TIME COURSE OF THE ELECTRORETINOGRAM OF ISOLATED FROG AND TURTLE RETINAS EXPOSED TO REPETITIVE PHOTIC STIMULATION AND INDUCED LIPID PEROXIDATION

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Recent investigations have shown that illumination of the outer segments of the rods (OSR) of the retina leads to a considerable increase in the rate of accumulation of lipid peroxidation (LPO) products and that α-tocopherol inhibits this process [2]. In the bovine retina a high level of α-tocopherol has been found [4], and it could take part in the reaction of free radical quenching [6]. The presence of glutathione peroxidase has also been demonstrated in the vertebrate retina. For instance, bovine OSR possess high glutathione peroxidase activity [5]. Electrophysiological investigations on the isolated retina of different animals under conditions of induced LPO, when a decrease is observed in both the *a* and b waves of the electroretinogram (ERG), indicate that α-tocopherol and selenium compounds can restore the above-mentioned parameters of the ERG to some degree [3].

In the present investigation, to make a fuller study of the effect of LPO on retinal function, the time course of changes in the ERG of the isolated frog and turtle retina exposed to repetitive photic stimulation was compared under conditions of induced LPO.

EXPERIMENTAL METHOD

The retina of dark-adapted frogs (*Rana ridibunda*) and turtles (*Testudo horsfieldi*) was removed from the eye in weak red light. The preparation was placed on filter paper with its receptors uppermost and kept in a special continuous-flow chamber with two platinum recording electrodes. The retina was immersed in freshly oxygenated Ringer's solution, pH 7.5 (NaCl, 110 mM; KCl, 1.8 mM; $CaCl_2$, 1.1 mM; $NaHCO_3$, 2.4 mM). The temperature in the chamber was stabilized by means of a thermostatic control system. LPO in the retina was activated by addition of $FeSO_4$ and ascorbic acid (10^{-5} and 10^{-4} M, respectively) to the Ringer's solution. An alcoholic solution of α-tocopherol in concentrations of 10^{-3} to 10^{-5} M and a solution of sodium selenite in Ringer's solution in a concentration of 0.01% were used as LPO inhibitors.

Photic stimulation was applied from a photostimulator with xenon tube, giving flashes with an energy of 0.15 J and with a frequency of stimulation of between 5 and 50 Hz. The ERG was recorded by means of a UBP-1-02 amplifier and photographed from the screen of an S1-69 oscilloscope. Rhythmic oscillograms were obtained by gradually reducing the frequency of stimulation (from 50 to 5 Hz) and by recording the ERG throughout the period of falling frequency. The smoothness of the fall in frequency with a rate of 2.5 Hz/sec was ensured by means of a dc electric motor connected to the stimulation frequency control of the photostimulator. The rhythmic ERG of the isolated retina of the above-mentioned animals also was studied at frequencies of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 Hz. The duration of stimulation at the above-mentioned frequencies was 4 sec.

EXPERIMENTAL RESULTS

The isolated frog and turtle retinas, when perfused with freshly oxygenated Ringer's solution, responded to the flashing light. With a frequency of stimulation of 5 Hz, the rhythmic ERG of the isolated frog retina revealed a decrease in the response to the second stimulus, but with effect from the third stimulus the response amplitude averaged 70% of the amplitude of the initial wave, and remained at this level throughout the period of subsequent stimulation. With an increase in the frequency of stimulation a change took place in the

