The mechanism of action of cordycepin is that, since it possesses affinity for poly(A)-polymerase, this inhibitor (3'-deoxyadenosine) is incorporated into the growing poly(A) chain, which terminates the synthesis of that chain because it has no OH group at the 3'-atom of ribose [4, 10]. The formation of the 3'-terminal poly(A) segment of hnRNA however, takes place through consecutive addition of several transformed oligo(A) to the 3'-oligo(A)-"primer" [2, 10]. The use of a small dose of cordycepin had the result that by no means all of the free oligo(A) nucleotides are "attacked" by cordycepin. As a result the cordycepin-containing oligo(A) [3'-deoxy-oligo(A)], the number of which is small, are incorporated mainly into the growing chains of those poly(A) segments whose synthesis requires the greatest number of acts of growth of thechain, i.e., into poly(A) with the largest "final" size.

The low relative content of hnRNA with short poly(A) segments in total poly(A^+)-hnRNA (Table 1) is also evidence in support of our suggested model of the molecular mechanism of selectivity of the effect of small doses of cordycepin: a decrease in the number of hnRNA molecules with small "final" size of their poly(A) segments reduces the likelihood that 3'-deoxy-oligo(A) segments, the content of which in the reserves of unmodified oligo(A) is very small, will take part in the biosynthesis of these segments.

LITERATURE CITED

1. O. V. Podobed, V. L. Leitin, E. V. Brykina, et al., Mol. Biol., 7, 343 (1973).
2. S. T. Jacob, F. J. Roe, and K. M. Rose, Biochem. J., 153, 733 (1976).
3. P.-E. Mansson and S. E. Harris, Biochemistry (Washington), 18, 2073 (1979).
4. C. Millcarek, R. P. Price, and S. Penman, Cell, 1, 1 (1974).
5. C. M. Palatnik, R. V. Stotti, and A. Jacobson, J. Mol. Biol., 128, 371 (1979).
6. S. Penman, M. Rosbach, and M. Penman, Proc. Natl. Acad. Sci. USA, 67, 1878 (1970).
7. A. Person and G. Beaud, J. Virol., 25, 11 (1978).
8. A. Person and G. Beaud, Eur. J. Biochem., 103, 85 (1980).
9. D. Sheiness, L. Puckett, and J. E. Darnell, Proc. Natl. Acad. Sci. USA, 72, 1077 (1975).
10. B. Sieliwanowicz, Acta Biochim. Pol., 25, 239 (1978).
11. A. E. Sippel, N. Hynes, B. Groner, et al., Eur. J. Biochem., 77, 141 (1977).
12. J. Spieth and A. H. Whitely, Dev. Biol., 79, 95 (1980).

ENDOGENOUS PHOSPHOLIPASE HYDROLYSIS IN THE CEREBRAL CORTEX DURING DEVELOPMENT OF EPILEPTIC ACTIVITY IN RATS

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The development of epileptic activity (EA) in the CNS is considered to be associated with structural changes in neuron membranes [5]. Structural and functional changes in biomembranes are induced by changes in the physicochemical state of their lipid component [15]. Lipid peroxidation (LPO) is one of the processes that determines the properties of membrane lipids [1]. The writers previously found activation of LPO in membrane fractions of the cerebral cortex during the development of EA [5, 6] and they showed that uncompensated intensification of LPO plays a pathogenetic role [5, 7].

Besides peroxidation, another factor determining the chemical composition and phase state of membrane lipids is the intensity of endogenous phospholipase hydrolysis (PLH) [8]. There

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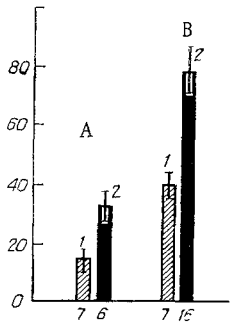

Fig. 1. FFA content in cerebral cortical homogenate of control rats and rats with EA. A, B) Bemegride- and penicillin-induced EA respectively. 1) Control; 2) rats with EA. Abscissa, number of animals; ordinate, FFA concentration (in μmoles/mg protein).

TABLE 1. Content of Various FFA Fractions (in % of total) in Cerebral Cortex of Normal Rats and Rats Developing Epileptic Activity (M ± m)

Experimental conditions	Fractions of fatty acids									K
	$C_{22:4}$	$C_{20:4}$	$C_{18:3}$	$C_{18:2}$	$C_{18:1}$	$C_{18:0}$	$C_{16:0}$	$C_{14:0,\ 12:0}$	$C_{<10}$	
Control	3,8±1,6	11,3±1,6	2,3±0,3	8,6±0,7	39,3±2,6	9,5±0.9	14,8±1,1	2,1±0,1	8,1±1,2	1,9
Bemegride	4,2±0,5	14,5±1,5	2,2±0,1	7,1±0,9	37,6±2,4	15,3±0,9*	16,8±0,4	0,4±0,05*	1,8±0,03*	1,9

Legend. K) Ratio of content of unsaturated FFA, in %, to content of saturated FFA. *P < 0.001 compared with control. Number of animals investigated 9-12.

is evidence that these two processes are interconnected [3, 10]. Interconnection between LPO and PLH may perhaps be preserved during the onset of EA. The possibility likewise cannot be ruled out that changes in endogenous phospholipase activity occupy a definite place in the pathogenesis of EA. However, no research in this direction has yet been undertaken.

