LIPID PEROXIDATION AND ENDOGENOUS DNA-POLYMERASE ACTIVITY OF ISOLATED CHROMATIN FRACTIONS FROM RAT LIVER

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Different lipid fractions in the composition of nuclear chromatin may play a structural and regulatory role in the molecular organization and function of the genetic apparatus of the cell [1, 3]. It has been claimed that the factor modifying activity of the genome is peroxidation of phospholipids bound with chromatin proteins [3]. Since the xenobiotic tetrachloromethane is one of the classical agents activating lipid peroxidation in hepatocyte membrane structures [5, 6], it was interesting to study the action of tetrachloromethane on the peroxidation of lipids in the composition of nuclear chromatin, and also on the functional activity of the genome. Preparations of nuclei of liver cells are known to be able to metabolize chloroalkanes, interacting with proteins and lipids of the chromatin [11].

In connection with the facts described above, it was decided to study the action of tetrachloromethane on lipid peroxidation (LPO) and on total endogenous DNA-polymerase activity of repressed and actively transcribable chromatin of rat liver.

EXPERIMENTAL METHOD

Chromatin fractions were obtained from the liver of 3-month-old female rats by the method described previously [8]. LPO of chromatin (uninduced, NADPH- and ascorbate-dependent – NDP and ADP, respectively) was estimated by measuring accumulation of malonic dialdehyde (MDA) and its derivatives after incubation of the samples for 2 h at 37°C [4]. Total endogenous DNA-polymerase activity in the chromatin fractions was determined before and after induction of LPO in them, by the method described previously [7]. The tetrachloromethane was dissolved in 96% ethanol (1:5) and added in a volume of 1 µl to 1 ml of incubation medium. Control samples were subjected to similar treatment, but using water instead of tetrachloromethane. The experimental results were subjected to statistical analysis by nonparametric tests.

EXPERIMENTAL RESULTS

Incubation of preparations of isolated chromatin fractions in control samples (without tetrachloromethane) and under the corresponding conditions led to stimulation of LPO (Table 1). Under these circumstances inhibition of DNA-polymerase activity also was observed, falling sharply in the fraction of repressed chromatin in the case of ADP and without induction ($p \leq 0.01$ and $p \leq 0.05$, respectively). In the fraction of actively transcribable chromatin there was a tendency for the degree of NDP to increase compared with the fraction of repressed chromatin, and under these circumstances the DNA-polymerase activity in active chromatin decreased during stimulation of this type of LPO after incubation ($p \leq 0.05$). Thus in this case there was an inversely proportional relationship between LPO of the chromatin fractions and its endogenous DNA-synthesizing activity.

Activation of nuclear prepartions of rat liver cells by tetrachloromethane and chloroform, with the formation of metabolites binding with chromatin lipids, like the effect observed in the microsomal fraction of the liver [13], was demonstrated in [11]. In our own experiments on incubation of tetrachloromethane with chromatin preparations, significant stimulation of

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TABLE 1. Effect of Tetrachloromethane on Intensity of LPO and on Endogenous DNA-Polymerase Activity of Chromatin Fraction (n = 5)

Parameters	Control						Tetrachloromethane					
	repressed chromatin			actively transcribable to chromatin			repressed chromatin			actively transcribable to chromatin		
	NDP	ADP	uninduced control	NDP	ADP	uninduced control	NDP	ADP	uninduced control	NDP	ADP	uninduced control
MDA, mmoles/mg protein	1 188**	470**	196	1 933**	376**	61	895**	164	281	1 489**	648**	148
DNA-polymerase activity, cpm/mg DNA; before incubation, after incubation for 2 h at 37°C	89 727 86 092*	76 955 3 027*	70 536 28 926	89 019 62 40 752 19	028 249*	69 379 47 517	80 113 40 25 722 2	420 549*	44 874 11 249	73 426 28 821	47 747 17 467	57 506 39 475

Legend. *$p \leq 0.05$, **$p \leq 0.01$ compared with uninduced control.

uninduced LPO ($p \leq 0.05$) and inhibition of ADP ($p \leq 0.01$) in repressed chromatin were observed, by contrast with results obtained for the microsomal fraction of the liver [13]. Under these circumstances inhibition of DNA-polymerase activity was observed in the tests with uninduced LPO and in NDP compared with control samples without tetrachloromethane. Reduction of DNA-polymerase activity began to appear immediately after addition of chromatin to the incubation medium containing tetrachloromethane (a decrease in ADP compared with analogous tests without tetrachloromethane, ($p \leq 0.05$). The smallest change in DNA-polymerase activity was observed under the influence of tetrachloromethane before incubation in NDP. After incubation for 2 h at 37°C, inhibition of DNA-polymerase activity by tetrachloromethane was intensified even more. Just as in the case of LPO, a greater fall of activity was observed in the repressed chromatin fraction. Incubation led to a significant lowering of DNA-polymerase activity in both chromatin fractions ($p \leq 0.05$ and $p \leq 0,01$ for NDP and ADP, respectively).

