SENSITIVITY OF THE RETINAL PIGMENTED EPITHELIUM TO THE MELANOTROPIC ACTION OF ACTH IN NORMAL AND MUTANT RATS WITH HEREDITARY DYSTROPHY OF THE RETINA

O. G. Stroeva, A. D. Bibikova, I. A. Ostapenko, and R. N. Étingof

UDC 591.35 + 591-8.085.23

KEY WORDS: retinal pigmented epithelium; melanogenesis; ACTH; cell proliferation; hereditary dystrophy of the retina; rats.

Hereditary dystrophy of the retina, known as "retinitis pigmentosa," in man is a composite group of various hereditary diseases with a similar end result — blindness due to death of the outer layers of the retina [2, 14]. Mutant lines of laboratory animals with hereditary dystrophy of the retina are carriers of a certain type of retinitis pigmentosa and they therefore may be used as objects for the experimental analysis of the etiology of this disease. The first stage of the lesion in rats is found in the retinal pigmented epithelium (RPE) and is connected with its inability to carry out phagocytosis of the desquamated disks of the outer processes of the photoreceptors [8, 11]. As a result the disks collect between the retina and interfere with the supply of metabolites from the vascular membrane to the retina, with consequent degeneration of the retina. In poorly pigmented RCS rats with hereditary dystrophy of the retina, under conditions of cyclic illumination (12-h day — 12-h night) destruction of the retina begins in the central zone from the 18th day after birth, and later spreads to the whole of the periphery [7, 9, 10]. If these rats are kept in permanent darkness, and also in congeneic pigmented RCS rats under whatever conditions they are kept (in darkness or in cyclic light) destruction of the retina begins later — on the 10th day in the center and on the 30th-35th day in the dorsal half of the eye. However, in the ventral half of the eye the retina is destroyed just as quickly as in poorly pigmented RCS rats under conditions of cyclic illumination [12]. These experiments showed that internal zonal differences determining the rate of its destruction exist in the retina of rats with hereditary dystrophy, and it was suggested that properties of the RPE are responsible for this [12]. The origin of such differences in eye development in rats has not yet been explained.

The results of a study of the functional role of postnatal cellular proliferation in the RPE of normal rats suggested that passage of RPE cells through a reproduction cycle during 2-5 days of the postnatal period is essential for completion of their differentiation, that melanotropic hormones are the active agent, and that it is at this time that the zonal differences in the properties of RPE which were discovered in RCS rats are formed. This suggestion is based both on coincidence of the zones of highest proliferative activity in RPE of normal rats [3] with zones of greatest resistance to the development of retinal destruction in RCS rats [12] and on discovery of a long G_2 phase of the mitotic cycle at this time [3]; by analogy with melanoma cells [13], this last fact attracted our attention to melanotropic hormone as the possible differentiating agent, for which dividing cells constitute the target. If this coincidence is not accidental and is causally connected not only with melanogenesis [5], but also with maturation of the phagocytic power of the RPE, postnatal proliferative activity in rats with heredity dystrophy of the retina ought to be abnormal, and this in fact was discovered: In rats of two lines with hereditary retinal dystrophy the number of dividing cells in RPE in the critical period of its development was below normal, and indeed, it was lower in Campbell than in Hunter rats [4]. In agreement with the hypothesis [3] the RPE of these rats ought to have reduced sensitivity to melanotropic hormones. The aim of the

Laboratory of Cell Differentiation, M. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. Laboratory of the Biochemical Basis of Reception, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 87-89, March, 1982. Original article submitted October 14, 1981.

 0007-4888/82/9303-0334$07.50

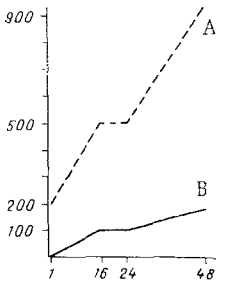

Fig. 1. Incorporation of [^{3}H]-leucine (A) and [^{14}C]thiouracil (B) into RPE of normal 3-day-old rats in organ culture without hormone. Abscissa, duration of culture (in h); ordinate, number of counts per microgram DNA.

present investigation was a comparative study of the sensitivity of the RPE of mutant Hunter rats [1, 4, 6, 15] and of normal brown rats (BR) to the melantropic action of ACTH.

