ANTIOXIDANT ACTION OF STEROID HORMONES ON THE PEROXIDATION OF MITOCHONDRIAL MEMBRANE LIPIDS IN VIVO AND IN VITRO

V. M. Gukasov, P. V. Sergeev,
R. D. Seifulla, and Yu. A. Vladimirov

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The action of several steroids on lipid peroxidation in the mitochondrial membranes of the rat liver in the presence of Fe^{++} ions was studied by recording the very weak chemiluminescence. All the hormones investigated had an antioxidant action, but the mechanism of this action varied. Removal of the ovaries and adrenals gave effects opposite to those of injection of estrogens and glucocorticoids in vitro.

KEY WORDS: steroid hormones; peroxidation of lipids; liver mitochondria; chemiluminescence; glucocorticoids.

Considerable experimental evidence of the mechanism of action of steroid hormones on the cell has now been obtained. Steroid hormones have been shown to affect the permeability of membranes [7], the activity of enzyme systems [10], and the genetic mechanism of synthesis of biopolymers [8, 10]. It is postulated that some of these effects can be explained by the ability of steroids to depress the peroxidation of membrane lipids [1], compounds known to affect the permeability of membranes, enzyme activity, mitotic activity in the cells, proliferative processes in organs, and so on [2, 5, 9]. The possibility cannot be ruled out that the study of the mechanism of the antioxidant action of steroids could provide a basis for the understanding of many aspects of the action of steroid hormones.

In the investigation described below an attempt was made, by using a sensitive method of determining antioxidant activity, to study the antioxidant action of steroid hormones.

EXPERIMENTAL METHOD

Mitochondria were isolated from rat liver in a medium containing 0.25 M sucrose and 2.5 mM tris-HCl, pH 7.4. The protein of the suspension was measured by Lowry's method. A suspension of mitochondria containing 1 mg protein was added to each sample; the volume was made up to 10 ml with incubation medium. The medium for measuring the luminescence was a solution containing 105 mM KCl and 20 mM tris-buffer. The chemiluminescence of the mitochondria was recorded on a special apparatus in which the element receiving the very weak luminescence was a sensitive photoelectronic multiplier (FÉU-39A). To determine the antioxidant activity the method suggested by the writers previously was used [1]; it is based on measuring the power factors δ in the equation describing the kinetics of the initial, exponential stage of the slow burst of luminescence. The antioxidant action of the hormones on lipid peroxidation was also characterized by measuring the latent period of the chemiluminescence (τ – time after addition of $FeSO_4$ during which the amplitude of the slow burst of luminescence reached half its maximal value). The concentration of malonic dialdehyde (MDA) in the sample; was determined by the reaction with thiobarbituric acid (TBA). The rate of accumulation of the peroxidation products was determined by the indices

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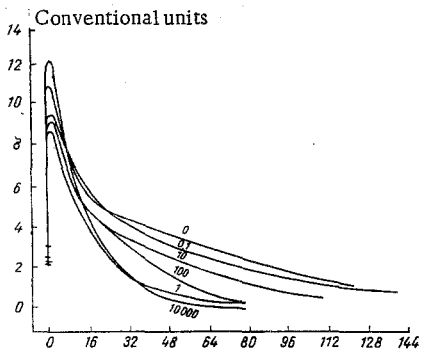

Fig. 1. Effect of estradiol on intermediate burst of chemiluminescence. Numbers by curves denote estradiol concentration (in nmoles). Abscissa, time of chemiluminescence (in sec); ordinate, intensity of chemiluminescence (in conventional units).

TABLE 1. Effect of Steroid Hormones on Some Parameters of Lipid Peroxidation in Mitochondrial Membranes

Name of hormone	C_1 (M^{-1})	$\frac{\tau}{\tau_0}$%	C_2 (M^{-1})	$\frac{\delta_0}{\delta}$%	$\frac{\Delta MDA_h}{\Delta MDA_0}$, %
Estrone	10^{10}	200	10^{11}	150	36
Estradiol	10^8	200	10^8	150	36
Estriol	$3 \cdot 10^6$	200	10^6	150	45
Corticosterone	$5 \cdot 10^9$	200	$5 \cdot 10^{10}$	50	44,3
Cortisone	10^8	200	10^8	50	56,6
Androsterone	10^3	200	$5 \cdot 10^4$	50	82,3
Progesterone	$5 \cdot 10^4$	200	10^5	50	100

Legend: C_1) concentration of steroid causing 100% change in duration of latent period (τ_0); C_2) concentration of steroid causing 50% change in value of δ_0; δ) tangent of angle of slope of straight line showing relationship between logarithm of intensity of luminescence and time to initial, exponential stage of slow burst; δ_0, τ_0) parameters of reaction without addition of hormones to samples.

ΔMDA_h and ΔMDA_0 – the mean difference between the MDA content after addition of 10^{-6} M $FeSO_4$ twice within a period of 5 min in the presence and absence respectively of 10^{-4} M of the hormone.

EXPERIMENTAL RESULTS

The regulation of lipid peroxidation in the cell is determined by the state of the Fe ions in the system (their binding, reduction, or oxidation) [11]. The main reactions taking place during peroxidation of lipids in the presence of Fe ions have been studied [1, 3]. Interaction between Fe^{++} ions and radicals participating in the peroxidation of lipids has been found to have a decisive influence on the kinetics of peroxidation. In the early stage of peroxidation these radicals could be alkoxyl radicals RO˙, formed by decomposition of hydroperoxides and participating in the branching of the peroxidation chains [2]. This hypothesis is based on observation of the intermediate burst of chemiluminescence (Fig. 1), possibly caused by the accumulation of these radicals, which was inhibited by an excess of Fe^{++} ions and by low concentrations of steroid hormones. Inhibition of RO˙ formation is evidently the basic process in the action of physiological concentrations of natural antioxidants, which include the steroid hormones. In fact, under conditions in which an intermediate burst of chemiluminescence was observed in the control, very low concentrations of steroid hormones acted as antioxidants. This action was manifested as a twofold increase in the latent period (Table 1). As Table 1 shows, the mechanism of the antioxidant action was common to all steroid hormones.

