BIOGERONTOLOGY

Retinoprotective Effect of Epithalon in Campbell Rats of Various Ages

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We studied the retinoprotective effect of Epithalon administered to the offspring of Campbell rats during postnatal ontogeny and to mothers before mating and during pregnancy. After this treatment the morphological structure and functional activity of the retina were preserved for a longer period compared to control rats (by 2 times) and to the animals receiving the peptide only during postnatal ontogeny (by 30%).

Key Words: *peptides; Epithalon; pigmentary retinal degeneration; Campbell rats*

Hereditary degenerative changes in the retina in mammals (cats, rats, dogs, *etc*.) and humans include progressive atrophy of photoreceptive cells and usually develop in the early period of postnatal ontogeny [2].

We previously evaluated the effect of Epithalon (Ala-Glu-Asp-Gly) on the retina in Campbell rats with hereditary pigmentary atrophy. Epithalon treatment from the first days of life increased functional activity of the retina and prolonged the period for preservation of its morphological structure by 43.9 and 75.6%, respectively, which was associated with retinoprotective properties of the peptide [1,5,6]. Pigmentary retinal degeneration is a genetically determined disease. Atrophy of the retina can result from the influence of adverse factors during intrauterine development of the fetus (embryopathies) [3,4].

Here we studied the retinoprotective effect of Epithalon administered not only to the offspring of Campbell rats during postnatal ontogeny, but also to mothers before mating and during pregnancy.

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MATERIALS AND METHODS

The experiments were performed on Campbell rats with hereditary pigmentary atrophy of the retina.

We used 43 females and 140 rat pups. The experiment was performed in 2 stages. In series I Epithalon (1.0 μg in 0.2 ml sterile 0.9% NaCl) was injected intraperitoneally to females of the main group (n=25) 3 weeks before mating and during pregnancy. Control rats (n=18) intraperitoneally received 0.2 ml sterile 0.9% NaCl. Series II was performed on the offspring of rats. Rat pups of the main group (n=78) intraperitoneally received Epithalon (0.2 ml) in doses of 0.5 (days 5-35 of life) and 1.0 μg (up to day 81). The injections were performed 5 days a week with 1-week intervals. Control animals (n=62) intraperitoneally received 0.2 ml sterile 0.9% NaCl.

Retinoprotective activity of Epithalon was studied electrophysiologically in rat offspring. Electroretinogram (ERG) is a graphic representation of changes in bioelectric activity of retinal cells in response to light stimuli. ERG reflects activity of most retinal elements and depends on the number of normal cells. This is the major method allowing evaluation of functional activity of the retina and prediction of pathological chan-

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ges at the subclinical level. ERG was recorded in rats placed in a shaded shielded chamber using a 3-channel electrophysiological device (paper rate 25 mm/sec). An universal amplifier with filters (1-30 Hz) served as a preamplifier of ERG potentials. For the final amplification we used an amplifier of an H-3273 ink-jet recorder. Standard photostimulator FS-02 for encephalogram recording (L'vov Factory of Electromedical Devices) served as a flash source. The intensity and duration of a light flash were 500 cd/m^2 and 500 msec, respectively. ECR was recorded using a chlorine-silver spring electrode. A small button at the end of this electrode was carefully applied to the cornea using a micromanipulator. The reference electrode (sharp needle) was fixed on the skin of the head. ERG was simultaneously recorded with an oscillograph.

The animals were anesthetized with urethane (150 mg/kg parenterally). The rat was immobilized in a stereotactic device equipped with a head holder. The upper and lower eyelids were pulled with threads. Light flashes were delivered stochastically after 10-min adaptation to darkness (no less than 1 stimulus per 5 sec). ERG was recorded in control and experimental animals from day 17 (period of eye opening) to the moment when they did not react to stimulation. The recordings were analyzed manually. The average amplitude of waves "a", "b", and "c" was determined by calibration of amplification. The results reflected total bioelectric activity of the retina. This value reflected total receptor potentials of fast wave 1 (wave "a") and changes in the membrane potential of glial and Muller cells (slower and largest wave "b") and pigmented epitheliocytes (wave "c").

Experimental and control animals were subjected to morphological examination. The rats (n=4-5) were decapitated. Enucleation was performed on days 23, 35, 41, 53, 65, 71, and 81. The eyeball was fixed in neutral formalin. Deparaffinized sagittal sections of the eyeball were stained with hematoxylin and eosin. Morphometric examination of preparations included measurements of the width of the inner plexiform layer, inner and outer nuclear layers, and receptor layer (×700).

The results were analyzed by means of Statistica software.

RESULTS

In control and experimental rats total bioelectric activity on day 23 remained unchanged (Table 1).

Bioelectric activity of the retina in control rats progressively decreased starting from day 35 of life and was undetectable on day 53. In experimental animals the amplitude of ERG remained high on day 41 of postnatal ontogeny and decreased only on day 70. Since the observations were performed for 81 days, we did not determine the term of ERG disappearance in experimental animals.