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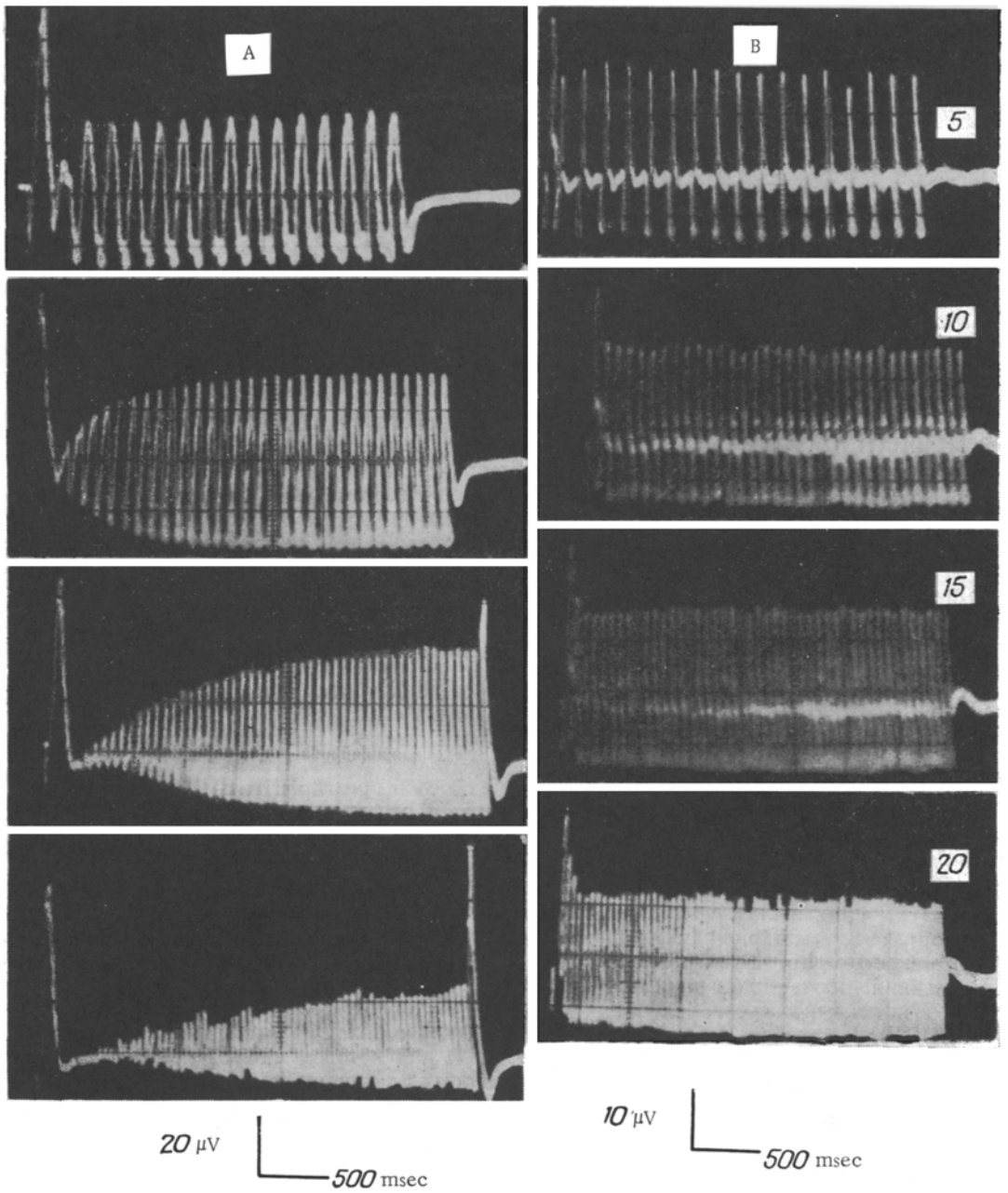

Fig. 1. ERG of isolated frog (A) and turtle (B) retina obtained by repetitive photic stimulation, at fixed frequencies (numbers on right).

shape of the rhythmic ERG (Fig. 1A). For instance, at a frequency of stimulation of 10 Hz, a period of stabilization appeared in the initial stage of the rhythmic ERG, when a gradual increase in amplitude of the responses was observed up to a certain value, after which the amplitude of the oscillations remained stable during the subsequent period of stimulation. The time for stabilization of amplitude during stimulation with a frequency of 10 Hz averaged 2-2.5 sec. A further increase in the frequency of stimulation led to a fall in amplitude of the rhythmic ERG and to a considerable increase in the time of stabilization of the responses, which ceased to be analyzed after a frequency of 20 Hz. The maximal amplitude in this case was measured 4 sec after the beginning of stimulation. When photic stimulation was stopped, the ERG showed a peak which appeared at relatively high frequencies and which increased with an increase in the frequency of stimulation.

The rhythmic ERG of the isolated turtle retina (Fig. 1B) has a number of special features which distinguish it substantially from the rhythmic ERG of the isolated frog retina. The first point to note is a difference in the overall amplitude of the ERG of the frog and turtle retinas, which, according to our observations, is 2-2.5 times greater in the frog than in the

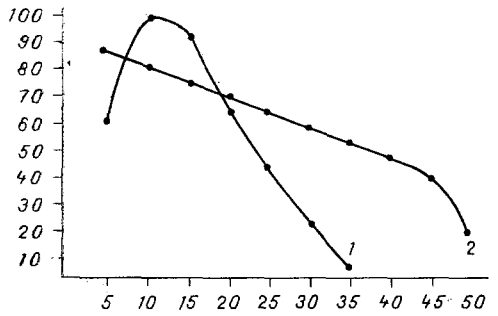

Fig. 2. Dynamics of changes in overall amplitude of rhythmic ERG depending on frequency of photic stimulation. Abscissa, frequency (in Hz); ordinate, ratio of maximal amplitude of rhythmic ERG to response to a single flash (in %). 1) For isolated frog retinas; 2) for isolated turtle retinas. Each point represents mean value of 12 experiments.

