CALCIUM AND LIPID PEROXIDATION IN MITOCHONDRIAL AND MICROSOMAL MEMBRANES OF THE HEART

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It has recently been shown that in lesions of the heart of varied etiology (myocardial infarction, ischemia, severe stress) the key stages in the pathogenetic chain of irreversible changes in the myocardium are two simultaneously occurring processes: activation of peroxidation in phospholipids of membranous structures of the cardiomyocytes and damage to enzyme systems responsible for transmembrane transport of Ca^{++} [2, 3, 6].

Considering the known facts on disturbance of the barrier function of membranes for ions during induction of lipid peroxidation (LPO), on the one hand [1], and also data on the effect of Ca^{++} on the efficiency of LPO, on the other hand [7, 10], it was decided to investigate to what extent activation of LPO and disturbance of calcium homeostasis can influence one another in cases when they take place simultaneously in heart cells. Accordingly, in the present investigation we studied, first, the action of LPO on enzyme systems for calcium ion transport in membranes of the microsomal fraction of the heart and, second, the effects of different concentrations of calcium ions on development of LPO processes in membranes of the microsomal and mitochondrial fractions when enzymic (pyridine nucleotide-dependent) and nonenzymic (Fe^{++} + ascorbate) methods of induction of LPO were used.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 140-180 g were used. After decapitation of the animal the heart was removed, washed, and minced in a homogenizer of "Polytron" type for 3 × 30 sec on ice, with a ratio of weight of the heart to 1.15% KCl solution of 1:6. After filtration through two layers of gauze the homogenate was centrifuged for 10 min at 800g. The mitochondrial fraction was sedimented first (30 min, 10,000g) from the resulting supernatant, followed by the microsomal fraction (90 min, 105,000g). All procedures were conducted at 0-4°C.

The Ca^{++}-transporting activity of the microsomes was recorded as accumulation of $^{45}Ca^{++}$. LPO in membrane fractions was induced in medium of the following composition: 50 mM phosphate buffer (pH 7.4, 37°C), 0.5 mM ascorbate, 0.05 mM Fe^{++}. Activation of LPO was recorded as accumulation of malonic dialdehyde (MDA) spectrophotometrically at 535 nm ($\varepsilon = 1.56 \cdot 10^5\ M^{-1} \cdot cm^{-1}$) [9].

EXPERIMENTAL RESULTS

Enzyme systems for Ca^{++} transport in membranous structures of the heart are exceptionally highly sensitive to LPO. Even slight accumulation of LPO products in membranes of the microsomal fraction of the heart led to complete inhibition of Ca^{++}-transporting ability (Table 1), on account of complete uncoupling of ATP hydrolysis from calcium transport. Antioxidants (4-methyl-2,6-di-tert-butylphenol, α-tocopherol) prevented accumulation of LPO products and protected the membranes of the microsomal fraction against the inhibitory action of LPO on Ca^{++} transport.

In the next series of experiments the action of Ca^{++} on LPO was studied in membranes of the microsomal and mitochondrial fractions of the heart; LPO was induced both by enzymic

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TABLE 1. Disturbance of Ca^{++}-Transporting Ability of Microsomal Fraction of Heart during Ascorbate-Induced LPO (M ± m)

Experimental conditions	Incubation time, min	MDA, nmoles/mg protein	Rate of transport, nmoles Ca^{++}/min/mg protein
Control		1,2±0,1	37,2±4,0
Fe^{++} + ascorbate	5	3,8±0,3	22,5±4,2
Fe^{++} + ascorbate	15	5,0+0,4	0
Fe^{++} + ascorbate + 4-methyl-2,6-di-tert-butylphenol ($5 \cdot 10^{-5}$ M)	15	1,1±0,1	36,8±3,6
Fe^{++} + ascorbate + α-tocopherol ($5 \cdot 10^{-5}$ M)	15	1,8±0,2	30,1±5,1

