ROLE OF PHOSPHOLIPIDS IN DESTABILIZATION OF LYSOSOMAL MEMBRANES IN CHRONIC ALCOHOL POISONING

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In connection with the decisive role of phospholipids (PL) in the structural organization and functioning of biological membranes, the study of changes in the qualitative and quantitative composition of PL taking place under the influence of endogenous phospholipases, which are a feature of the pathogenesis of many diseases, merits great attention. One factor which merits further study is the membrane-bound Ca^{++}-stimulated phospholipase A_2, with pH-optimum in neutral and alkaline media, and found in many subcellular formations: microsomes [6], mitochondria [3], etc. However, its presence in lysosomal membranes (LM) is still disputed [6, 11].

The aim of this investigation was to study changes in the PL spectrum and possible activity of membrane-bound phospholipase A_2 in lysosomal membranes from albino rat liver under conditions of the normally metabolizing tissue and during long-term alcohol poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g, kept under standard conditions in the animal house. Animals of the experimental group received 26% ethanol solution only to drink for 30 days. Four days before sacrifice, the animals were given an intraperitoneal injection of the nonpolar detergent WR-1339 in a dose of 85 mg/g body weight. Liver tissue was fractionated by the method in [8].

Lysosomal membranes were obtained after freezing and thawing 10 times, by centrifugation at 100,000g for 30 min. Membrane PL were separated by two-dimensional thin-layer chromatography (TLC) [12].

Changes in stability of the lysosomal membranes were determined [7] by measuring nonsedimented acid phosphatase (AP) activity. The substance 1-acyl-2-[1-^{14}C]-oleoyl-phosphatidylcholine (^{14}C-PCh) was synthesized by an enzymic method [13], using as the substrate 1-acyllysophosphatidylcholine [9], purified by the method in [4] from residual phospholipase A_2 activity, and [1-^{14}C]-oleoyl-CoA (specific activity 57 μCi/mmole; from Amersham Corporation, England).

Phospholipase A_2 activity was determined in an incubation medium of Tris-maleate buffer (0.1 M, pH 8.0) containing 20 nanomoles [^{14}C]-PCh, 8 mM $CaCl_2$, and about 100 μg protein (final volume 1.5 ml). At the end of incubation, carried out at 37°C for 30 min, lipids were extracted [1] and hydrolysis products were fractionated by one-way TLC in a system of chloroform–methanol–acetic acid–water (14:6:0.5:1.0 by volume). The spots were developed in iodine vapor and the degree of their radioactivity was determined in Bray's fluid on an SL-4221 scintillation spectrophotometer (from Roche-Bioélectronique, France).

The purity of the lysosomal fraction was verified relative to activity of the marker enzymes: monoamine oxidase (MAO) [10] and AP [2].

EXPERIMENTAL RESULTS

The results showed that alcohol poisoning with a duration of 30 days is characterized by outflow of AP from the lysosomes (Fig. 1A).

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TABLE 1. Changes in PL Fractions (in μg lipid phosphorus/mg protein) in Lysosomal Membranes of Rat Liver during Chronic Alcohol Poisoning (M ± m)

PL fraction	Control	Alcohol poisoning
PC	5,41±0,18	9,53±0,69
LPC	1,12±0,11	1,87±0,21†
PE	3,13±0,11	4,34±0,4 †
LPE	Trace	1,41±0,16*
SPM	7,11±0,26	3,14±0,28*
PS	3,50±0,1	2,50±0,11*
PI	2,44±0,2	1,74±0,09 ‡
LBPA	2,96±0,28	3,22±0,3

*P < 0.001, †P < 0.002, ‡P < 0.05 compared with control.

