MECHANISMS OF THE ANTIOXIDATIVE ACTION OF SCREENED PHENOLS IN BIOLOGICAL MEMBRANES. EFFECTS OF IONOL DERIVATIVES ON LUMINOL-DEPENDENT CHEMILUMINESCENCE

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Radicals of oxygen and lipids are intermediates in the initiation and development of reactions of free-radical oxidation [3-6]. Correspondingly, the action of inhibitors of free-radical oxidation can be realized through their interaction with radicals of both types.

It is considered that the source of information about oxygen radicals generated by membrane and cytosol enzymes may be luminol-dependent chemiluminescence (LDCL) [4, 5, 7]. However, it is not quite clear which oxygen radicals (superoxide anion-radicals or hydroxyl radicals) are responsible for chemiluminescence in the presence of luminol [4, 5, 7]. The role of lipid radicals as possible "inducers" of LDCL likewise has not been established. This last question can be answered with the aid of lipid-soluble scavengers of free radicals, interacting weakly with oxygen radicals in the aqueous phase.

Previously the writers studied a series of hydrophobic derivatives of ionol (4-methyl-2,6-di-tert-butylphenol), exhibiting an antioxidative action, of varied efficacy, in biological membranes [1]. In the investigation described below the action of ionol derivatives (Fig. 1) on LDCL was studied during the generation of oxygen radicals only (in the presence of potassium superoxide) and in the presence of (Fe^{2+} + NADPH) and (Fe^{2+} + ascorbate)-dependent systems of induction of peroxidation in the microsomal fraction of rat liver.

EXPERIMENTAL METHOD

Microsomal membranes were obtained from rat liver by differential centrifugation [2]. LDCL was recorded as described in [7, 8]. The incubation medium contained: NADPH/ascorbate

TABLE 1. Values of Constant K_7, Partition Coefficients in Heptane/Water System, and Constants of Inhibition of Enzymic (Fe^{2+} + NADPH) and Nonenzymic (Fe^{2+} + Ascorbate) LPO for Ionol and Its Derivatives

Compound	K_7, 60 °C		Fe^{2+} + NADPH	Fe^{2+} + ascorbate	
				microsomes	synaptosomes
I	$4{,}42 \cdot 10^4$	0,01	$1{,}26 \cdot 10^{-8}$ M	$2{,}40 \cdot 10^{-7}$ M	—
II	$6{,}28 \cdot 10^4$	0,10	$>10^{-3}$ M	$>10^{-3}$ M	$>3 \cdot 10^{-3}$
III	$5{,}07 \cdot 10^4$	99	$2{,}50 \cdot 10^{-5}$ M	$2{,}30 \cdot 10^{-5}$ M	$3 \cdot 10^{-4}$
IV	$5{,}07 \cdot 10^4$	99	$4{,}27 \cdot 10^{-6}$ M	$1{,}45 \cdot 10^{-5}$ M	$3 \cdot 10^{-4}$
V	$5{,}90 \cdot 10^4$	0,01	$3{,}90 \cdot 10^{-5}$ M	$1{,}55 \cdot 10^{-4}$ M	10^{-3}
VI	$5{,}61 \cdot 10^4$	0,01	$>10^{-3}$ M	$>10^{-3}$ M	$>3 \cdot 10^{-3}$
VII	$1{,}33 \cdot 10^4$	0,01	$4{,}80 \cdot 10^{-10}$ M	$2{,}60 \cdot 10^{-7}$ M	$2 \cdot 10^{-6}$
VIII	$8{,}86 \cdot 10^4$	—	$1{,}10 \cdot 10^{-6}$ M	$2{,}27 \cdot 10^{-6}$ M	—
IX	$2{,}13 \cdot 10^4$	0,02	$4{,}17 \cdot 10^{-7}$ M	$1{,}74 \cdot 10^{-6}$ M	10^{-4}
X	$2{,}22 \cdot 10^4$	—	$3{,}02 \cdot 10^{-7}$ M	$2{,}19 \cdot 10^{-6}$ M	—
XI	$2{,}65 \cdot 10^4$	0,02	$2{,}57 \cdot 10^{-10}$ M	$2{,}63 \cdot 10^{-7}$ M	$3 \cdot 10^{-6}$
XII	$3{,}13 \cdot 10^4$	—	$4{,}47 \cdot 10^{-10}$ M	$1{,}38 \cdot 10^{-7}$ M	—
XIII	$1{,}52 \cdot 10^4$	—	$1{,}10 \cdot 10^{-6}$ M	$5{,}25 \cdot 10^{-7}$ M	$3 \cdot 10^{-5}$
XIV	$2{,}73 \cdot 10^4$	—	$4{,}79 \cdot 10^{-7}$ M	$4{,}79 \cdot 10^{-8}$ M	$6 \cdot 10^{-5}$
XV	$4{,}25 \cdot 10^4$	0,01	$3{,}35 \cdot 10^{-7}$ M	$5{,}87 \cdot 10^{-7}$M	$6 \cdot 10^{-6}$

