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Effects of Sex Hormones on Plasma Lipid Peroxidation *In Vitro*

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The effects of sex steroid hormones on the level of plasma lipid peroxidation are studied *in vitro*. Estradiol shows pronounced antioxidative activity; progesterone and testosterone slightly suppress lipid peroxidation only when used in high concentrations.

Key Words: *lipid peroxidation; sex hormones; blood plasma; chemiluminescence*

Sex steroid hormones are a component of endocrine regulation system of cardiovascular activity [8]. Estrogen, gestagen, and androgen imbalance plays an important role in the pathogenesis of many diseases [3,8,10]. On the other hand, experimental and clinical reports indicate that cardiovascular diseases involve intensification of free-radical processes [1,2]. Moreover, steroid compounds are known to possess antioxidant activity, which explains their antiatherogenic and anti-ischemic action; their efficacy in prevention and correction of the extreme states has been reported [3,8,10]. However, the role of sex hormones in the regulation of free-radical oxidation in the blood is so far unclear.

Our purpose was to compare the effects of estradiol, progesterone, and testosterone on the level of lipid peroxidation (LPO) in blood plasma of experimental animals.

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MATERIALS AND METHODS

Estradiol, progesterone, and testosterone were from Sigma. LPO was assessed in the plasma of adult outbred albino rats of both sexes. Plasma was prepared by centrifugation of whole blood at 600*g* for 15 min. Antioxidant activity of the preparations was assessed from a decrease in the intensity of Fe^{2+}-induced chemiluminescence (CL) and the content of product acummulation reacting with thiobarbituric acid (TBA). Antioxidant activity of sex hormones was assessed in suspension of multilayer liposomes from yolk lipoproteins [4]. The kinetics of CL and time course of LPO products in this series of experiments was studied by the methods described previously [4,5]. Registration of "fast flash" of plasma CL was described in detail [6]. Plasma concentration of TBA-reactive products was measured as described elsewhere [9]; in order to study the effect of steroids on the time course of LPO products accumulation, the tested compounds in concentrations 10^{-4}-10^{-9} M were added to the reaction mixture instead of ionol.

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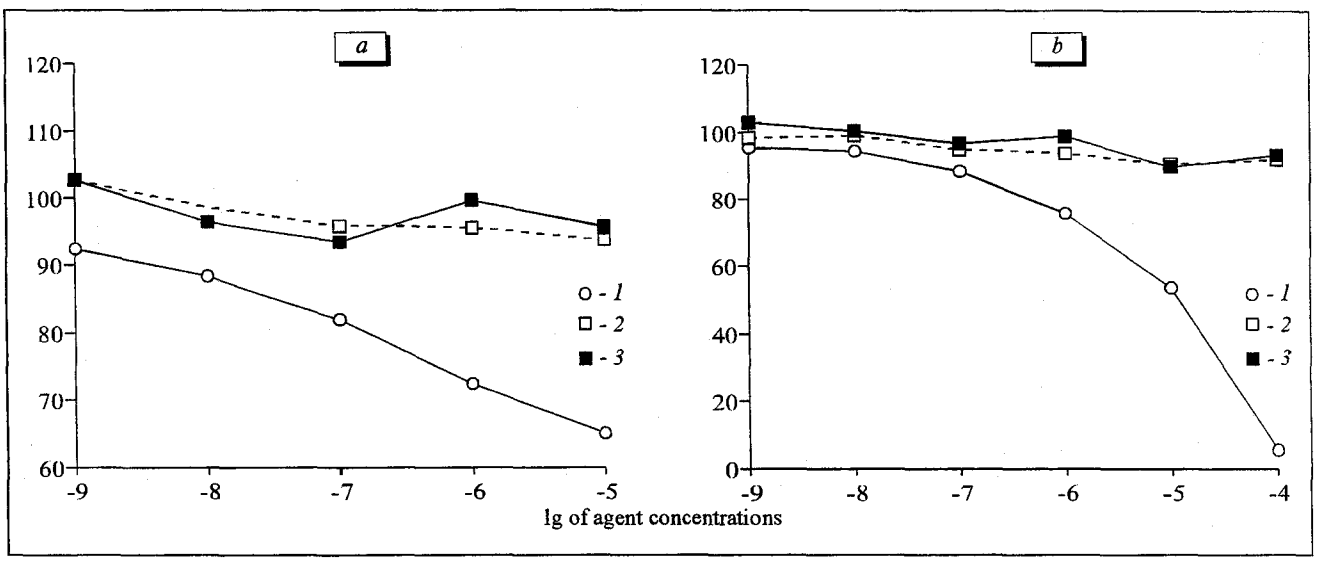