EXPERIMENTAL METHOD

RPE of 3-day-old Hunter and BR rats were cultured by the method in [5] in the scleral sector of the eye in order to preserve the original topographic relationships of its cells. Immediately after enucleation the anterior part of the eye was resected under aseptic conditions and discarded together with the lens. The retina was removed from the scleral part to provide better contact between RPE and medium. Each explant was placed in a glass dish 20 mm in diameter, containing 2 ml of medium, and cultured at 37.0°C for 48 h, the medium being changed every 16 h and constantly aerated with a gas mixture containing 5% CO_2 and 95% air. The viability of the explants was verified by incorporation of [^{3}H]leucine (Amersham, England), with specific activity 40 Ci/mmole; 10 μCi/ml) and melanin synthesis was determined from the incorporation of [^{14}C] thiouracil (Hungary, specific activity 20 mCi/mole; 5 μCi/ml). The two precursors were constantly present in the medium. ACTH (Kaunas), present in the medium (5 i.u./ml) for the first 16 h of culture, was used as melanotropic hormone. One eye from each animal was placed in medium with hormone (experiment), the other eye in medium without hormone (control), and the results for each activity were expressed as the ratio of experiment/control (E/C). All numerical results were subjected to statistical analysis. The data for incorporation of labeled precursors were obtained on isolated RPE [5] by the liquid scintillation method on a type SD-30 counter, working with double labeling, and were expressed as the number of counts per microgram DNA. Eight pairs of eyes from each line of rats were used. The experiment was carried out twice on Hunter rats and three times on BR rats.

EXPERIMENTAL RESULTS

The highest total level of proliferative activity during the postnatal development of genotypically normal rats was observed in RPE of animals aged 3 days [3]; during culture of RPE taken at this stage without hormone, the highest synthetic activity of its cells with respect to both parameters was observed 24 and 48 h after the beginning of the experiment (Fig. 1). Accordingly this stage (3 days) and the 48-h point of culture were chosen for the measurements in the present investigation. The results are summarized in Table 1 for BR and in Table 2 for Hunter rats. It must be noted in particular that in the present investigation absolute values of incorporation of labeled precursors into RPE of different animals could not be compared, either within the same group or between the different lines of mice because of possible differences between individuals as a result of differences in their number in the litter, birth trauma, maternal age, etc. The use of E/C ratio in cases when both eyes belonged to the same animal enabled errors of this sort to be reduced. According to incorporation of [^{3}H]leucine into RPE of rats of the two lines the E/C ratio was not affected by the hormone and its average value was 1. Since the condition of age identity was observed for each pair of eyes, deviations from this mean statistical value could be affected chiefly by individual injuries to explants during their preparation for culture and during changing of

TABLE 1. Incorporation of [^{14}C]Thiouracil and [^{3}H]Leucine (in counts/μg DNA) into Cells of RPE of 3-Day-Old Normal BR Rats after 48 h in Culture with (experiment) and without (control) ACTH

No. of animal	[^{14}C]thiouracil		E/C	[^{3}H]leucine		E/C
	experimental	control		experimental	control	
1	2434,0	1089,0	2,2	2353,0	2133,0	1,1
2	1567,2	536,1	2,9	—	—	—
3	356,4	163,6	2,2	822,4	927,1	0,9
4	225,0	478,3	0,4	173,1	329,2	0,5
5	667,5	648,2	1,0	149,7	107,9	1,3
6	1052,1	430,3	2,4	401,3	193,3	2,0
7	638,6	569,9	1,1	168,7	269,2	0,6
8	843,8	306,9	2,7	130,8	212,0	0,6
	$\bar{x}$=2,0±0,2			$\bar{x}$=1,0±0,15		

TABLE 2. Incorporation of [^{14}C]Thiouracil and [^{3}H]Leucine (in counts/μg DNA) in Cells of RPE of 3-Day-Old Mutant Rats (Hunter) after Culture for 48 h with (experiment) and without (control) ACTH

No. of animal	[^{14}C]thiouracil		E/C	[^{3}C]leucine		E/C
	experimental	control		experimental	control	
1	461,4	856,1	0,5	84,2	160,0	0,5
2	1234,2	814,3	1,5	196,2	146,0	1,3
3	309,6	731,4	0,4	99,3	147,0	0,6
4	594,0	649,0	0,9	135,0	106,0	1,2
5	885,0	873,0	1,1	131,0	103,0	1,2
6	114,2	200,1	0,5	71,2	56,0	1,2
7	137,6	218,5	0,6	40,0	69,0	0,5
8	322,0	249,0	1,2	129,0	86,0	1,5
	$\bar{x}$=1,0±0,2			$\bar{x}$=1,0±0,15		

the medium: Severe trauma to the explants in the experimental group would reduce, and in the control group would increase this ratio. Rats of the two lines did not differ from each other with respect to these characteristics. It was a different matter with melanin synthesis. Whereas the E/C ratio for RPE of Hunter rats according to incorporation of [^{14}C]thiouracil averaged 1 and, for each pair, it virtually reproduced the E/C ratio for labeled leucine, melanin synthesis in BR was on average twice as high in the experimental as in the control group. These results, in accordance with the hypothesis [3] and with the results of a previous investigation showing a decrease in the number of dividing cells in RPE of mutant rats [4], are evidence of the insensitivity of the RPE of Hunter rats to the melanotropic action of ACTH at the stage when the RPE of genotypically normal pigmented rats is most sensitive to the action of ACTH. Further investigations will show to what extent hormones are involved in the maturation of the phagocytic function of the RPE in rats.