The second manifestation of the antioxidant action of Fe^{++} ions was delay in the development of the slow burst of chemiluminescence. Only the action of estrogens on peroxidation was found to lead to a decrease in the value of δ, i.e., to an increase in the antioxidant action at the stage of the slow burst, while the other groups of hormones, on the other hand, increased the value of this index (Table 1). The reason for the difference between the action of nonestrogenic steroids on the intermediate and slow burst could be connected with the fact that they react only with RO˙ radicals and not with RO_2˙ radicals.

Very few investigations have been carried out with the aim of detecting the products of lipid peroxidation in vivo [6], and there are virtually no data on the hormonal mechanisms regulating this process in the body.

In the present experiment, endocrine organs producing steroid hormones (the adrenals and ovaries) were successively removed in order to study the role of the endocrine background of steroid hormones in vivo. The antioxidant action of the hormones was studied after the endocrine background had been lowered in this way. As might be expected, removal of each endocrine component in rats gave an effect opposite to that observed after administration of the corresponding hormones in vitro (Table 2). For instance, removal of the ovaries led to an increase in the index δ, whereas removal of the adrenals, by contrast, led to a decrease in this index and a simultaneous shortening of the latent period and an increase in the TBA-active peroxidation products. After administration of small doses of hormones to the ovariectomized and adrenalectomized animals, the effect of the antioxidant action corresponded to the results obtained in vitro. For instance, the action of estrogens given in vivo was manifested as a rule as an increase in all the parameters of antioxidant action: lengthening of the latent period, decrease in the value of the TBS-active peroxidation products, and a decrease in the index δ.

TABLE 2. Effect of Removal of Some Endocrine Organs and Replacement Administration of Estrogens of Some Parameters of Chemiluminescence of Peroxidation and its Products (M ± m)

Character of experiments	τ	δ	MDA (in μ moles/mg protein)
Normal	5,6±0,27	3,7±0,14	6,5±0,4
Ovariectomy	13,0±0,34	4,33±0,15	3,3±0,08
P	<0,001	<0,05	<0,001
Ovariectomy + adrenalectomy	9,7±0,6	2,6±0,2	4,6±0,1
P	<0,02	<0,001	<0,001
Ovariectomy + adrenalectomy + estrone	13,8±1,1	1,97±0,09	2,8±0,09
P	<0,02	<0,01	<0,001
Ovariectomy + adrenalectomy + estriol	13,7±0,7	2,6±0,96	3,1±0,18
P	<0,002	<0,01	<0,001

Note. Estrogens injected in a dose of 0.1 μg/g intraperitoneally 24 h before decapitation. Remainder of legend, see Table 1.

These results show that all the investigated hormones possess some degree of antioxidant action, although the mechanism of this action on the mitochondrial membranes varies. Estrogens and glucocorticoids have the strongest antioxidant action. Estrogens act as powerful inhibitors of the radicals responsible for branching of the chains at the intermediate burst stage, and they also give rise to parallel inhibition of the radicals responsible for the rate of rise of the slow burst. Glucocorticoids, however, act only as inhibitors of the first type of radicals and they do not inhibit the development of the slow burst. It is not quite clear whether this action of the glucocorticoids is connected with their ability to stabilize the membranes and thus to affect the course of the chain oxidation reactions in the lipid phase indirectly. As regards the androgens and progesterone, while they have very weak antioxidant action, manifested as absence of inhibition of the accumulation of peroxidation products such as MDA (Table 1), in high concentrations they nevertheless lengthen the latent period in the development of the process (Table 1). This points to the important role of the membrane-stabilizing effect of these hormones in the observed kinetics of chemiluminescence and the rate of chain oxidation. Experiments carried out in vivo showed that steroid hormones produced by the body itself may also significantly affect the process of peroxidation. Judging from their effect on the parameters of peroxidation studied, the mechanism of the antioxidant action of these hormones is identical with that of their action in vitro.

LITERATURE CITED

1. Yu. A. Vladimirov, P. V. Sergeev, R. D. Seifulla, et al., Molekul. Biol., No. 7, 247 (1973).
2. Yu. A. Vladimirov and A. I. Archakov, Peroxidation of Lipids in Biological Membranes [in Russian], Moscow (1972).
3. Yu. A. Vladimirov, T. B. Suslova, and V. I. Olenev, Biofizika, 14, 836 (1969).
4. Yu. A. Vladimirov, T. B. Suslova, V. I. Olenev, et al., in: Mitochondria. Biochemical Functions in the System of Cell Organelles [in Russian], Moscow (1969), p. 203.
5. E. A. Korepanova, K. I. Trukhmanova, V. F. Antonov, et al., in: Biophysics of Membranes. Proceedings of a Symposium [in Russian], Kaunas (1971), p. 476.
6. E. A. Neifakh and V. E. Kagan, Biokhimiya, 34, 511 (1969).
7. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
8. A. S. Allison, in: Society for Endocrinology (Great Britain). Cell Mechanisms in Hormone Production and Action. Proceedings of a Symposium, London (1961), p. 42.
9. F. E. Hunter, Jr., J. M. Gebicki, P. E. Hoffsten, et al., J. Biol. Chem., 238, 828 (1963).
10. P. Karlson, Dtsch. med. Wschr., 86, 668 (1961).
11. P. Talalay, in: Biological Approaches to Cancer Chemotherapy, London (1961), p. 59.