14, activity is expressed in percentage free/total)

Treatment	Enzyme	Enzyme activity (free/total), %			
		male		female	
		derma	epidermis	derma	epidermis
None (control)	b–glucosidase	40.6±0.9	43.2±1.2	44.4±0.7	37.5±1.2
	b–galactosidase	50.8±0.9	50.2±1.5	60.4±1.3	58.0±0.2
	Cathepsin D	55.0±1.65	60.0±0.23	61.0±0.7	60.8±0.37
Adrenalectomy	b–glucosidase	36.5±0.1	28.6±1.5	50.9±1.3	38.5±1.18
	b–galactosidase	49.7±2.1	54.4±4.6	60.0±1.8	60.0±0.43
	Cathepsin D	81.0±2.0	86.6±1.4	67.8±2.8	68.0±2.9
Hydrocortisone	b–glucosidase	54.0±0.8	53.0±0.1	47.2±0.2	44.8±0.3
	b–galactosidase	62.07±0.1	60.8±0.03	70.5±0.3	62.9±0.7
	Cathepsin D	50.3±1.9	52.5±1.4	77.0±1.8	69.0±0.9

In adrenalectomized males a decrease in the carbohydrate cleavage by the studied glycosidases together with an increased catabolism of proteins by cathepsin D are observed in the skin, especially in the epidermis. In contrast, females exhibited a significant increase in the possibility of enzyme-substrate interaction and substrate degradation regarding both cathepsin D and the glycosidases, despite a decrease in the absolute values of enzyme activity.

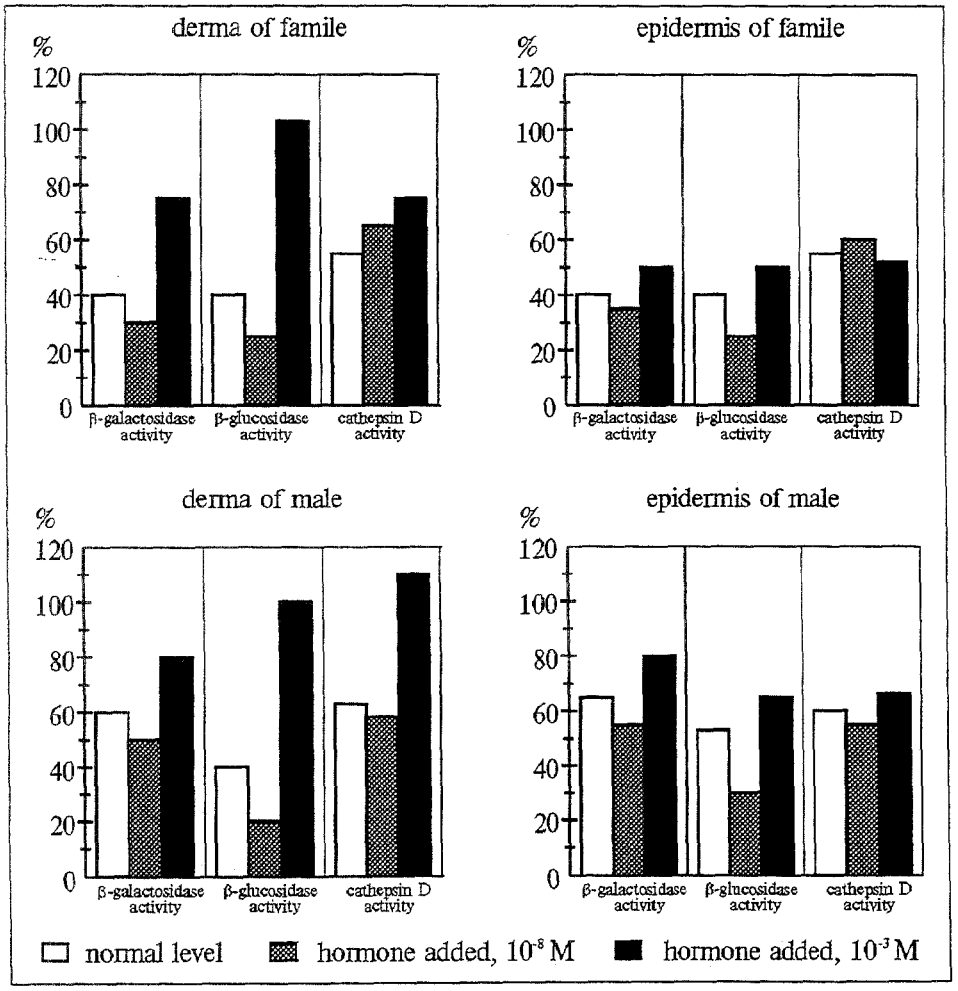

Fig. 1. Effect of hydrocortisone on free activity of skin lysosomal enzymes.

Administration of hydrocortisone in doses of 0.01 and 0.1 mg per 100 g body weight in experiments *in vivo* produced practically no effect on the lysosomal functional activity of the epidermis or derma, regardless of sex. However, in a dose of 1 mg per 100 g body weight (see Table 1) the hormone induced a significant release of lysosomal glycosidases from the epidermis and derma in animals of both sexes, and of cathepsin D in females. In males a decreased potential for cathepsin D-protein interaction was observed, especially in the epidermis.

A study of the effect of hydrocortisone on skin lysosomes *in vitro* (Fig. 1) revealed that when given in a concentration of 10^{-8} M, the hormone causes a decrease in the enzyme-substrate interaction of all studied enzymes in the derma of females and in both skin layers of males. The epidermis of females proved an exception,

as no significant changes in enzyme activity were found.

On the other hand, in a concentration of 10^{-3} M hydrocortisone induced an increase in the potential for enzymatic degradation of substrates (both proteins and carbohydrates) in the derma of females and males. In the epidermis an increase in both the free and total activity of cathepsin D, though without a change in the fraction of the latter, was also registered.

The unequivocal effect of pharmacological doses of hormone (respectively, 1 mg per 100 g body weight *in vivo* and 10^{-3} M *in vitro*) on the lysosomes, regardless of the sex of rats, seems to evidence a direct interaction of hydrocortisone with the lysosomal membrane [2]. Differences in hormone-induced enzymatic changes in derma and epidermis may result from: a) differences in the functional organization of the lysosomal membranes in these skin compartments (the presence or absence of hormone receptors, their specificity, etc.) and b) differences in the composition and/or hormone-dependent "transitions" of the lysosomal membrane phospholipids, which are known to effect the function of membranes and cells [6]. Validation of one or another hypothesis and, consequently, the gathering of new information about the mechanisms underlying the side effects of endocrine therapy call for further detailed study.

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Role of Nicotinic Acid and Its Analogs in the Regulation of Hemostasis

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According to numerous data in the literature, nicotinic acid (NA) and its precursor pyridine produce a complex effect on hemostasis [6,8,12]. They have been shown to be able to increase the fibrinolytic and thrombolytic activity of the blood [3], to reduce platelet aggregation [1,8], and to suppress thromboxane synthesis [9].

Despite NA belonging to the thrombolytics, its wide application in hemostasiology is restricted, because it has no effect on the activity of a number of the most important enzymes regulating platelet formation, such as cyclooxygenase and prostaglandin

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