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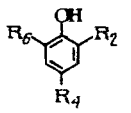
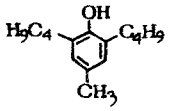



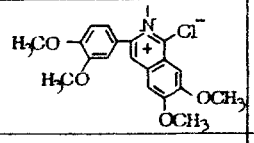
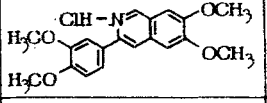
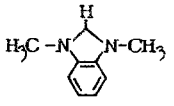
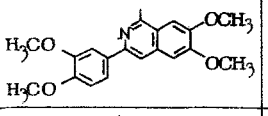
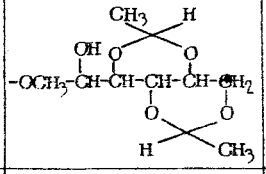
	R_2	R_4	R_6
I	-OH	$-C_4H_9$	$-C_4H_9$
II	$-OC_{16}H_{33}$	$-C_4H_9$	$-C_4H_9$
III	$-OCH_2COONa$	$-C_4H_9$	$-C_4H_9$
IV	$-OCH_2COOH$	$-C_4H_9$	$-C_4H_9$
V	$-C_4H_9$	$-CH_2-CH(NH_2)-COOH$	$-C_4H_9$
VI	$-OC_{12}H_{25}$	$-C_4H_9$	$-C_4H_9$
VII	$-C_4H_9$	$-CH_2-CH(C_2H_5)(COOC_2H_5)_2$	$-C_4H_9$
VIII	$-C_4H_9$		$-C_4H_9$
IX	$-O-CH_2-CH_2-N$ 	$-C_4H_9$	$-C_4H_9$
X	$-C_4H_9$		$-C_4H_9$
XI	$-C_4H_9$		$-C_4H_9$
XII	$-C_4H_9$		$-C_4H_9$
XIII	$-C_4H_9$	$-CH_2-C(NHCOCH_3)(COOC_2H_5)_2$	$-C_4H_9$
XIV		$-C_4H_9$	$-C_4H_9$
XV	$-C_4H_9$	$-CH_3$	$-C_4H_9$

Fig. 1. Structural formulas of ionol and its derivatives.

$5 \cdot 10^{-4}$ M, 10 mM $FeCl_2$, 50 mM luminol (pH 12.0) in 0.1 M K-Na-phosphate buffer. The protein concentration was 0.5 mg/ml. In the absence of microsomes the incubation medium contained: 50 mM luminol, 50 mM $FeCl_2$, 3 mM KO_2. The homologs of ionol were synthesized in the Rostov Institute of Organic and Physical Chemistry [1].

EXPERIMENTAL RESULTS

Typical curves of LDCL, induced by the addition of Fe^{2+} ions to the buffer in the absence (a) and in the presence (b, c) of microsomal membranes, are given in Fig. 2. It will be seen that on the addition of Fe^{2+} to the buffer a burst of chemiluminescence was recorded, its origin probably due to the appearance of superoxide anion-radicals as a result of reduction of molecular oxygen by bivalent iron. This chemiluminescent response disappears if superoxide dismutase (SOD) is added to the buffer. In the presence of ascorbate, no Fe^{2+}-induced burst of chemiluminescence was observed, although superoxide generation, as we know

