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ACTION OF RETINOIC ACID ON LIPID PEROXIDATION IN RAT LIVER MICROSOMES *in Vitro*

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Despite much research, the biochemical mechanisms of the systemic function of retinol, which plays a role in growth, reproduction, cell proliferation and differentiation, and maintenance of an adequate immunologic status of the body, are insufficiently understood [4, 9, 15]. It can be postulated that these mechanisms are "multivalent" in character and are determined by the effect of retinol on several metabolic reactions, including microsomal oxidation and lipid peroxidation (LPO) [4]. It was shown previously that retinol *in vitro*, and also if given in large doses to experimental animals *in vivo*, causes marked depression of LPO in rat liver microsomes [6, 12]. Later these results were confirmed [10, 11]. However, many aspects of the antioxidant action of vitamin A have not been adequately studied. This is true in particular of the presence of antioxidant properties in other representatives of the vitamin A group and, in particular, of trans-retinoic acid, which can perform many of the functions of retinol (except its visual and reproductive functions), and which in some cases exhibits higher physiological and pharmacological (including antitumor) activity than retinol itself [7, 8, 13, 15].

The aim of this investigation was to compare the effects of retinol and trans-retinoic acid *in vitro* on enzymic and nonenzymic LPO by rat liver microsomes.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar rats weighing 250-300 g, kept on a natural balanced diet. The microsomal fraction was isolated by differential centrifugation, as described previously [6, 12], from liver homogenates prepared in the ratio of 1:3 (w/v) in 25 mM Tris-HCl buffer, pH 7.4, containing 0.175 M HCl. The microsomes were resuspended in isolation medium, so that 1 ml of suspension contained microsomes isolated from 0.2 g of liver. To 10 ml of the suspension 0.2 ml of a solution of retinol or trans-retinoic acid (both from Serva, West Germany) in ethanol was added to a final concentration of 7×10^{-5} M for retinol and from 3.5×10^{-5} to 2.8×10^{-4} M for retinoic acid. The samples were thoroughly mixed and preincubated for 30 min at 37°C on a water bath with constant stirring. After the end of preincubation the samples were quickly transferred to an ice bath and materials were taken for investigation of the LPO capacity of the microsomes, which was estimated from accumulation of malonic dialdehyde (MDA) in the microsomes after incubation for 60 min at 37°C on a water bath (with constant stirring), either without the addition of pro-oxidants (spontaneous LPO) or in the presence of Fe^{++} and ascorbate—ascorbate-dependent LPO (ADP), or of NADPH—dependent LPO (NDP), in the medium [1]. The MDA concentration in the samples was determined by the method in [1, 14] and expressed in nanomoles per milligram protein.

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TABLE 1. Effect of Retinol and Retinoic Acid on LPO in Rat Liver Microsomes *in Vitro* (in nanomoles MDA/mg protein, M±m)

Type of LPO	Control	Retinol	Retinoic acid
Spontaneous LPO	0,90±0,14	0,22±0,04*	0,63±0,07*
ADP	29,49±0,92	2,05±0,28**	30,22±1,12
NDP	10,09±1,04	2,46±0,41*	5,40±0,32*

Legend. Concentration of antioxidants 7×10^{-5} M. *P < 0.01, **P < 0.001 compared with control. Mean data of 10-14 experiments shown.

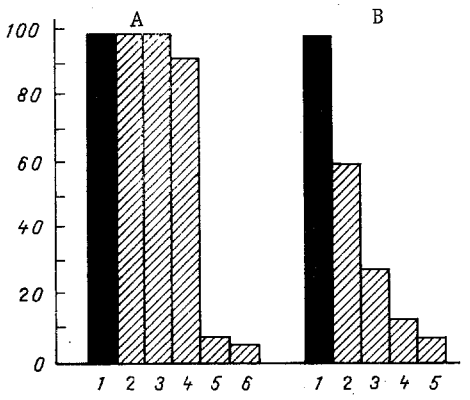

Fig. 1. Dependence of antioxidant action of retinoic acid on its concentration in the medium. A) ADP, B) NDP. 1) Control; 2-6) concentration of retinoic acid 3.5×10^{-5}, 7.0×10^{-5}, 1.05×10^{-4}, 1.4×10^{-4}, and 2.8×10^{-4} M respectively. Ordinate, intensity of LPO (in percent of control).

EXPERIMENTAL RESULTS

Addition of retinol to the incubation medium led to marked depression of all types of LPO studied: to 25% of the control value in the case of spontaneous and enzymic LPO and to 7% in the case of ADP (Table 1), confirming previous observations [6, 12]. Addition of retinoic acid to the sample (in the same concentration as retinol) led to significant reduction of the ability of the microsomes to undertake spontaneous LPO (to 70% of the control), and to even greater depression of enzymic LPO (to 53%). Meanwhile retinoic acid, in a concentration of 7×10^{-5} M, did not affect the ADP capacity of the microsomes.

The results of this series of experiments thus indicate that retinoic acid exhibits its antioxidant properties primarily in relation to NDP, but also in relation to spontaneous LPO. The inhibitory action of trans-retinoic acid on LPO was weaker than the action of retinol (Table 1).

The next step was to study dependence of the action of trans-retinoic acid on ADP and NDP on its concentration in the medium. With an increase in the retinoic acid concentration in the medium the degree of inhibition of NDP was found to rise progressively, and when the concentration reached 2.8×10^{-4} M the intensity of NDP was only 6% of the control (Fig. 1). Meanwhile the intensity of ADP remained virtually unchanged when the retinoic acid concentration was increased from 3.5×10^{-5} to 1.05×10^{-4} M, it fell sharply (to 8% of the control) when the concentration was increased to 1.4×10^{-4} M, and then showed little change during a further increase of concentration to 2.8×10^{-4} M. Dependence of the antioxidant action of trans-retinoic acid on its concentration in the medium thus differed for enzymic and nonenzymic LPO: In the first case it was linear, in the second it was stepwise in character.

The causes of the differences in the antioxidant action of retinol and retinoic acid, and also in the action of the latter on enzymic and nonenzymic LPO are not yet perfectly clear and require further study. Meanwhile, on the basis of the hypothesis that the antioxidant effects of retinol are connected with its interference in electron transport processes in the course of the LPO reaction [12], it can be tentatively suggested that in its donor—acceptor properties retinoic acid is weaker than retinol, especially as regards its interaction with the nonenzymic LPO system.

The results thus indicate that one of the natural biologically active representatives of the vitamin A group, trans-retinoic acid, possesses antioxidant properties. These findings, together with information now published on the antioxidant properties of retinol [2, 4, 6, 10, 12], suggest that ability to inhibit LPO may reflect to some degree the physiological action of retinol and retinoic acid in the body, and it must undoubtedly be taken into account when their pharmacological action is analyzed. The differences discovered in the degree of the antioxidant effects of retinol and retinoic acid *in vitro* agreed both with data on the differences in the ultimate physiological effects of these compounds and also with information on differences in their action on metabolic processes [4, 5, 15].

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