Fig. 1. Effects of sex hormones on LPO in rat plasma. Ordinate: *a*) amplitude of fast flash of Fe^{2+}-induced chemiluminescence, % of control; *b*) concentration of products reacting with thiobarbituric acid, % of control. *1*) estradiol, *2*) progesterone, and *3*) testosterone.

The data were processed by standard methods of statistical analysis.

RESULTS

Effects of three hormone preparations on the intensity of free-radical oxidation in the blood were studied in the first series of experiments *in vitro*. The intensity of LPO was estimated from the amplitude of the flash of Fe^{2+}-induced CL in phosphate buffer containing trace amounts (10 μl) of blood plasma. The results of CL analysis were compared with the data of TBA test in which the intensity of formation of malonic dialdehyde, a secondary LPO product, is determined.

Estradiol showed the highest capacity for inhibiting LPO in the plasma; it is seen from a monotonous decrease of the CL flash amplitude to 65% of the initial level within the entire range of tested concentrations (10^{-7}-10^{-3} M) and of TBA-reactive products (to 5.5% of the control values, Fig. 1). It is noteworthy that decrease in the intensity of very weak luminescence was greater when the hormone was added to male plasma, although decrease in the concentration of TBA-reactive products was observed in animals of both sexes. The addition of estradiol in the final concentration of 10^{-4} M to the reaction media led to a decrease in the content of TBA-reactive products to 2.15 nmol of malonic dialde-

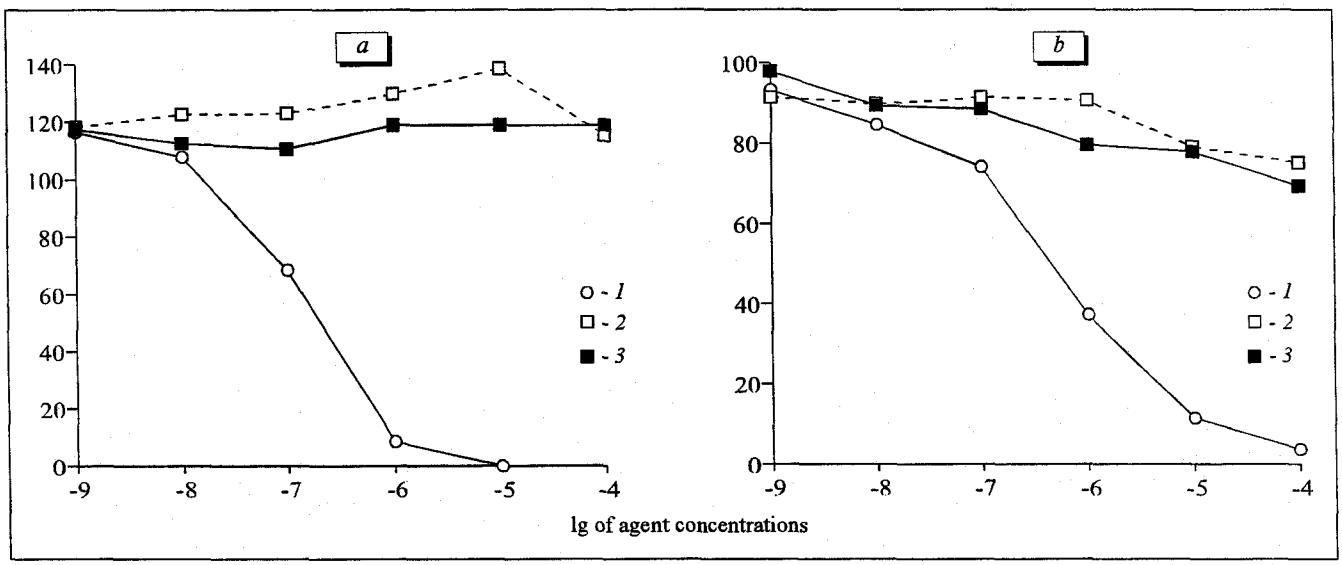

Fig. 2. Effects of sex hormones on LPO in suspension of multilayer liposomes from yolk lipoproteins. Ordinate: *a*) tangent of the slope angle of the slow flash of Fe^{2+}-induced chemiluminescence, % of control; *b*) concentration of products reacting with thiobarbituric acid, % of control. *1*) estradiol; *2*) progesterone; *3*) testosterone.

hyde/ml plasma, which corresponded to the level recorded by the standard methods [9]. This indicates that antioxidative activity of estradiol is comparable to that of ionol, a synthetic antioxidant used in experimental studies.

