EFFECT OF PROLONGED FUNCTION ON PROTEIN CONTENT IN GANGLION CELL NUCLEI IN THE MOUSE RETINA

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The dynamics of the protein content and concentration in the ganglion-cell layer of the mouse retina was studied by interference microscopy. In response to stimulation by a flashing light for 9 h, fluctuations developed in the content and concentration of proteins. Initially (first 2 h) these fluctuations were synchronous in all individuals studied, but during the course of stimulation the synchronization disappeared. The amplitude of the fluctuations and the protein content in the cells decreased gradually.

During adequate functional stimulation of neurons in the ganglionic layer of the vertebrate retina by a flashing light for 2 h, fluctuations are observed in the protein content of their nuclei [3-8]. If the population of ganglion cells in the central region of the retina was synchronized before the experiment with respect to the parameter studied, by keeping the experimental animals for several hours in darkness, the cells of this region reacted approximately equally to photic stimulation [1].

The object of the present investigation was to study changes in the protein content in the nuclei of ganglion cells in the central region of the mouse retina during adequate stimulation for 9 h.

EXPERIMENTAL METHOD

Experiments were carried out on 3-month-old F_1 (CBA $\times C_{57}Bl_6$) male mice whose ganglion cells give a clear and reproducible reaction to stimulation by a flashing light for 2 h consisting of a periodic change in the protein content in their nucleus. Before the beginning of stimulation the animals were kept in darkness for 24 h. Under these conditions relative synchronization of the cytochemical and electrophysiological properties of the ganglion cells takes place (the protein content in their nuclei remains unchanged and the level of electrical activity of the cells is low). As an adequate stimulus for the neurons of the ganglionic layer an electric light with a flash frequency of 2 Hz was used. This frequency of flashing was chosen because, compared with others (frequencies of 0.5 and 1 Hz also were tested) it yields fluctuations in the protein content of the nuclei with the greatest amplitude. Stimulation continued for 9 h. The brightness of the light at floor level (the mice were kept on the floor) was 80-100 lx. The animals were killed in pairs every 10 min, and their eyes were enucleated and placed in a mixture of formalin, absolute alcohol, and glacial acetic acid (3 : 1 : 0.3) for 1 h 45 min. The fixative preserved the tissue structure well, so that the volume of the cells showed relatively little change and their dry weight was hardly reduced [2, 4]. The material was passed through alcohols and xylol and embedded in paraffin wax. Series of sections about 5 μ in thickness were cut. The thickness of the sections was measured interferometrically [9]. Allowing for the thickness of the section, the protein content and its concentration in the nuclei of the ganglion cells in the central region of the retina were then determined interferometrically and the mean values of these parameters for each experimental mouse was calculated (see [4], pp. 266-289). The content and concentration of proteins in the cytoplasm could not be measured because of the very high nucleo-cytoplasmic ratio (about 10 : 1).

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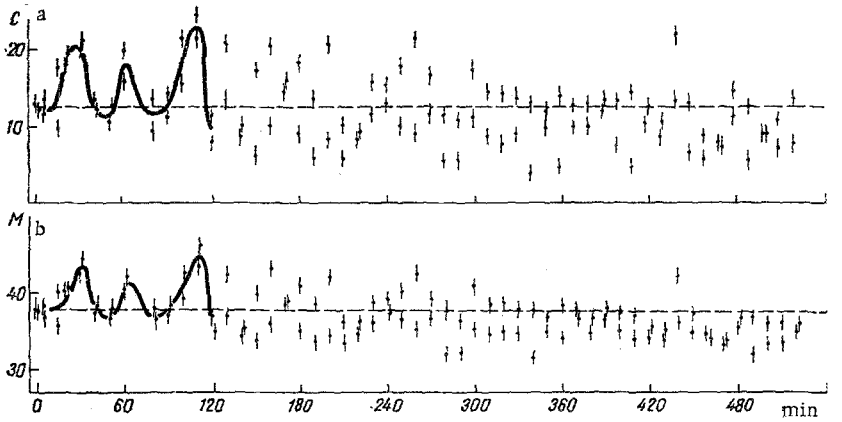

Fig. 1. Mean concentration (a) and mean content (b) of protein in nuclei of ganglion cells in central region of retina as functions of duration of continuous specific stimulation by a light flashing at 2 Hz. Each dot corresponds to one animal. Broken horizontal lines show approximate dark level of protein concentration and content in nuclei of neurons. For each dot the confidence limits corresponding to a 95% level of significance are shown. Abscissa, duration of illumination (in min); ordinate, value of corresponding cytochemical parameter (protein concentration or content in nuclei in relative units).

EXPERIMENTAL RESULTS

All cells of the retinal region examined reacted to functional stimulation in approximately the same way. During stimulation a change was observed in the mean protein concentration in the nuclei (this hypothesis was tested by Student's criterion), but there was no change in the character of distribution of the nuclei with respect to this feature. This distribution remained normal in this experiment, with no change in dispersion (the parametric criterion of normality of distribution and Fisher's criterion were used; see [10], pp. 142 and 164). Consequently, the change in the mean value took place simply because all the variants in the statistical series received approximately the same positive or negative increase, i.e., in the central region of the retina there were no significant groups of cells which differed sharply from the "average" cell in their reaction to stimulation by a light flashing at a frequency of 2 Hz.

The dynamics of the content and concentration of protein in the nuclei of the ganglion cells in the central zone of the mouse retina during continuous stimulation for 9 h by a flashing light is illustrated in Fig. 1. The differences between the mean values of these cytochemical parameters in different individuals before the experiment, i.e., after the animals had been kept in total darkness, were not significant. In response to stimulation the scatter immediately increased sharply, and some of the points were above the dark level while others were below it, indicating fluctuations in the content and concentration of protein in the nuclei during stimulation. For about the first 2 h of stimulation these fluctuations were apparently synchronous in all the animals. The mean values of the protein content and concentration in the nuclei of animals exposed to photic stimulation of equal duration evidently showed only small differences (usually not significant). Later fluctuations in the cytochemical parameters continued (as shown by the considerable scatter of their mean values), but they were not synchronous in the different mice (the difference between the mean values for animals sacrificed after stimulation of equal duration on the average was not less than the difference between the mean values for animals exposed to different durations of photic stimulation). Toward the end of the experiment the fluctuations gradually diminished. This was shown by the fact that the scatter between the mean values of the protein content and concentration in the nuclei became less for different animals and after different periods of stimulation.

During the first 3 h of the experiment the protein content in the nuclei of the ganglion cells was higher on the average than in darkness, but they gradually fell below the dark level. This is clearly seen in Fig. 1.

There is insufficient evidence as yet to allow a final answer to the question of the cause or meaning of the changes in the cytochemical parameters of the ganglion cells in the retina arising in response to stimu-

lation. It can only be conjectured that the metabolism of a cell brought at the beginning of stimulation from the stationary state in which it existed in darkness, gradually passes into a certain stationary state (the cell metabolism under these circumstances becomes adapted to its new level of functional activity).

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