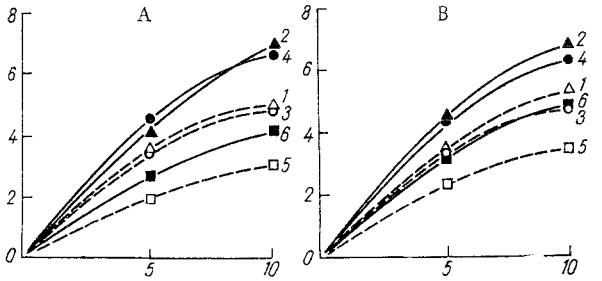

Fig. 1. Kinetics of MDA accumulation in mitochondrial (A) and microsomal (B) fractions of heart during induction of LPO by different methods and addition of Ca^{++} (5×10^{-5} M). 1) Fe^{++} + ascorbate + Ca^{++}, 2) control; 4) Fe^{++} + NADH + Ca^{++}, 3) control; 6) Fe^{++} + NADPH + Ca^{++}, 5) control. Abscissa, incubation time (in min); ordinate, MDA (in nmoles/mg protein).

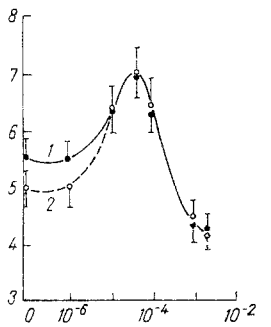

Fig. 2. Dependence of MDA accumulation in microsomes (1) and mitochondria (2) of heart on Ca^{++} concentration during ascorbate-dependent LPO. Abscissa, Ca^{++} concentration (in M); ordinate, MDA (in nmoles/mg protein); incubation time 10 min.

pyridine nucleotide-dependent (NADPH or NADH) systems and by a nonenzymic (Fe^{++} + ascorbate) system. The efficiency of LPO inducers for the microsomal and mitochondrial fractions was found to decrease in the following order: ascorbate > NADH > NADPH (Fig. 1). During LPO induced by the Fe^{++} + ascorbate system, the curves of accumulation of MDA in mitochondrial and microsomal fractions from the heart as a function of Ca^{++} concentration in the medium were extremal in character (Fig. 2). In both cases, with concentrations of between 10^{-6} and 5×10^{-5} M, Ca^{++} had a stimulating action on LPO, but if its concentration was increased from

TABLE 2. MDA Accumulation (in nmoles/mg protein) During Enzymic NADH and NADPH-Dependent and Nonenzymic Ascorbate-Dependent LPO Induction in the Presence of Different Ca^{++} Concentrations in Fractions of Mitochondria and Microsomes of the Heart (M ± m)

Membrane fraction	Experimental conditions	MDA		
		without Ca^{++}	$5 \cdot 10^{-5}$ M Ca^{++}	$2 \cdot 10^{-3}$ M Ca^{++}
Mitochondria	Fe^{++} + ascorbate	5,10±0,33	6,95±0,62	4,15±0,45
	NADH + Fe^{++}	5,05±0,40	6,55±0,55	4,20±0,38
	NADPH + Fe^{++}	3,15±0,30	4,10±0,35	2,40±0,20
Microsomes	Fe^{++} + ascorbate	5,30±0,45	6,90±0,65	4,30±0,41
	NADH + Fe^{++}	4,80±0,42	6,40±0,58	3,80±0,35
	NADPH + Fe^{++}	3,60±0,40	4,85±0,48	2,85±0,33

5×10^{-5} to 2×10^{-3} M, Ca^{++} inhibited LPO. Starting with a concentration of 10^{-3} M, LPO activity was lower than in the absence of exogenous calcium. A similar character of action of Ca^{++} on LPO in microsomes and mitochondria also was found during induction by the enzymic method in the presence of NADPH or NADH (Table 2). Effects of Ca^{++}-dependent stimulation of LPO were weak and on average did not exceed 30-35% (Table 2; Fig. 2), but when the action of Ca^{++} on intracellular LPO is assessed it must be remembered that under physiological conditions intracellular Ca^{++} concentrations in heart muscle vary from 10^{-7} M in diastole to 10^{-5} M in systole [5], whereas in the extracellular medium they are about 1 mM. The Ca^{++} concentrations present inside the cell ought therefore to have a stimulating action on LPO, whereas the external Ca^{++} concentrations should inhibit LPO.

