Effect of Estradiol on the Peroxidation of Skin Lipids

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Free radical oxidation (FRO), in particular that of lipids (FROL), is involved in the development of various pathological states. It is also part of the mechanism whereby side effects are produced by some medicinal preparations [1,2]. Tissue resistance to FRO is determined by the state of the antiperoxide enzymatic system of the tissues and by the reserves of water- and fat-soluble antioxidants [3].

In the present work the intensity of FROL and the antioxidative activity of the dermis and epidermis in rat skin were determined in relation to the animals' sex, and the effect of estradiol on them was investigated.

MATERIALS AND METHODS

Experiments were carried out on 120 rats of both sexes weighing 100-130 g each. After the animals were killed under mild ether anesthesia, a piece of skin from the ventrolateral part of the abdomen was removed and the dermis was separated from the epidermis. The tissue was homogenized according to a known method [4]. The female rats were determined to be in the phase of diestrus [5]. The intensity of FROL was determined from the amplitude of the gradual increase in the reaction rate [7] and from the concentration of thiobarbituric acid products [8]. The antioxidative activity (AOA) of the water-soluble antioxidants and the antioxidation potential (AOP) of the tissues were determined from the effect of inhibition of FROL on a model system [6].

RESULTS

On the basis of Fe^{2+}-induced chemiluminescence (CL) it was shown that the FROL concentration in the dermis was greater than in the epidermis (Table 1). Analogous results were obtained in the determination of the products of the thiobarbituric acid reaction during both induced and spontaneous FROL. The

TABLE 1. Effect of Estradiol on Amplitude of Gradual Increase in Reaction Rate of Fe^{2+}-Induced Chemiluminescence in Dermis and Epidermis in Male and Female Rats (in Arbitrary Units per mg Protein)

Concentration of estradiol, M	Epidermis		Dermis	
	female	male	female	male
0 (control)	1.2±0.3	1.6±0.2	1.8±0.4	2.2±0.4
10^{-9}	0.9±0.3	1.3±0.3	1.4±0.3	1.7±0.4
10^{-7}	0.6±0.4	0.8±0.3*	1.0±0.3	1.2±0.3*
10^{-5}	0.4±0.3*	0.6±0.3*	0.6±0.3*	0.8±0.4*

Note: The average data given here and in Table 2 are based on 6-8 experiments. Asterisk: denotes the values for which $p<0.05$ as compared with the control animals.

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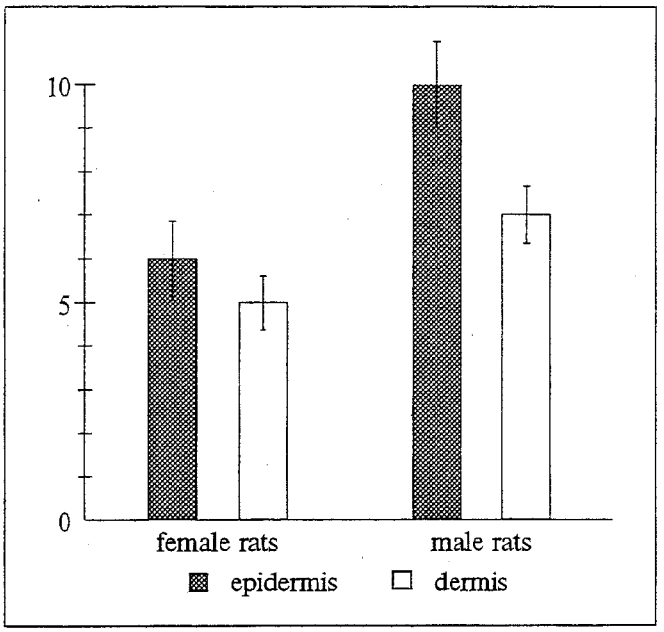

Fig. 1. Antioxidative activity of water–soluble antioxidants in the dermis and epidermis in male and female rats (ml^{-1}).

higher FROL concentration in the dermis may be due to its structural-functional properties (the qualitative and quantitative composition of the lipids, blood supply and so on).

The relatively small accumulation of peroxide radicals in the dermis and epidermis in rats of both sexes (the amplitude of the gradual increase in the oxidation rate was greater in the case of the male rats than in the case of the females) can be regarded as a consequence of high AOA. Separate measurements showed the presence of strong antioxidative inhibition in both the male and female rats. The correlation between the concentration of FROL and the magnitude of AOP of the tissues was negative (Tables 1 and 2; Fig. 1), and this depended on the tissue and the sex of the animals. Thus, the activity of the water-soluble antioxidants in the dermis was greater than in the epidermis; the ratio of this activity in the dermis to that in the epidermis in the female and male rats was 1.2 and 1.5, respectively (Fig. 1). The same regularities were observed in an investigation of the AOP of tissues: in the female rats the magnitude of the potential for the dermis and epidermis was 5.1 and 2.6 ml^{-1}, respectively, while in the males it was 3.2 and 2.2 ml^{-1}, respectively (Fig. 2). The sex-dependent differences in the AOA can be attributed to the specific nature of the hormonal status of the males and females, as well as to the interactions between the hormones.

