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ELECTRON-MICROSCOPIC AND MORPHOMETRIC INVESTIGATION OF THE ACTION OF ENKAD ON RETINAL PHOTORECEPTOR CELLS OF CAMPBELL RATS WITH HEREDITARY RETINAL DEGENERATION

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Retinitis pigmentosa (RP) is a heterogeneous group of hereditary diseases leading to blindness. On average this disease is found in one of every 3500 persons in the world. The features of RP are distinguished by their great variability, but nocturnal blindness, a circular scotoma, and narrowing and loss of peripheral vision with longer preservation of central vision are always observed [14]. As the disease progresses, reduction of amplitude and disappearance of the electroretinogram (ERG) are observed. Blindness arises on account of degradation of the outer layers of the retina, and in particular, of the photoreceptor layer [14]. The hereditary character of RP was established in the middle of last century. Since then the view has been held that this disease is incurable. In 1971, however, the use of the preparation enkad [8], which is a mixture of ribonucleotides obtained by enzymic hydrolysis of yeast RNA, was suggested in the Soviet Union. Clinical trials of this preparation have revealed a group of patients with a form of retinitis pigmentosa in whom it was possible to obtain temporary restoration or improvement of vision or to delay progression of the disease, sometimes with a prolonged effect, if repeated courses of injections of enkad were given [1-4, 6-9]. As early as 5-8 weeks after the beginning of treatment the eyesight of these patients began to improve, and the improvement reached a maximum after 2-3 weeks. It could include widening of the peripheral field of vision, reduction of the circular scotoma, increased dark adaptation, and enhanced visual acuity. In some cases the ERG was restored. The improvement of dark adaptation was found to be the least stable feature and reverted to its initial level during the few months immediately after the end of treatment [6-8]. As a rule repeated treatment gave more lasting results, and nowadays a second course is given 6-8 months after the first [4]. These studies served as a prototype for a successful attempt to treat RP in the USA, with similar results. As therapeutic preparation there, a mixture of 5′-nucleotides was used, followed by oral administration of their precursors, namely inositol and orotic acid [13]. The positive results of clinical treatment with enkad shed some light on a hitherto unknown aspect of the metabolic disturbance in patients with hereditary RP, namely a disturbance of nucleotide metabolism, which the name of dysnucleotidosis has been given [1].

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It has been shown by laboratory investigation that 5′-nucleotidase activity is depressed [5] in the retina of mutant Campbell rats with hereditary retinal degeneration. Evidence of a disturbance of purine metabolism in these animals is given by the raised uric acid level in the urine and blood serum. The same excess of uric acid has been found in the blood of randomly chosen patients with RP [10]. These data may have the basis of the hypothesis that RP develops on account of a disturbance of purine and RNA synthesis, due to depressed 5′-nucleotidase activity [9]. The authors of this hypothesis regard the therapeutic effect of enkad as replacement therapy. However, if the replacement action of enkad applies only to metabolism, the therapeutic effect would be very short-lived, but in fact it lasts for months. This suggests the possibility of reinforcement of the effect at a certain structural or functional level. It was necessary to find which retinal cells respond first to the action of enkad, for which purpose an adequate laboratory model was needed. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

As a guide for selection of a cell system on which enkad conjecturally may act, we used observations by clinicians [4, 8, 11], who on the basis of the time course of dark adaptation at the beginning and end of treatment, postulated that enkad stimulates the rod system of the retina. As object we chose Campbell rats, for the excess of uric acid found previously in their blood serum was evidence of a common feature of the metabolic disturbances in these animals with hereditary retinal degeneration and patients with RP [10]. The Campbell rats were provided by Professor R. N. Etingof (of the Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad), and previously she had obtained them from Professor Reading (Edinburgh University). They are now being bred in the animal house of the Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, on the basis of inbred mating and they are kept in a cycle of 12 h daylight and 12 h darkness. Animals aged 1 week after birth, when the retinal cilia are transformed into the outer segments of the rods (OSR), and this process has not yet been complicated by pathological changes [15], were used for the experiments. We suggested that during the action of enkad intensification of morphogenesis of OSR could be expected. An injection of 0.1 ml of 3.5% enkad in the experiment (three animals) and of physiological saline in the control (3 animals) was given subcutaneously in the abdominal region to young rats from one litter, daily for 6 days starting with the 1st day after birth. The duration of this course of injections corresponded to the minimal duration of the clinical course of treatment with enkad after which the first signs of improvement of vision are observed in patients with RP. The animals were killed on the 7th day and the eyes prepared for electron microscopy by the standard method. One eye was taken from each rat, and used to prepare three blocks from different points of the central zone of the optic fundus. Foci of membranogenesis were counted on the screen of a Tesla electron microscope, after examination of 200 fields from transverse sections of each block (magnification 14,000). A focus of membranogenesis was taken to be a concentration of disks in the field of vision between the apical microvilli of the pigmented epithelium and the inner segments of the photoreceptors. Morphogenesis was evaluated on a 4-point system, reflecting categories of conventional area of the field of vision occupied by membrane disks: O) no disks in field of vision, 1) disks occupy one-third or less of the field of vision, 2) disks occupy half of the field of vision, 3) disks occupy two-thirds of the field of vision or more. The relative percentage of each category (N) was subtracted from the total area of the fields counted, and this was multiplied by the conventional area (M). The sum of these products in the experiment (S_{ex}) and control (S_c) reflects the relative fraction of area, in per cent, occupied by disks of OSR in the central zone of the retina. The efficacy of the stimulating action of enkad was estimated by the ratio $(S_{ex} - S_c)/S_c$ and expressed as a percentage. According to this assessment, in the central zone of the retina in Campbell rats aged 7 days, disks of OSR occupy on average 14%. In the retina of rats from different litters, these values vary within limits of the normal distribution curve with extreme points 5 and 19%, and with small differences between animals from the same litter. This composes the condition that animals from the same litter be used in the control and experiment. The experiments were repeated 4 times.

