Effects of Sex Hormones and Antihormones on the Activity of Redox System Enzymes of Human Erythrocyte Glutathione

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Effects of estradiol and testosterone and of the antiandrogens cyproterone acetate, niftolide, and antiestrogen tamoxifen on the activities of human erythrocyte glutathione peroxidase and glutathione reductase were studied *in vitro*. In contrast to hormone preparations, antihormones in high concentrations (10^{-4}-5×10^{-4} M) modified the enzyme activities. Cyproterone acetate and tamoxifen increased the activity of glutathione reductase, while tamoxifen stimulated glutathione reductase and inhibited glutathione peroxidase. Niftolide inhibited both enzymes.

Key Words: *glutathione peroxidase; glutathione reductase; sex hormones; antihormones; erythrocytes*

Steroid hormones, primarily estradiol, and antihormones possess antioxidative activity; they can be regarded as free radical interceptors and membrane stabilizers [1,10]. The effects of sex hormones and their antagonists on endogenous systems of antioxidative defense are less known. There are virtually no published data on their effects on the glutathione redox system, which regulates free-radical oxidation and other important functions, for example, the maintenance of intracellular redox balance as a result of coordinated activities of the redox enzymes glutathione peroxidase (GP) and glutathione reductase (GR) [2]. Recent findings suggest that along with other thiol-containing compounds, glutathione can modulate the genomic effects of steroid hormones [5]. We studied the effects of steroid hormones and their antagonists on the activities of erythrocyte glutathione-related enzymes *in vitro*.

MATERIALS AND METHODS

Blood samples from healthy volunteers were examined using testosterone, estradiol, the antiandrogen cyproterone acetate (6-Chloro-1β,2β-dihydro-17-hydroxy-3'H-cyclopropa[1,2]-pregna-1,4,6-triene-3,20-dione acetate), the antiestrogen tamoxifen ([Z]-1-[p-Dimethylaminoethoxyphenyl]-1,2-diphenyl-1-butene) (Sigma), and the antiandrogen niftolide — 2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide (Institute of Organic Chemistry of the Ukrainian Academy of Sciences). Erythrocyte GP and GR activities were assayed as described [9]. The results were processed by standard methods of statistical analysis. The significance of differences between experimental groups was evaluated using the nonparametrical Wilcoxon—Mann—Whitney's *U* test.

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RESULTS

Erythrocytes were chosen for studies of the effects of hormone and antihormone preparations on glutathione redox system enzymes because these cells can be readily obtained and because it is possible to reproduce the time course of enzyme activity in various cellular diseases and test different drugs [3,4,6,7].

Normal donor GP activity is 26.91±1.62 IU/g hemoglobin, individual values varying from 19.43 to 38.95 IU/g hemoglobin (n=12), which is in line with the results of other scientists [6,9].

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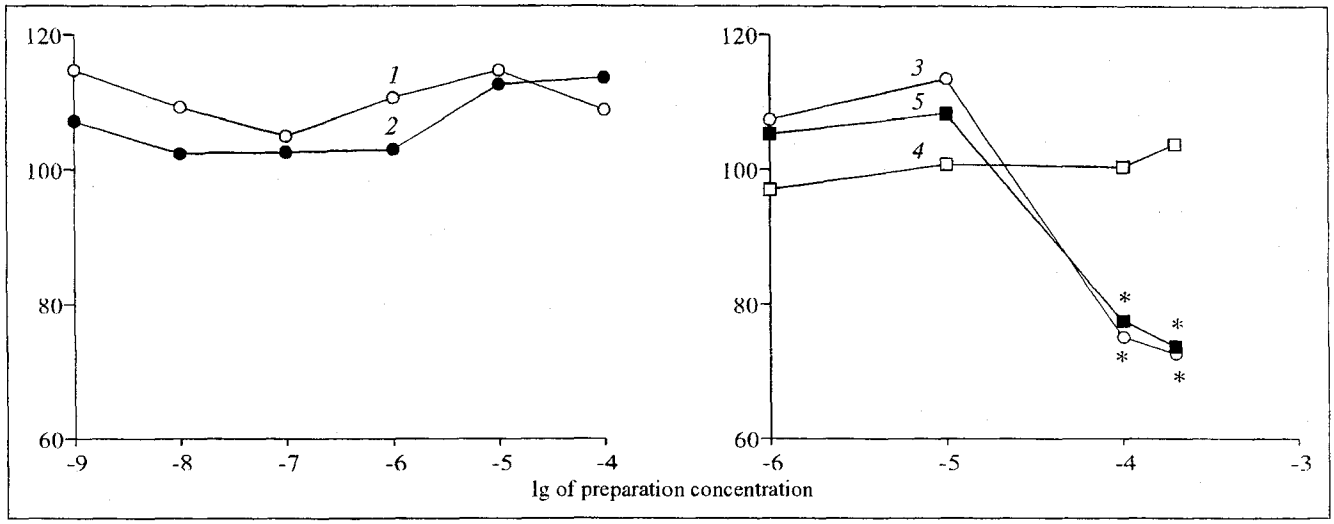

Fig. 1. Effects of sex hormones and antihormones on the activity of human erythrocyte glutathione peroxidase. Ordinate: erythrocyte glutathione peroxidase activity, % of control. Here and on Fig. 2: *1*) estradiol; *2*) testosterone; *3*) niftolide; *4*) cyproterone acetate; *5*) tamoxifen. *$p<0.05$ *vs.* the control.