We compared histological preparations of the retina in rats of the main and control group. In animas receiving Epithalon morphological characteristics of the retina were preserved on day 35. In these animals the layer of photoreceptors was brightly stained, other layers had more definite boundaries compared to the control. In control animals the examined retinal layers were narrowed (nuclear layer, photoreceptive layer, and outer plexiform layer containing synapses of rods and cones with horizontal and bipolar cells). The inner nuclear layer containing amacrine, bipolar, and horizontal cells was positioned in close proximity to the outer nuclear layer of rods and cones. These changes developed progressively. Morphological examination showed that structural characteristics of the retina differed in control and experimental animals on day 41 of life. In control rats we revealed destruction of retinal layers, while morphological characteristics in Epithalon-treated animals remained unchanged. We examined preparations of the retina from Epithalon-treated rats on days 71 and 81 of life (Fig. 1, *b*, *c*). On day 71 retinal layers were partially destructed (narrowing of the outer plexiform layer and closeness of the outer and inner nuclear layers). However, the partially narrowed layer of photoreceptors was preserved and strongly stained. It should be emphasized that several functional elements of the retina undergoing destructive changes were preserved even on day 81. Histological examination of the retina in control and experimental rats showed that Epithalon prolonged the period for preservation of its morphological structure at least by 2 times.

Thus, administration of Epithalon to rats with hereditary pigmentary retinal degeneration prolonged the period for preservation of the morphological structure

TABLE 1. Bioelectric Activity of the Retina in Campbell Rats (Total Amplitude of Waves in ERG, μV)

Group	Days						
	23	35	41	53	65	71	81
Control	90.5±11.2	80.6±9.2	38.4±7.4	0	0	0	0
Experiment	94.5±12.1	106.6±13.3*	107.1±12.8*	91.4±11.7	68.3±9.5	54.1±8.4	21.6±5.8

Note. *p<0.05 compared to the control.

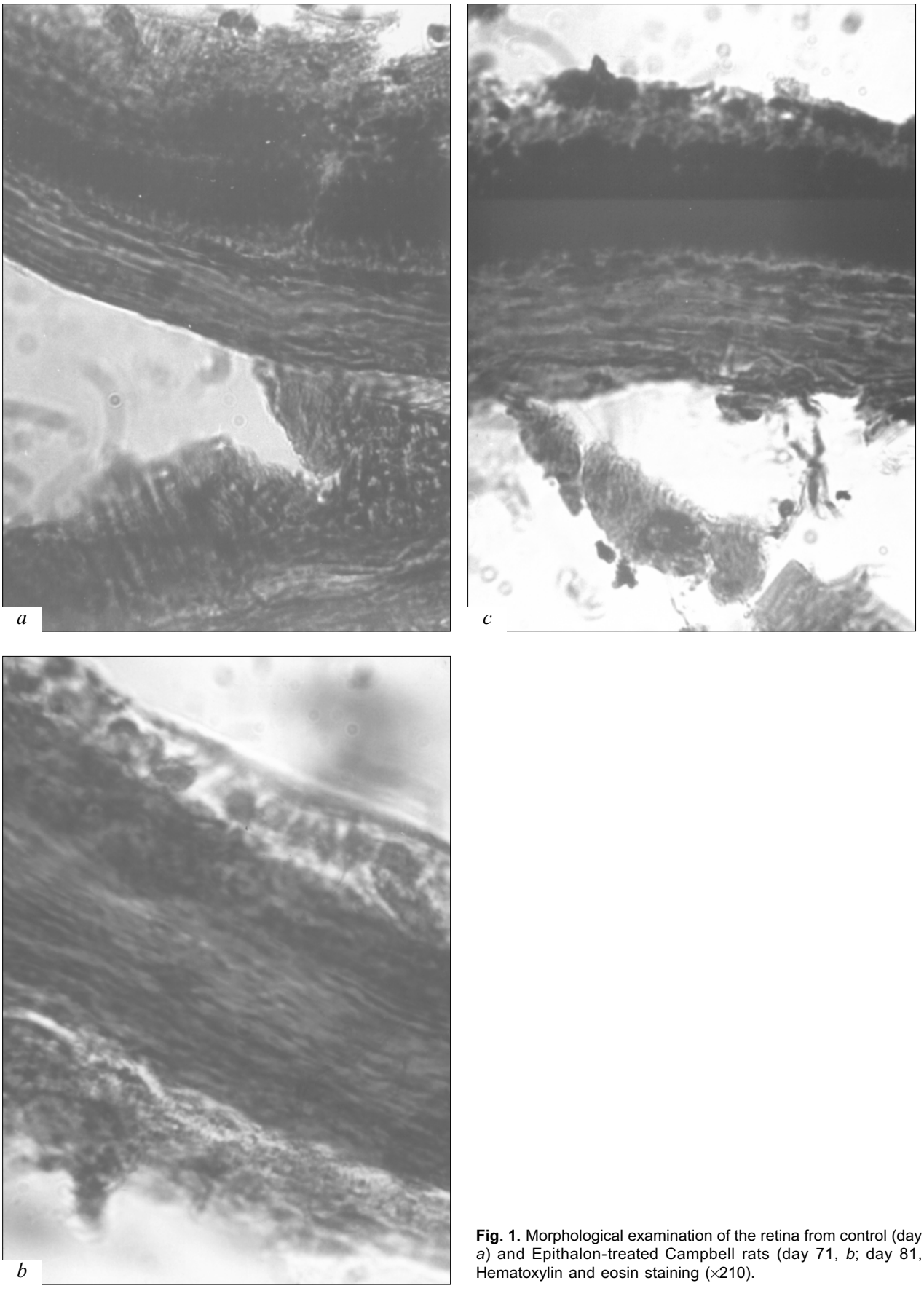

Fig. 1. Morphological examination of the retina from control (day 41, *a*) and Epithalon-treated Campbell rats (day 71, *b*; day 81, *c*). Hematoxylin and eosin staining (×210).

of the retina and its functional activity compared to control animals (by 2 times) and animals receiving the peptide only during postnatal ontogeny (by 30%). Injection of Epithalon not only to the offspring, but also to mothers before mating and during pregnancy potentiates retinoprotective activity of the peptide.

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