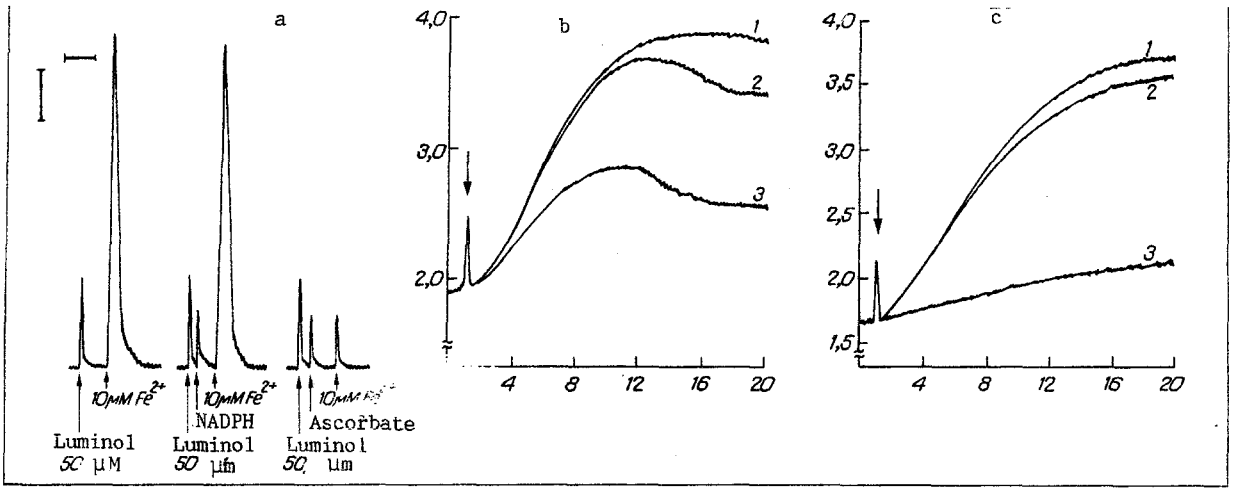


Fig. 2. Effect of NADPH and ascorbate on luminol-dependent chemiluminescence induced in a system of Fe^{2+} + O_2 + phosphate buffer (a) and ionol and its homologs on luminol-dependent chemiluminescence, induced in rat liver microsomes in the presence of NADPH (b) and ascorbate (c). 1) Control; 2) ionol II; 3) ionol XV, arrow indicates NADPH (b) and ascorbate (c). Calibration: a) 0.5 mV; b, c) mV × 1000. b, c) In min. Abscissa, time; ordinate, intensity of chemiluminescence.

[7], is enhanced. It can be postulated that ascorbate, which is a quencher of luminescence, prevents recording of interaction of the superoxide anion-radicals with luminol. NADPH has no action on Fe^{2+}-induced luminescence in solution. In the presence of microsomes (Fig. 2b, c) the addition of ascorbate and Fe^{2+} leads to a slow decrease in the intensity of chemiluminescence (in the course of 15-20 min; Fig. 2b, curve 1), after which the intensity of the chemiluminescent response begins to fall. In other words, in the presence of microsomes, ascorbate does not prevent the recording of chemiluminescence. It can be tentatively suggested that this is due to inaccessibility of the centers of photon emission in the membrane for water-soluble ascorbate. A similar chemiluminescent response is caused by the addition of Fe^{2+} and NADPH to microsomes (Fig. 2c, curve 1).

On the addition of antioxidants to the incubation medium containing microsomes and inducers of lipid perioxidation (LPO), inhibition of chemiluminescence was observed (Fig. 2b, c, curves 2 and 3) during both enzymic and nonenzymic LPO. The efficacy of the inhibitory action of the ionol derivatives differed greatly (Table 1) in very close correlation with the ability of these compounds to inhibit accumulation of LPO products in the presence of both ascorbate and NADPH (Fig. 3). Since both oxygen radicals and radicals of lipids are involved in the development of LPO, it cannot be concluded from the results with which of them the antioxidants used interact either predominantly or exclusively.

To solve this problem separate experiments were undertaken to study the action of ionol derivatives on LDCL in a system of generation of the superoxide anion-radical, and not containing sources of lipid radicals (i.e., absence of microsomes). For this purpose, KO_2 was dissolved in a mixture of water and dimethylsulfoxide (DMSO), the minimal quantity of water being used so that the intensity of LDCL was sufficiently high and on a stationary level (Fig. 4).

These conditions are satisfied by a mixture of water and DMSO in which the concentration of water was 2-5 M. Control measurements of pH showed that when KO_2 was added to this mixture, alkalification of the medium did not take place.

Both SOD and catalase, if sufficiently high activites were used, caused marked inhibition of KO_2-induced chemiluminescence (Fig. 5). A combination of the two enzymes also could inhibit the intensity of chemiluminescence to 85% of the control level. This indicates that the chemiluminescence which we recorded owed its origin mainly to interaction between luminol and oxygen radicals.

