COMPARISON OF SPHINGOMYELINASE ACTIVITY IN LIVER CELL NUCLEI AND IN THE BRAIN

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The sphingomyelinases (sphingomyelin-cholinophosphohydrolases) are lipolytic enzymes that are involved in sphingolipid metabolism [11]. These enzymes are present in large quantities in the liver, brain, spleen, and kidneys. Disturbance of sphingolipid metabolism as a result of a sharp decline in tissue sphingomyelinase activity leads to the accumulation of sphingomyclin in them and is accompanied by the development of severe pathological states, such as the disturbance of mental activity in Niemann–Pick disease [6, 15].

Two types of enzymes are found in liver cells: acid sphingomyelinase, with an optimum of activity at pH 4.8, and neutral, active at pH 7.2-7.4 [8, 12]. There is evidence of their subcellular distribution [9, 10]. Neutral sphingomyelinase is found in plasma membranes and microsomes of liver and brain cells [9, 10]. Activity of this enzyme in the liver, spleen, and kidneys amounts to 1.2, 0.6, and 4.6% of brain sphingomyelinase activity, respectively [9]. The acid form of the enzyme is localized in liver and brain cells in the lysosomes. No sphingomyelinases are found in the mitochondrial fraction or cytosol. Despite intensive study of the distribution of sphingomyelinase activity in the cell there is no information in the literature about its presence in nuclei.

Previous investigations showed that the quantity of sphingomyelin fluctuates considerably in nuclear structures in the course of cell proliferation [1]. At the moment of stimulation of DNA and RNA synthesis in the nucleus the sphingomyelin content rises rapidly from 12 to 30% of the total content of phospholipids in the chromatin and from 50 to 80% in the nuclear matrix [3]. After the completion of nucleic acid synthesis the sphingomyelin content returns to its initial level.

The possibility cannot be ruled out that endogenous sphingomyelinases of the cell nucleus participate in these rapid fluctuations in the phospholipid composition of nuclear structures. It was accordingly decided to study the level of sphingomyelinase activity in cell nuclei of the intact rat liver at different pH values and to compare it with sphingomyelinase activity both of liver homogenate and also of brain homogenate, in which the activity of this enzyme is maximal.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats. Nuclei were extracted from the liver cells by the method of Blobel and Potter. To remove nuclear membranes a 1% solution of Triton X-100 was used. Sphingomyelinase activity was determined by the method in [10]. The incubation mixture consisted of 50 mM Tris-HCl (pH 7.1-7.8), 1 mM EDTA, and 10 mM $MgCl_2$; or 50 mM Tris-acetate buffer, pH 5.8, containing 1 mM EDTA, and 10 mM $MgCl_2$, or 50 mM Na-acetate buffer, pH 5.0. All samples also contained 2 mg/ml of Triton X-100, 2 mM N-methyl-[^{14}C]-sphingomyelin (specific activity 0.2 mCi/mole, "Amersham," England), and 3-4 mg/ml protein, of the nuclear fraction. The volume of each fraction was 0.7 ml. The mixture was incubated at 37°C for 20 min with regular mixing. Sphingomyelinase activity was judged by passage of the label in the [^{14}C]-choline phosphate form, into the aqueous phase after extraction of lipids from the volume of the sample when the reaction was complete. Lipids were extracted by the method of Bligh and Dyer. An aliquot of the aqueous phase was added to a solution of ZhS-8 scintillator and its radioactivity was determined on a Mark II liquid scintillation counter (USA).

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TABLE 1. Comparison of Sphingomyelinase Activity of Liver Cell Nuclei and Liver and Brain Homogenates ($M \pm m$)

Test object	pH of incubation medium			
	7-1		5,0	
	pulses/mg protein	pulses/mg sphingomyelin	pulses/mg protein	pulses/mg sphingomyelin
Liver nuclei	165±23	$(2,0\pm0,3)\cdot10^4$	50±6	$(6,3\pm1,1)\cdot10^3$
Liver homogenate	360±35	$(3,9\pm0,7)\cdot10^4$	—	—
Brain homogenate	4500±870	$(4,4\pm0,9)\cdot10^4$	630±26	$(6,1\pm0,9)\cdot10^3$

The sphingomyelinase activity was studied in these experiments in whole nuclei of rat liver cells, in nuclei from which the nuclear membrane had been removed, and in liver and brain homogenates. Sphingomyelinase from *B. Cereus*, used as the standard, came from "Boehringer" (West Germany). The experiments were conducted at pH values of 5.0, 5.8, 7.1, and 7.8. The purity of the nuclear fraction was verified by electron microscopy.