DNA-polymerase activity in samples treated and not treated with tetrachloromethane decreased under the influence of this compound in the repressed chromatin fraction after incubation for 2 h at 37°C (for NDP and uninduced LPO $p \leq 0.05$). Treatment with tetrachloromethane also led to a chagne in the intensity of LPO in this chromatin fraction. The inhibitory effect of tetrachloromethane on endogenous DNA-polymerase activity in this case was thus probably connected, at least partially, with its effect on LPO processes. However, complete agreement was not observed in this case. Tetrachloromethane may possibly have a direct inhibitory action also on enzymes, and may also modify the properties of the template, and this could be reflected in DNA-polymerase activity. The possibility of such an action is indicated by the fact of binding of labeled tetrachloromethane with chromatin DNA and proteins [12].

The results of the investigation as a whole are evidence of the effect of a change in the intensity of LPO processes on activity of the genome (in this case on endogenous DNA-polymerase activity of chromatin): induction of LPO in control samples leads to inhibiton of DNA polymerase activity whereas tetrachloromethane induces changes in the intensity of LPO in the repressed chromatin fraction and an even greater lowering of DNA-polymerase activity in this chromatin fraction after induction of LPO. The selective effect of tetrachloromethane on the parameters of the repressed chromatin fraction studied may perhaps be linked with differences in the lipid composition of fractions of the latter.

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CHANGES IN SOME PARAMETERS OF LIPID METABOLISM IN ERYTHROCYTE MEMBRANES DURING DEVELOPMENT OF ALLOXAN DIABETES

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KEY WORDS: alloxan diabetes; erythrocyte membranes; lipid peroxidation; phospholipids; α-tocopherol; cholesterol.

Cell membranes are kept in the intact state by many factors, among which the most important are normal functioning of certain membrane-bound lipid-dependent enzymes, on the one hand, and a continuous process of lipid renewal through maintenance of a strictly limited level of lipid peroxidation (LPO), on the other hand [2-4, 7, 11, 13].

The aim of this investigation was to study the molecular mechanisms of the action of LPO products on the functional state of a biological membrane during alloxan diabetes (AD). The general principles governing development of changes in the qualitative and quantitative composition of phospholipids (PL), concentrations of total, free, and esterified cholesterol (TCh, FCh, and ECh, respectively) and of α-tocopherol (TP), and the cholesterol/phospholipid (Ch/PL) ratio in the erythrocyte membrane (EM) were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male albino rats weighing 180-200 g, kept on an ordinary diet. AD was induced by the usual method: intraperitoneal injection of alloxan in a dose of 15 g/kg body weight. Blood taken for investigation was stabilized with oxalate. The blood glucose concentration was determined by the orthotoluidine method. EM were isolated, purified, and identified by Limber's method [14], and PL were determined by one-way ascending chromatography on Filtrak FN-11 paper (East Germany), impregnated with silicic acid [6].

The intensity of LPO was judged by the accumulation of malonic dialdehyde (MDA) in enzymic and nonenzymic systems of LPO [3]. Concentrations of TCh, ECh, and FCh were determined by a unified method [10], TP and EM by Duggan's method [12], and protein by Lowry's method [15].

EXPERIMENTAL RESULTS

All periods of development of AD were characterized by appreciable activation of LPO in EM (Table 1). Release of LPO products definitely predominated on the 7th day of the disease in the NADPH-dependent system (by about 66%), and there was a smaller shift (by about 43%) in the ascorbate-dependent system of LPO. On the 14th day of the disease, release of MDA in the above peroxidation systems continued to rise, up to about 145 and 106%, respectively. On the 21st day after injection of alloxan a tendency was noted for the intensity of LPO to decrease, although as before the MDA level in the two systems was higher than that in the control animals. It can thus be concluded from the results that lipid peroxidation takes place with high activity in EM of albino rats with AD, determined at all times of observation.

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