It was accordingly decided to study the state of endogenous PLH in the rat cerebral cortex during the development of EA.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 180-200 g. Models of primary generalized and focal cortical EA were used.

Primary generalized EA was induced by intramuscular injection of bemegride [6]. The animal was decapitated 15-20 min after the development of convulsions, the brain was removed, and a sample of 7-8 mg was taken from the sensomotor cortex. A cortical focus of EA was induced by application of the sodium salt of penicillin to the sensomotor cortex [5]. The sample (2-3 mg) was removed from the region of the focus 30-35 min after the appearance of the first epileptic fit. The material obtained was homogenized in a glass homogenizer with Teflon pestle (2.5 mg of cortex to 1 ml physiological saline) at 0-4°C.

The intensity of LPH was estimated by the level of free fatty acids (FFA) in the cortical homogenate. The FFA content was determined by the reaction of copper salts of FFA with 1,5-diphenylcarbazide [13]. To 0.4 ml of homogenate 2 ml of extraction mixture (chloroform—hexane—methanol, 1:1:0.0048 by volume) and 0.6 ml of copper reagent (to 1 ml of saturated NaCl solution, in mmoles: $Cu(NO_3)_2$ 50, triethanolamine 100, NaOH 100, pH 8.0 at 22°C) were added. The tube was tightly closed with a lid and shaken for 3 min. The mixture was then centrifuged for 15 min at 5000 rpm on the TsLU-1 centrifuge. Samples of 1 ml of the top phase were taken and 0.1 ml of a solution of 1,5-diphenylcarbazide in ethanol (4 mg/ml) was added. In the control 1 ml of extraction mixture was used. The intensity of the color of the complex thus formed was measured every 15 min on a Specord UV VIS (East Germany) spectrophotometer at 550 nm. Steric acid was used for calibration. The protein concentration was determined by Lowry's method.

The composition of the FFA was analyzed by gas-liquid chromatography. To 2 ml of homogenate of rat cerebral cortex (25 mg cortex to 1 ml physiological saline) were successively added: 50 ml physiological saline, 60 ml diethyl ether, 10 ml hexane; the mixture was shaken for 3 min and poured into a separating funnel. After 10 min the top phase — an ethereal solution of FFA — was removed. Solutions obtained from three animals were pooled and evaporated on a rotary vaporizer to a volume of 1-2 ml. Methylation of the FFA was carried out with a saturated solution of diazomethane [2]. Methyl esters of the FFA were purified from phospholipids on a column with Al_2O_3 (activity of the II degree according to Brockmann). The first 5 ml was withdrawn and the solvent evaporated. The residue was dissolved in 0.1 ml hexane. The composition of the FFA was determined on a CHROM-4 chromatograph. The stationary phase consisted of 10% PEGA, the length of the column was 2500 mm, and its internal diameter 3 mm. The carrier gas was nitrogen. A flame-ionization detector with a sensitivity of 1:200 was used. The temperature of the vaporizer was 300°C. Fractionation was carried out with temperature programming: 4 min at 60°C, followed by an increase in temperature with a speed of 5 rpm to a final temperature of 200°C. Peaks were identified by means of internal standard The volume of the sample was 1-5 µl in hexane.

EXPERIMENTAL RESULTS

The FFA level in homogenate of the sensomotor cortex of the control rats was 14 ± 3 µg/mg protein. Development of primary generalized EA led to an increase in the FFA concentration in the cortical homogenate to 35 ± 4 µg/mg (Fig. 1A). The appearance of a cortical focus of EA was accompanied by a twofold increase in the FFA content in cortical homogenate taken from the region of the focus of EA (Fig. 1B). In this case the FFA level in the control animals was 40 ± 5 µg/kg, and in rats with focal EA it was 75 ± 10 µg/mg. The increase in the initial FFA concentration was evidently due to the traumatic nature of the operation [5] which preceded the application of penicillin. Operations were performed on both control and experimental animals.

The appearance of primary generalized EA and of a cortical focus of EA thus led to a considerable increase in the FFA concentration in the rat cerebral cortical homogenate. The comparatively rapid and considerable increase in the FFA concentration in the cerebral cortex could have arisen through activation of LPO, of lipase hydrolysis, and of PLH. However, lipase has low specificity relative to fatty acids with different degrees of saturation and length of their carbon chain [9]. Activation of this enzyme ought to be accompanied by a uniform increase in the content of all fractions of FFA. The activation of LPO, observed during development of EA [5, 6], on the other hand, ought to lead to changes in the FFA spectrum toward a decrease in the relative content of unsaturated and an increase in the fraction of short-chain fatty acids. Meanwhile the results of gas-liquid chromatography point to accumulation chiefly of stearic acid in the cerebral cortex during the development of EA, a considerable fall in the fraction of short-chain FFA, and a tendency for the content of arachidonic acid to increase (Table 1). It follows from these data that activation of lipase hydrolysis and LPO must evidently be excluded from the main causes of elevation of the FFA level in the cortex during EA. The most likely process whose activation is responsible for the observed increase in the FFA level and the change in the relative proportions of individual fatty acids among them is evidently endogenous PLH.