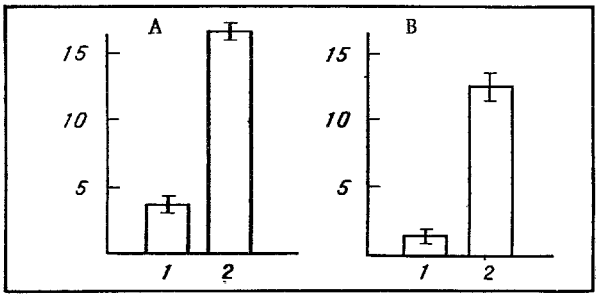

Fig. 1. Action of 30-day alcohol poisoning on AP (A) and phospholipase A_2 (B) activity. 1) Control; 2) alcohol poisoning. Ordinate, enzyme activity: AP) in μg/P_i/mg protein; phospholipase A_2) in nanomoles 1-^{14}C-oleic acid/mg protein/h.

Investigation of the PL spectrum of the lysosomal membranes showed an increase in the concentration of lysophosphatidylethanolamines (LPE) and lysophosphatidylcholines (LPC), which have marked membranolytic properties [14], compared with normal, with a parallel increase in the concentration of diacyl forms of these compounds (Table 1). A reduction also was found in the concentrations of sphingomyelins (SPM) and basic PL of acid nature, namely phosphatidylserines, (PS) and phosphatidylinositols (PI). Consequently, chronic alcohol poisoning was accompanied by profound changes in PL fractions of the lysosomal membranes, which could be the cause of their destabilization, leading to increased outflow of AP.

The lysosomal fractions obtained had a high level of relative specific AP activity (from 30 to 50) but no activity of MAO, a marker enzyme of destroyed mitochondrial membranes. Besides the absence of phosphatidic acid (a characteristic lipid for mitochondria), a fairly high level of lyso-bis-phosphatidic acid (LBPA), a marked lipid of lysosomes, and which served, in particular, as an objective parameter of the purity of the lysosomal fractions obtained, was noted in the PL spectrum of the lysosomal membranes.

The investigation thus revealed that lysosomal membranes contain phospholipase activity of type A_2, which is Ca^{++}-dependent, functions at pH 8.0, and is increased many times over by the action of alcohol poisoning (Fig. 1B).

These results also are evidence that labelization of lysosomal membranes, observed under conditions of alcohol poisoning, is associated with an increase in the concentration of lyso-forms of PL in the membranes which, in turn, is due to activation of phospholipase A_2.

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THE RECOVERY PROCESS AFTER PROLONGED MUSCULAR WORK

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The process of recovery after muscular work involves not only replenishment of the energy reserves, but also their supercompensation (over-restoration) [5]. In this situation phasic changes of working capacity and of the state of various systems of the body are observed [2]. Intensification of protein synthesis [4, 10] but, at the same time, increased excretion of 3-methylhistidine (3-MH), indicating enhanced degradation of contractile proteins [15], are observed in the recovery period.

The aim of this investigation was to determine the state of protein metabolism and correlation between exchanges and replenishment of the glycogen reserves and with adrenocortical activity.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 170-200 g. The rats were made to swim in water (33 ± 1°C) for 10 h. The animals were anesthetized superficially with ether (for 2 min) 1, 2, 6, 24, and 48 h after the end of swimming (4-5 rats in each group), and blood was taken from the heart and pieces of tissue from the quadriceps femoris muscle and the liver. The concentrations of corticosterone [12] and tyrosine were determined in the blood plasma and of 3-MH [7], free tyrosine [14], glycogen [8], and protein [9] in muscle homogenate. The glycogen concentration also was determined in liver homogenate. In animals used in the experiment 24 and 48 h after swimming, and also in the control rats, kept in special cages, 24-hourly samples of urine were collected and the 3-MH excretion was determined [11].

EXPERIMENTAL RESULTS

The total protein concentration 1 h after the end of a 10-h period of swimming showed no significant change in working muscles, calculated per gram wet weight of tissue. The protein concentration per gram dry weight of tissue was increased, which was due to a decrease in the percentage of dry residue of the muscle, and indicated increased hydration of the muscle tissue. The total protein concentration in the working muscles was increased 2 h after the end of work, when calculated per gram both wet and dry weight of tissue. (Fig. 1). It remained raised 6 and 24 h after work, and returned to its initial level after 48 h. The ob-

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