If as a result of induction of LPO the intracellular depots and enzymic mechanisms of Ca^{++} transport are damaged, Ca^{++} ions released into the sarcoplasm will stimulate LPO and so facilitate further outflow of calcium and an increase in its concentrations up to values close to those maximally effective for LPO activation (10^{-5} M). The processes of LPO activation and Ca^{++} release from the intracellular depots may thus prove to be mutually potentiating cascades of the pathogenetic mechanism, responsible as a whole for the exceptionally high effectiveness of damage to intracellular structures.

In various pathological states, namely hypertrophy of the heart, stress or hypoxic injuries of the heart — disturbance of relaxation is regularly observed, the duration of systole is increased, the Ca^{++} concentration in the sarcoplasm rises and, consequently, the stimulating action of Ca^{++} on LPO is enhanced. This vicious circle, formed between excess of Ca^{++} on the one hand and LPO activity on the other, is evidence of the desirability of combined administration of antioxidants and inhibitors of slow calcium channels in heart diseases.

Meanwhile, high Ca^{++} concentrations in the extracellular medium, inhibiting LPO, as it were, localize the foci of LPO to a single cell, and do not allow the process to spread to other cardiomyocytes. It is not surprising, therefore, that during LPO activation, induced initially by an excess of calcium, such as under the influence of catecholamines, microfocal myocardial lesions may arise. This hypothetical possibility corresponds to the real facts which are that after exposure to stress the lesions which are essentially adrenergic are microfocal in character [4], and also to data indicating that in infarction the zone of ischemia is separated from the adjacent myocardium by a clear line of demarcation [8].

The concept of relations between LPO processes and the dynamics of changes in the Ca^{++} concentrations thus bring us closer to an understanding of some of the characteristic features of lesions of the heart due to ischemia and stress.

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CHANGES IN THE PRIMARY STRUCTURE OF DNA IN SOME RAT ORGANS DURING AGING

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Aging is essentially the summation of changes arising at different levels of organization, including the biochemical level. It is generally accepted that the metabolically most stable component of the cell is DNA. However, even in nondividing cells, extracopying of DNA fragments from individual regions of the genome can take place [4]. In ontogeny the level of DNA methylation changes [2] and the DNA undergoes injuries, which are removed in the course of reparative synthesis [12], the effectiveness of which diminishes with age [15]; this evidently leads to the accumulation of mistakes in the genetic apparatus [8]. An important role in this situation is played by free-radical processes [7], the inhibition of which lengthens the duration of life [6].

The object of this investigation was to study the organ specificity of age changes in the primary structure of DNA.

EXPERIMENTAL METHOD

Noninbred albino rats aged 1, 12, and 30 months were used. The animals were decapitated, the organs quickly removed, the nuclei isolated in the presence of EDTA [10], and DNA was isolated from the nuclei [11, 14]. The DNA was hydrolyzed to pyrimidine fragments of different lengths — blocks or isopliths [5]. The isopliths were separated according to length and composition by thin-layer chromatography [9]. For subsequent quantitative analysis, thin-layer disks were scanned on a chromatogram spectrophotometer (Opton, West Germany) at 270 nm, i.e., at the isobestic point of pyrimidines, determined by analysis of UV reflection spectra. The composition of the DNA bases also was determined by thin-layer chromatography. The relative percentages of the different bases were calculated by multiplying the areas of peaks on the densitograms by coefficients determined empirically for each base, and on the basis of direct correlation between the quantity of material in the spot and the area of the corresponding peak on the densitogram, provided that the quantity of material in the spot did not exceed 1 μg. The chromatograms were scanned in reflected UV light using a slit measuring 12 × 0.2 mm, the velocity of the disks relative to the beam was 10 mm/min and the tape winding speed of the automatic writer was 30 mm/min. The coefficients for bases dispersed in an alkaline solvent [3] were as follows: adenine (A) — 1.00, guanine (G) — 1.56, cytosine (C) — 1.96, thymine (T) — 1.51, 5-methylcytosine (5-MC) — 1.37. Scanning was carried out at 260 nm to analyze the ordinary bases and at 290 nm to determine the 5-methylcytosine content.

EXPERIMENTAL RESULTS

As Table 1 shows, a general tendency was observed in all investigations for the size of the pyrimidine blocks to decrease with age, as shown by an increase in the number of mono-

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