LITERATURE CITED

1. V. I. Govardovskii, I. A. Ostapenko, M. E. Shabanov, et al., Neirofiziologiya, 9, 527 (1977).
2. M. V. Oizerman, Usp. Sovrem. Biol., 81, 365 (1976).
3. O. G. Stroeva and I. G. Panova, Ontogenez, 11, 571 (1980).
4. O. G. Stroeva, I. G. Panova, and A. D. Bibikova, Zh. Obshch. Biol., 42, 99 (1981).
5. O. G. Stroeva, I. G. Panova, V. A. Poplinskaya, et al., Zh. Obshch. Biol.,

6. M. E. Shabanova, O. D. Tereshchenko, and I. A. Ostapenko, Byull. Eksp. Biol. Med., No. 2, 167 (1978).
7. D. Bok and M. O. Hall, J. Cell Biol., 49, 664 (1971).
8. D. Bok and R. W. Young, in: The Retinal Pigment Epithelium (M. F. Marmor and K. M. Zinn, eds.), Cambridge (1979), p. 148.
9. J. E. Dowling and R. S. Sidman, J. Cell Biol., 14, 73 (1962).
10. W. L. Herron, B. M. Riegel, and M. L. Rubin, Invest. Ophthalmol., 10, 54 (1971).
11. M. M. La Vail, in: The Retinal Pigment Epithelium (M. F. Marmor and K. M. Zinn, eds.), Cambridge (1979), p. 356.
12. M. M. La Vail and B. A. Bettelle, Exp. Eye Res., 21, 167 (1975).
13. A. B. Lerner, G. Moellemann, J. M. Varga, et al., in: Hormones and Cell Culture (G. H. Sato and R. Ross, eds.), New York (1979), p. 187.
14. S. Merin and E. Auerbach, Surv. Ophthalmol., 20, 303 (1976).
15. C. M. Yates, A. J. Dewar, H. Wilson, et al., Exp. Eye Res., 18, 119 (1974).

DESTRUCTIVE AND REPAIR PROCESSES IN THE HIPPOCAMPUS AFTER PROLONGED EXPOSURE TO NONIONIZING MICROWAVE RADIATION

V. S. Belokrinitskii

UDC 616.831.314-001.28-003.9

KEY WORDS: nonionizing microwave radiation; hippocampus; destructive and repair processes.

Data in the literature on disturbance of CNS functions in subjects exposed to nonionizing radiation [4-7] and the results of the writer's previous investigations [1-3] have necessitated special studies of the pathogenesis of these disturbances and their clinical manifestation.

This paper describes a study of the ultrastructural changes in the brain following prolonged exposure to nonionizing microwave radiation (NIMR) of low intensity. The hippocampus, one of the principal formations of the limbic system which is concerned in many functions of the body including memory and behavior, was chosen as the test object.

EXPERIMENTAL METHOD

Male rats were exposed to the action of NIMR (wavelength 12.6 cm) for 40 min, 3 times a day, with intervals of 3-4 h, and always at the same times of day, for 2 months. The animals of different groups were exposed to NIMR of the following intensity: group 1) 1000 $\mu W/cm^2$, 2) 50 $\mu W/cm^2$, 3) 25 $\mu W/cm^2$, 4) 10 $\mu W/cm^2$. The animals were irradiated on the Luch-58 apparatus with dosimetric control in an anechoic chamber, in which the rats were kept in special cages. A group of intact rats served as the control. Each group consisted of 100 animals, in which various physiological, biochemical, and histochemical parameters were determined at different times.

For the electron-microscopic investigations the brain was perfused, and thereafter subjected to the normal procedures for investigation of the ultrastructure of nerve tissue. Three layers of the hippocampus (Ammon's horn) were subjected to electron microscopic analysis: the alveus layer, the layer of polymorphic cells, and the layer of pyramidal cells.

EXPERIMENTAL RESULTS

In animals exposed to NIMR with an intensity of 1000 $\mu W/cm^2$ the state of the structural elements of most fibers differed from the control in the alveus layer, which consists mainly of myelinated and unmyelinated nerve fibers conducting impulses to and from the hippocampus.

A. N. Marzeev Kiev Research Institute of General and Communal Hygiene, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 89-92, March, 1982. Original article submitted November 4, 1981.