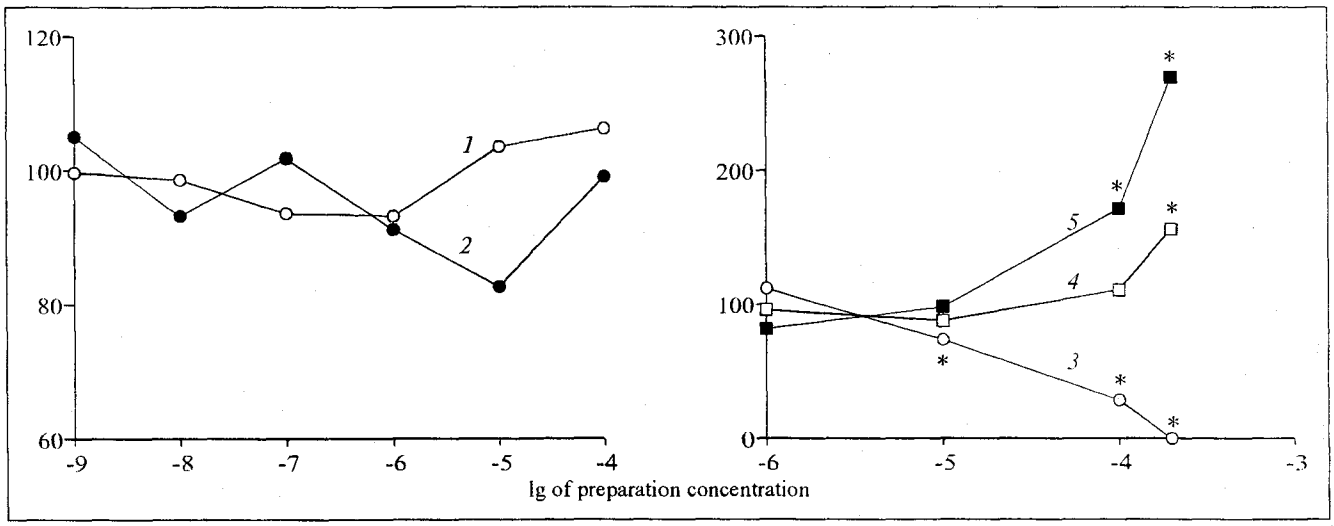

Fig. 2. Effects of sex hormones and antihormones on the activity of human erythrocyte glutathione reductase. Ordinate: erythrocyte glutathione reductase activity, % of control.

Our results indicate that estradiol and testosterone in relatively low concentrations (10^{-9}-10^{-6} M) do not modify the activity of human erythrocyte GP. In higher concentrations (10^{-5}-10^{-4} M) these agents increased the enzyme activity by 10-15%, but this reaction was statistically insignificant (Fig. 1).

The antiandrogen cyproterone acetate had no effect on GP: the enzyme activity remained on the control level in the entire range of studied concentrations (10^{-6}-5×10^{-4} M). In contrast to cyproterone acetate, niftolide and tamoxifen inhibited erythrocyte GP. The enzyme activity decreased by 25-30% in the presence of these agents in concentrations 10^{-4}-5×10^{-4} M (Fig. 1).

GR activity in erythrocytes is 8.35±0.58 IU/g hemoglobin, the values varying from 3.24 to 12.05 IU/g hemoglobin (n=12), which is in line with published reports [6,9].

During incubation of erythrocytes with hormone preparations the activity of GR did not change (Fig. 2). Antihormones in low concentrations virtually did not change the enzyme activity. Niftolide, added to the reaction medium in a concentration of at least 10^{-4} M, sharply decreased GR activity, while cyproterone acetate and tamoxifen increased it considerably (by 1.3-2.7 times) in comparison with the control (Fig. 2).

It can be concluded that estradiol and testosterone virtually do not change the activities of glutathione enzymes. In contrast, sex steroid antagonists modify the activities of both GP and GR, the intensity and direction of the effects of the three studied

agents varying within a wide range. Niftolide inhibited both enzymes. Cyproterone acetate increased the activity of GR, but not of GP. Tamoxifen inhibited GP as niftolide and activated GR as cyproterone acetate.

Enhancement of free-radical reactions leads to the so-called "peroxidative stress;" one of its signs is changed ratio between reduced and oxidized glutathione forms in favor of the latter [8]. The antioxidative effects of niftolide and tamoxifen revealed previously can hardly be mediated by their effect on the antioxidative defense glutathione enzymes. Presumably, cyproterone acetate exerting no direct antioxidative effects can be used to correct the consequences of peroxidative stress by stimulating enzymatic reduction of oxidized glutathione.

From our results it can be concluded that among the studied compounds, only drugs possessing antihormonal activity modify the activity of erythrocyte glutathione redox system enzymes; as a rule, their effects are manifested at relatively high concentrations. Thus, our studies provided new details about the molecular mechanisms of the effects of sex hormone antagonists.

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