The addition of progesterone and testosterone to the reaction medium changed the CL intensity less significantly. The flash amplitude decreased by 14-19% only if the content of steroids in the reaction medium was high, the effect being higher in female blood plasma (Fig. 1, *a*). Both preparations did not appreciably change the content of TBA-reactive products, no matter what the animal sex was (Fig. 1, *b*).

Obviously, the total effect of sex hormones on LPO level in the blood is determined by their pro- or antioxidant properties and their influence on the activity of endogenous systems of antioxidative defense. Therefore, in order to assess the degree of direct antioxidative effects of sex hormones, the concentration dependence of LPO values was studied in a model system containing a suspension of multilayer liposomes from yolk lipoproteins. Estradiol caused a monotonous decrease of the rate of intensification of the "slow flash" of Fe^{2+}-induced CL in concentrations 10^{-7}-10^{-5} M (Fig. 2, *a*); when the preparation was added in the maximum concentrations, zero values of the studied parameter were recorded, indicating a complete inhibition of the formation of free radicals in the model system. The "fast flash" amplitude remained at the control level (10^{-9}-10^{-6} M), and only at high concentration of estradiol a tendency to its moderate decrease was observed. This was paralleled by a substantial decrease in the accumulation of TBA-reactive products (Fig. 2, *b*). The concentration dependence of changes in this parameter corresponded to that for the rate of the "slow flash" increment.

In contrast to estradiol, progesterone and testosterone did not change appreciably the LPO values in the model system. The rate of the slow flash intensification increased by 11-38% for all tested concentrations, which was paralleled by a tendency to a decrease in the fast flash amplitude and the rate of LPO product formation (Fig. 2); the latter effect was the highest in the presence of high concentrations (10^{-4}-10^{-5} M) of the agents in the model system. Thus, progesterone and testosterone can decrease the intensity of radical degradation of lipid peroxides in the presence of divalent iron in high concentrations, but as the ferrixyl ions oxidized, the rate of free radical generation in the model system tended to increase. The TBA test showed that the total final effect of the hormones consisted in stable intensity of LPO or its slight decrease, which, according to CL analysis, was apparently not due to the radical capturing activity. Some scientists [1] believe that the membrane-stabilizing properties of progesterone and testosterone are responsible for their inhibitory effect on the level of free radical oxidation.

Thus, our results indicate that out of the three tested hormones, only estradiol possesses pronounced antioxidative activity. Its value estimated routinely [5] is 1.2×10^7 M^{-1}. Progesterone and testosterone slightly influence LPO level only when used in high concentrations.

Normally, LPO level in the plasma is regulated mainly by α-tocopherol, glutathione, ceruloplasmin, enzymes extinguishing the active oxygen forms, and some metabolites; the contribution of steroid hormones to the total antioxidative activity is relatively small [7]. From our findings it can be hypothesized that steroid drugs are capable of regulating free-radical processes in the blood, and their antioxidative effects are partially determined by interactions with endogenous hormones. However, the mechanisms inhibiting free-radical processes during interactions between exo- and endogenous hormones require further investigation.

Thus, our results show that estradiol, progesterone, and testosterone affect LPO *in vitro*, the antioxidant activity of estradiol being much higher than that of progesterone and testosterone which inhibit free-radical oxidation in the plasma only at high concentrations in the reaction system, certain level of endogenous hormones in the plasma, at individual (but not all) stages of LPO cascade, etc.; this effect is not due to capture of radicals by these agents. At present, sex hormones, primarily estradiol and its derivatives, are widely used for preventing cardiovascular diseases and for treating extreme states. Therefore, our results can be used for validating the efficacy and developing the optimal schemes for their treatment and prevention.

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