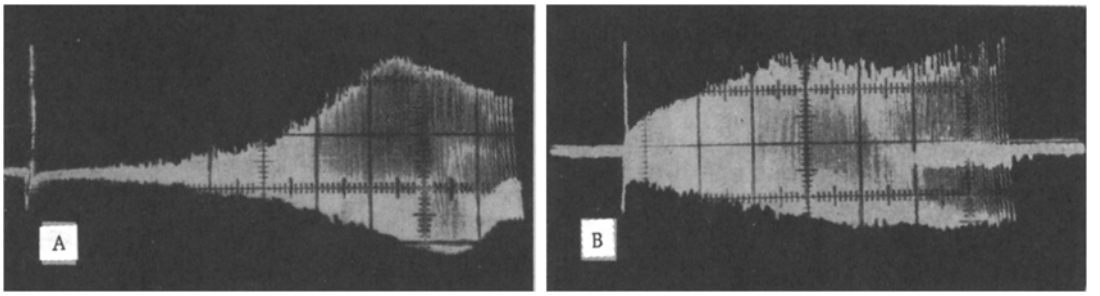

Fig. 3. Rhythmic traces obtained by frequency drop method. A) For isolated frog retina; B) for isolated turtle retina.

turtle retina. On the rhythmic ERG of the isolated turtle retina the period of stabilization characteristic of the rhythmic ERG of the frog retina could not be detected. The amplitude of the rhythmic ERG in this case, at corresponding frequencies of stimulation, was stable throughout the period of stimulation. The decrease in amplitude of oscillations of the rhythmic ERG of the isolated turtle retina with an increase in the frequency of stimulation took place much more slowly than in frogs (Fig. 2). Comparison of the ERG traces of the isolated frog and turtle retinas obtained by the frequency drop method also revealed significant differences (Fig. 3). Whereas on the trace of the isolated turtle retina oscillations were observed at a frequency of stimulation of 50 Hz, and increased in amplitude steadily with a decrease in frequency, on the trace of the isolated frog retina oscillations appeared at frequencies of 30-35 Hz, and a further increase in amplitude of the oscillations with a decrease in frequency showed two peaks: the first, positive, at a frequency of 15-20 Hz, and the second, negative, at a frequency of 10-15 Hz.

Induction of LPO by the $FeSO_4$—ascorbic acid system led after 12-15 min to a decrease in amplitude of both the single and rhythmic ERG of isolated frog and turtle retinas. Under these circumstances, as we know, the rate of accumulation of LPO products in the retina rises [1]. Addition of an alcoholic solution of α-tocopherol to the perfusion medium in the concentrations given above did not affect restoration of the amplitude of the rhythmic ERG. The amplitude of the single response was virtually unchanged. However, rinsing the isolated retinas of the experimental animals with Ringer's solution containing sodium selenite in a concentration of 0.01% led to definite restoration of the amplitude of both the single and the rhythmic ERG.

The investigations thus indicate a significant difference in the dynamics of changes in the rhythmic ERG of the isolated retinas of frogs and turtles, depending on the frequency of stimulation, and also the possibility that LPO may play a role in the regulation of retinal function.

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EFFECT OF HYDROCORTISONE ON BLOOD CLEARANCE OF INERT COLLOIDS BY CELLS OF THE RETICULOENDOTHELIAL SYSTEM

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Responses of the reticuloendothelial system (RES) and of its main component — the system of mononuclear resident phagocytes — to glucocorticoids have been studied in several investigations. However, different results have been obtained. This is evidently because the effects of hydrocortisone (HC) and of other glucocorticoids are dependent on the original functional state of the RES, which as a rule was not verified in these investigations. In addition, after administration of pharmacologic and physiologic doses of the same hormone, it is natural to expect both similar and diametrically opposite effects. After injection of HC in pharmacologic doses into cortisone-sensitive animals (rats and mice) the ability of the RES to cleanse the blood of inert colloids was most frequently observed [13]. Meanwhile, after injection of HC in a physiological dose (3 mg/kg) into adrenalectomized animals no change was found in the phagocytic activity of the RES [4]. According to some data, adrenalectomy itself causes depression [10], according to others — stimulation of the ingestive power of the RES [12]. It likewise is not yet clear how the behavior of the RES differs after single and repeated stimulation of HC.

It is important to study not only the response of the RES to HC, but also the course of recovery of functions of the system after the abrupt resetting of endocrine regulation in response to a large dose of the hormone.

EXPERIMENTAL METHOD

Experiments were carried out on 85 female Wistar rats weighing 240-280 g. HC was injected intraperitoneally into some rats in a dose of 125 mg/kg and blood clearance of inert colloid was tested after 2, 24, and 48 h and 1 and 3 weeks, and into other rats it was injected daily at 9-10 a.m. for 1, 2, and 3 weeks in a sessional dose of 12.5 mg/kg. Function of the RES was determined 24 h after the final injection of the hormone. In the control, 0.85% NaCl was injected at all times of the investigation. To study blood clearance, 2 ml of a suspension of colloidal carbon (from Günther Wagner, West Germany) was added to 3 ml of a

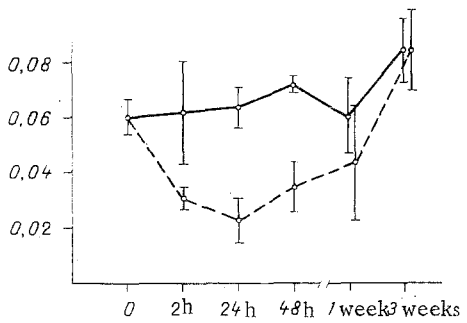

Fig. 1. K-indices after injection of hydrocortisone (125 mg/kg). Continuous line, control; broken line, experiment. Abscissa, K-indices; ordinate, time.

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