Typical chemiluminescent responses to addition of KO_2 in the control and also on the addition of certain ionol derivatives are shown in Fig. 6. Clearly the effects of the antioxidants were manifested as reduction of the amplitude of the flash and lowering of the steady-state level of luminescence.

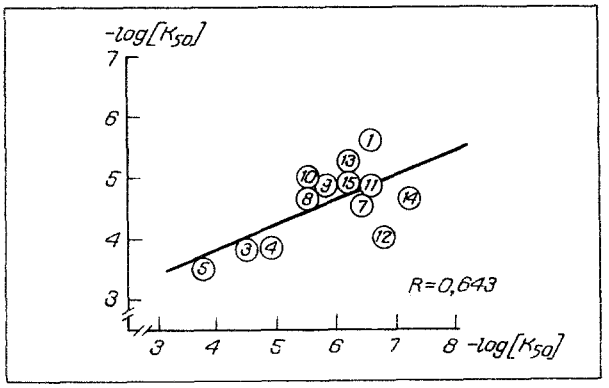


Fig. 3

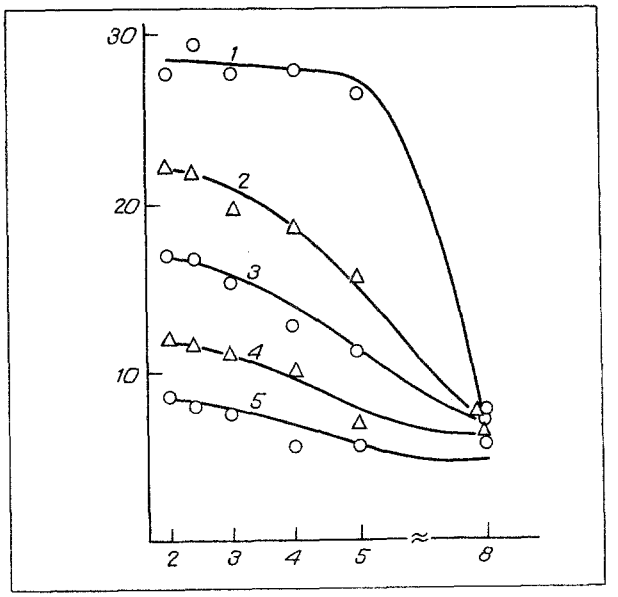


Fig. 4

Fig. 3. Comparison of efficacy of ionol and its derivatives as inhibitors of LPO and quenchers of luminol-dependent chemiluminescence in presence of Fe^{2+} + ascorbate. R) Coefficient of correlation. Ordinate, inhibition of chemiluminescence; abscissa, inhibition of LPO.

Fig. 4. Effect of water on intensity of luminol-dependent chemiluminescence induced by KO_2. Abscissa, H_2O concentration (in M); ordinate, intensity of chemiluminescence (in mV).