EXPERIMENTAL RESULTS

Enkad was shown to stimulate orthogenesis of membrane disks of OSR significantly. When single daily injections were given, stimulation after 6-7 days reached 12-20% of the control level (Table 1). In the period of formation of OSR, enkad stimulated this process in the cilia, in which disk formation had already begun, but it did not increase the number of centers of membranogenesis. This was shown by the fact that differences in the integral value for the O category in the experiment and control were not statistically significant.

TABLE 1. Morphometric Parameters of Stimulating Effect of Enkad in Campbell Rats Aged 7 Days

Series of experiment	Category of field of vision	Prevalence of categories	Conventional area occupied by disks (M)	Total area occupied by disks (N.m)	Effectiveness of stimulation, $\%(S_{ex}-Sc)/S_c$
I:					
Control	0	46,13	—	—	
	1	46,88	1/3	15,63	
	2	6,44	1/2	3,22	
	3	0,56	2/3	0,38	
				$S_c=19,23$	
					20,28
	0	41,48	—	—	
Experiment	1	41,76	1/3	13,92	
	2	11,75	1/2	5,87	
	3	5,01	2/3	3,34	
				$S_{ex}=23,13$	
II:					
Control	0	49,79	—	—	
	1	42,87	1/3	14,29	
	2	6,81	1/2	3,40	
	3	0,53	2/3	0,36	
				$S_c=18,05$	
					12,08
Experiment	0	50,72	—	—	
	1	32,28	1/3	10,76	
	2	11,17	1/2	5,58	
	3	5,83	2/3	3,89	
				$S_{ex}=20,23$	

Thus the experimental morphological investigation revealed that enkad, injected subcutaneously, stimulates morphogenesis of OSR in the developing retina of mutant rats with hereditary degeneration of the retina. As a result of correlation between the rhodopsin concentration, length of OSR, and the number of membrane disks in them, on the one hand, and the time of appearance of the ERG on the other hand [12], the data obtained explain the improvement in the visual functions after administration of enkad in clinical practice as being due primarily to improvement of dark adaptation and of the ERG. Constancy of renewal of the membrane disks of OSR explains why stimulation of their morphogenesis takes place in patients of all ages provided that viable photoreceptor cells are preserved in the retina.

Thus an experimental morphological study showed that enkad, if injected subcutaneously, stimulates morphogenesis of OSR in the developing retina of mutant rats with hereditary degeneration of the retina. As a result of correlation between the rhodopsin concentration, length of OSR, and number of membrane disks in them, on the one hand, and the time of appearance of the ERG, on the other hand [12], the results explain the improvement of the visual functions after clinical use of enkad as being primarily due to improvement of dark adaptation and of the ERG. The constancy of renewal of the membrane disks of OSR explains why their morphogenesis can be stimulated in patients of all ages, provided that viable photoreceptor cells are preserved in the retina.

This biological model proved to be sufficiently sensitive to detect the first signs of action of enkad on the retina, and also to distinguish between active and inactive batches of this preparation.

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REPAIR PROCESSES IN THE POSTISCHEMIC CEREBRAL CORTEX IN THE EARLY POSTRESUSCITATION PERIOD*

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After total ischemia pathological changes develop in the brain which frequently lead to so-called postresuscitation encephalopathies. In these conditions higher nervous processes are disturbed to some extent, and in man this affects his principal social quality, namely his intellect. The problem of restoring brain functions completely in the postresuscitation period is one of the most important aspects of modern resuscitation practice [3, 4, 7]. It will be quite evident that before a system of scientifically based measures for the treatment and prevention of these encephalopathies can be worked out, precise and reliable data are needed on the pathogenetic mechanisms lying at the basis of these diseases. Special attention here must be paid to repair processes in the postischemic brain in the early postresuscitation period, about which hardly anything was known until recently [2, 5]. This paper gives new data on reparative regeneration in the cerebral cortex on the 1st day of the postresuscitation period after total experimental ischemia in rats.

EXPERIMENTAL METHOD

Experiments were carried out on nine Norwegian male albino rats weighing 180-220 g, which were subjected to total ischemia for 10 min by cardiac arrest [1]. The animals were killed 1, 3, and 24 h after resuscitation, three animals in each group. Pieces of cortex were taken from the occipital, parietal, and frontal regions of the brain and fixed in 2% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.2. Subsequent treatment of the brain tissue for electron-microscopic study was carried out by the usual method. Ultrathin sections of the cerebral cortex were studied in the EM 10 CR electron microscope ("Opton," West Germany).

EXPERIMENTAL RESULTS

In the early postresuscitation period the brain tissues are exposed to the action of several unfavorable factors, of which the most important are hypoxia and endogenous toxins [6, 8], which leads primarily to pathological changes in cell membranes,

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