EXPERIMENTAL RESULTS

Dependence of sphingomyelinase activity in whole nuclei on the pH of the incubation medium showed that activity of this enzyme reached a maximum (165 ± 23 pulses/mg protein) at pH 7.1, indicating that the nucleus contained chiefly the neutral form of the enzyme; at pH 5.0, 5.8, and 7.8 it amounted to 50 ± 6, 46 ± 10, and 75 ± 10 pulses/mg protein, respectively. Acid sphingomyelinase activity was 3 times lower than neutral. Activity of the enzyme at neutral pH values fell sharply (down to 10% of its activity in intact nuclei) on treatment of the nuclei with 1% Triton X-100 solution. This effect may point to a predominant localization of sphingomyelinase in the nuclear membrane. The fact that sphingomyelinase is a membrane-bound enzyme is known from data showing that its isolation from the tissues requires the use of a detergent. In the absence of detergent, the aqueous buffer extracts only 50% of the enzyme from liver tissue. However, these data were obtained for acid sphingomyelinase, bound with lysosomes [7].

Another investigation [13] has shown that as a result of treatment of plasma membranes of rat hepatoma AH 7974 cells with Triton X-100, neutral sphingomyelinase activity falls, but it can be restored by the addition of phosphatidylserine, evidence that the enzyme is lipid-dependent: Triton did not remove the sphingomyelinase from the membrane, and its activity was depressed as a result of removal, by Triton, of the lipid environment in which its activity is manifested.

Comparison of the sphingomyelinase activity of the nuclei (calculated per milligram protein) with the sphingomyelinase activity of liver and brain homogenate indicates that activity of the enzyme in the liver virtually coincides with activity of the enzyme in liver homogenate and is an order of magnitude lower than the brain (Table 1). However, when the quantity of substrate (sphingomyelin) in the nuclei and in homogenate of liver and brain is taken into account, these values are found to be comparable. Activity of the enzyme is evidently determined by the amount of substrate (i.e., by sphingomyelin).

Comparison of the spylingomyelinase activity in the nuclei with the standard (sphingomyelinase of *B. cereus*) showed that activity of the neutral form of the enzyme in whole nuclei is 1.72 ± 0.24 IU/mole sphingomyelin. Thus the investigation demonstrated that mainly neutral sphingomyelinase activity was present in the nucleus. Changes in pH of the intranuclear medium and in the concentration of bivalent cations taking place in the cell nucleus during nucleic acid synthesis, may regulate sphingomyelinase activity and, ultimately, the sphingomyelin content in the nucleus. Sphingomyelin biosynthesis takes place in the smooth and rough endoplasmic reticulum at the peak rate of 43 pmoles/mg/h [14]. No direct experiments indicating that sphingomyelin biosynthesis takes place in the nuclei have yet been undertaken, and the mechanisms of sphingomyelin accumulation in the nucleus are not yet understood. It is therefore perfectly logical to suggest that sphingomyelinase may act as a factor controlling the sphingomyelin concentration in the nucleus. This suggestion is supported by data on the presence of phospholipases A_1 and A_2 in the cell nucleus and that these enzymes are involved in the regulation of its lipid composition and its functional activity [2, 4].

The sphingomyelinases of the cell nucleus may not only make a definite contribution to the rapid changes in the phospholipid composition of the cell nucleus during the change in its functional state, but may also facilitate destruction of the sphingomyelin-DNA- and sphingomyelin-RNA-nuclear matrix after completion of nucleic acid synthesis, in the same way as exogenous sphingomyelin, which the writers used previously in experiments in vitro [5].

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EFFECT OF EMOXYPIN ON BASAL CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY AND LATE RECEPTOR POTENTIAL OF THE ISOLATED RETINA

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In pathology of the organs of vision and, in particular, in hereditary degenerations and dystrophies [7, 9], disturbances in the cyclic nucleotide system are observed. It has been shown [4, 5] that emoxypin (a preparation of the 3-hydroxypyridine class) causes an increase in visual acuity in patients with hereditary degeneration of the retina (Stargardt's disease) and with central chorioretinal dystrophies, in whom total electrical activity is depressed. However, the mechanisms of action of emoxypin in the treatment of degenerations and dystrophies of the retina remain unexplained.

The writers showed previously [3, 8], that 3-hydroxypyridine (3-HP) derivatives, including emoxypin (2-ethyl-6-methyl-3-HP), inhibit cyclic nucleotide phosphodiesterase (PDE) in the rabbit heart and platelets. PDE inhibitors are known to cause an increase in amplitude and to delay the appearance of the receptor response of the rods in the isolated vertebrate retina, to raise their threshold sensitivity, and to shift the curve of response amplitude as a function of photic stimulus intensity toward lower levels of illumination, simulating the action of dark adaptation [2].

To determine the possible mechanism of the therapeutic action of emoxypin we studied its effect on the late receptor potential (LRP) of the isolated retina and on basal PDE activity of the outer segments of the retinal rods.

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