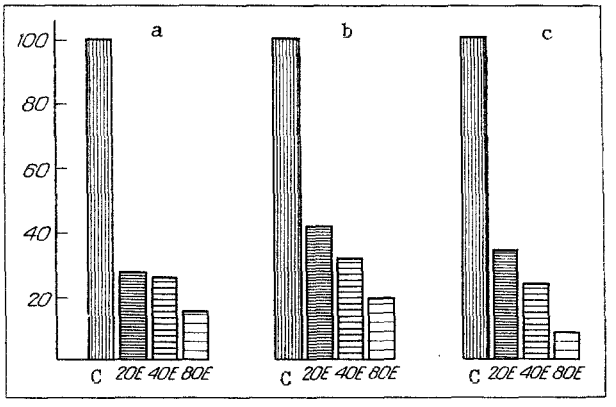


Fig. 5

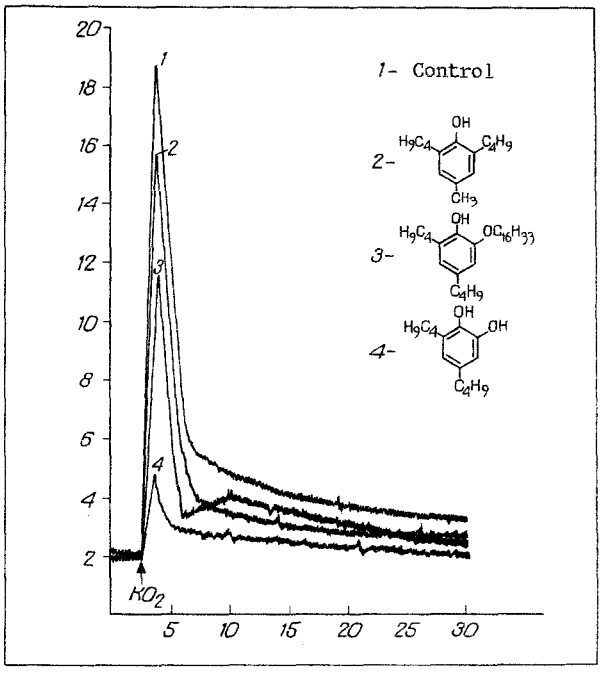


Fig. 6

Fig. 5. Action of catalase (a), SOD (b), and a combination of both (c) on luminol-dependent chemiluminescence. Abscissa, dose of enzyme; ordinate, intensity of chemiluminescence (in percent). C) Control.

Fig. 6. Effect of ionol and its derivatives on luminol-dependent chemiluminescence induced by KO_2. Abscissa, time (in sec); ordinate, intensity of chemiluminescence (in mV).

Data on inhibition of KO_2-induced chemiluminescnce by ionol derivatives, taken in equal concentration (10^{-5} M), are given in Table 1. Clearly the ionol derivatives used are weak inhibitors of chemiluminescence, induced by oxygen radicals. No correlation likewise was found between the effectiveness of inhibition of LPO in microsomes and the effectiveness of inhibition of KO_2-induced chemiluminescence by ionol derivatives.

From the results as a whole it can be concluded that inhibition of LPO by the hydrophobic ionol derivatives used is realized predominantly by interaction with lipid radicals, and chemiluminescence induced in a microsomal suspension arises mainly through interaction of luminol with lipid radicals (and not with oxygen radicals).

LITERATURE CITED

1. E. Serbinova, M. Kharfuf, and L. Ukhin, Byull. Éksp. Biol. Med.
2. A. Archakov, G. Bachmonova, and I. Karusina, Acta Biol. Med. Germ., 38, 229 (1979).
3. E. Cadenas, A. Boveris, and B. Chance, Biochem. J., 187, 131 (1980).
4. E. Cadenas, A. Boveris, and B. Chance, FEBS Lett., 112, 287 (1980).
5. B. Halliwell and J. M. C. Gutteridge, Free Radicals in Biology and Medicine, Oxford (1985).
6. A. M. Michelson, Biochimie, 55, 465 (1973).
7. W. Bors et al. (eds.), Oxygen Radicals in Chemistry and Biology, Berlin (1984).
8. A. Weimann, A. G. Hildebrandt, and R. Kahal, Biochem. Biophys. Res. Commun., 125, 1033 (1984).

IDENTIFICATION OF GLUCOCORTICOID-ACTIVATED Ca^{2+}-DEPENDENT NUCLEASES IN RAT THYMOCYTE NUCLEI

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Cell death in the course of embryo- and morphogenesis and of specific T-killing, as well as death of lymphoid cells under the influence of radiation and glucocorticoid hormones are examples of programmed cell death. The biochemical manifestation of programmed cell death is accumulation of chromatin fragments which are multiples of mononucleosomes [14]. These fragments are formed by activation of endogenous nucleases. A key in chromatin degradation is most probably played by Ca/Mg-dependent endonuclease [1, 3]. However, it is not yet clear whether endonucleolysis takes place as a result of the action of pre-existing enzymes or whether it depends on induction of synthesis of new proteins.

This paper describes an attempt to identify nucleases activated by glucocorticoids in thymocyte nuclei and to explain some mechanisms of the activation of these enzymes.

EXPERIMENTAL METHOD

Noninbred male albino rats were subjected to bilateral adrenalectomy and kept on a saline diet for 5-10 days. The animals were given dexamethasone ("Cadila," India) by intraperitoneal injection in a dose of 5 mg/kg. Thymocyte nuclei were isolated by the method in [2]. 0.6M NaCl-extracts of the nuclei were obtained by homogenization in buffer containing 0.6 M NaCl, 0.05 M Tris-HCl, pH 7.5, 0.5 mM PMSF, and 1 μM pepstatin A, and were subsequently centrifuged at 200,000 g for 30 min on an L8-70 ultracentrifuge ("Beckman," USA). Histones were removed from the extracts by ion-exchange chromatography on a column with CM-Sephadex C-50 ("Pharmacia," Sweden) [12].

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