The effect of different concentration of estradiol on the parameters mentioned above was investigated in a second series of experiments. Following the addition of estradiol *in vitro*, the amplitude of the gradual increase in the Fe^{2+}-induced CL and the concentration of the thiobarbituric acid reaction products decreased in direct proportion to the hormone concentration (Tables 1 and 2).

The correlation between the estradiol concentration and the drop in the level of oxidation of the tissues was disturbed in experiments where a concentration of 10^{-5} M was used. This may be due to a different mechanism of penetration of steroids into the cell. Being an antioxidant, estradiol, in penetrating the plasma membrane and subcellular organelle membrane, decreased FROL in these membranes.

Thus, the estradiol-induced lowering of the degree of oxidation and the increase in the AOP and

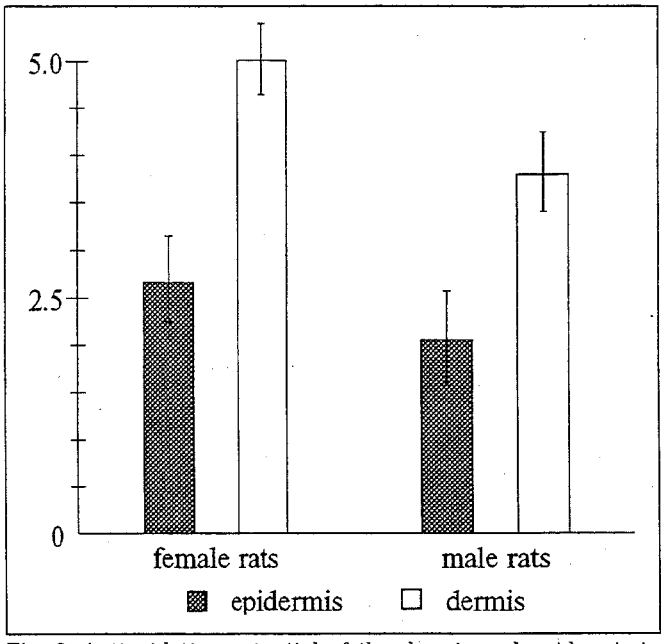

Fig. 2. Antioxidative potential of the dermis and epidermis in male and female rats (ml^{-1}).

TABLE 2. Effect of Estradiol on Concentration of Thiobarbituric Acid Reaction Products in Dermis and Epidermis in Male and Female Rats (nM/mg Protein)

Concentration of estradiol, M	Epidermis		Dermis	
	female	male	female	male
0 (control)	1.15±0.20	1.33±0.31	1.69±0.35	1.95±0.25
10^{-9}	0.85±0.25	1.20±0.30	1.20±0.25	1.52±0.30
10^{-7}	0.70±0.20	1.15±0.20	0.80±0.20*	1.12±0.30*
10^{-5}	0.60±0.20	0.85±0.25	0.70±0.20*	1.04±0.21*

AOA were more pronounced in the dermis than in the epidermis.

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Chemically Modified Hemoglobin-Based Oxygen-Carrying Blood Substitute

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There are various chemically modified hemoglobins which differ in their fractional composition and which contain pyridoxal-5-phosphate (MH-PP) as the regulator of reversible oxygenation. These hemoglobins are considered to be the most promising all-purpose oxygen carriers [2,7]. However, such compounds are nonuniform with respect to their structure and the functional groups which they contain [3,8]. Moreover, as their structure and functional groups are what determine their rheological and antigenic properties, this nonuniformity is one of the main obstacles to creating the corresponding medicinal preparations [6,8].

In order to select substances with optimal physicochemical and oxygen-carrying properties we obtained and studied a series of model MH-PP derivatives with different concentrations of the polymeric fraction. Various compositions of MH-PP were investigated with the aid of gel chromatography and SDS electrophoresis. In addition, an analysis was made of their functional characteristics, viscosity, colloidal and oncotic properties, and degree of immunogenicity.

MATERIALS AND METHODS

Samples of MH-PP were prepared by treating hemoglobin with glutaraldehyde and pyridoxal-5-phosphate according to a known procedure [1]. By varying the reaction conditions we obtained a series of hemoglobin derivatives containing from 5-10 to 70-75% of the polymeric fraction.

The distribution of MH-PP samples according to their molecular weight was determined by separation on a TSK-G-3000SW high-performance liquid chromatographic column (7.5×3000 mm) (LKB, Sweden), with an eluant flow rate of 0.5 ml/min and a 0.01 M phosphate buffer at pH 6.5. Detection was carried out at 400 nm, and the concentration of vari-

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