What are the relations between LPO and PLH in the cerebral cortex under normal conditions and during the development of EA? What is the role of activation of PLH in the mechanisms of development of EA?

It has been shown that LPO and endogenous PLH are activated parallel to each other during the development of certain pathological states and diseases [11, 12, 14]. There is evidence also of the interdependence and interaction of these processes [3, 10]. It has been suggested that endogenous phospholipases participate in repair processes in membranes whose lipids have undergone intensive peroxidation [3]. It may be that the work of the phospholipases leads to renewal of phospholipids by a monomolecular substitution mechanism [3] and accelerates elimination of peroxidized fatty acids from the membrane. At the same time, activation of PLH, leading to an increase in the content of FFA, is known to be accompanied by an increase in membrane permeability and inhibition of membrane-bound enzymes and, in particular, by disturbance of oxidative phosphorylation in mitochondria [12]. Consequently, the role of activation of PLH during the development of EA is not completely clear. Considering that preliminary injection of an antioxidant prevents both activation of LPO and activation of phospholipases, it can be tentatively suggested that activation of PLH takes place through activation of LPO in the membranes.

The appearance of a focus of EA, which is a generator of pathologically enhanced excitation [4], and of primary generalized EA in the cerebral cortex is thus accompanied by activation of endogenous phospholipase hydrolysis in the cortex.

LITERATUTE CITED

1. Yu. A. Vladimirov, T. B. Suslova, V. I. Olenev, et al., in: Progress in Science and Technology. Series: Biophysics [in Russian], Vol. 5, Moscow (1975), p. 56.
2. M. Kates, Techniques of Lipidology, [Elsevier (1972], Moscow (1975).
3. V. E. Kagan, A. A. Shvedova, and K. N. Novikov, Biofizika, No. 2, 279 (1978).
4. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
5. G. N. Kryzhanovskii, E. V. Nikushkin, V. E. Braslavskii, et al., Byull. Éksp. Biol. Med., No. 1, 14 (1980).
6. E. V. Nikushkin, V. E. Braslavskii, and G. N. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 12, 696 (1980).
7. E. V. Nikushkin, V. E. Braslavskii, and G. N. Kryzhanovskii, Zh. Nevropatol. Psikhiat., No. 6, 810 (1981).
8. É. D. Polyakova, in: Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981), pp. 120-128.
9. M. M. Rakhimov and N. R. Dzhanbaeva, Usp. Sovrem. Biol., 81, No. 3, 323 (1977).
10. R. D. Seifulla, N. A. Onishchenko, S. D. Artamonov, et al., Farmakol. Toksikol., No. 2, 157 (1979).
11. I. A. Serebrennikova, Radiobiologiya, 18, No. 6, 903 (1978).
12. V. I. Sorokovoi and Yu. A. Vladimirov, in: Molecular Pathology of Membrane Structures [in Russian], Moscow (1975), pp. 5-10.
13. V. A. Shaternikov and L. A. Savchuk, Lab. Delo, No. 10, 595 (1964).
14. G. V. Chernysheva, L. V. Stoida, G. G. Amarantova, et al., Byull. Éksp. Biol. Med., No. 5, 565 (1980).
15. O. Colard-Torquebiau, C. Wolf, and G. Bereziat, Biochim. Biophys. Acta G., 58, 587 (1976).

HYPERTHYROIDISM AND THE TRANSMEMBRANE POTENTIAL OF RAT LIVER MITOCHONDRIA

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There is as yet no general agreement regarding the effect of the thyroid hormone level *in vivo* on the energy-transforming functions of the mitochondria. It was shown previously that the content of esterified phosphate per unit quantity of oxygen consumed is reduced in mitochondria isolated from the tissues of hyperthyroid animals [8]. Other workers did not observe this uncoupling effect of an excess of thyroid hormones [7]. Bronk [6], moreover, observed acceleration of oxygen consumption by isolated mitochondria in medium with thyroxine without any decrease in the efficiency of oxidative phosphorylation, ruling out the possibility of any direct uncoupling of oxidation and phosphorylation by thyroid hormones. Recently it was shown in the same laboratory that the transmembrane potential (TMP) of rat liver mitochondria rose after a single injection of thyroxine into animals, and on this basis it was postulated that a thyroxine-induced increase in the efficiency of oxidative phosphorylation takes place in the mitochondria [11], a concept directly opposite to that of the uncoupling action of thyroid hormone. In the case of mitochondria whose physiological state is determined by several cytoplasmic factors [4] it is evidently difficult to interpret an unambigu-

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