FLUCTUATIONS IN INCORPORATION OF H^3-AMINO ACIDS INTO PROTEINS OF THE ISOLATED RETINA

V. Ya. Brodskii, A. M. Aref'eva, and I. M. Kunina

UDC 612.843.015.348-087.43

The rate of incorporation of amino acids into proteins of the mouse retina was measured during the development of dark adaptation and at various times of photic stimulation. The rhythms of the changes in dry weight of the retinal ganglion cells and of incorporation of amino acids into their proteins had a period of about 1 h.

KEY WORDS: rhythm of protein synthesis; retinal ganglion cells.

The rhythm of quantitative changes in cell proteins observed in various objects [1-4] including the retina has hitherto been determined during functional stimulation of differentiated cells.

The object of the present investigation was to measure the rate of incorporation of amino acids into proteins of the retina in the course of dark adaptation (i.e., when the cells were at relative rest) and at different times of photic stimulation.

EXPERIMENTAL METHOD

CBA × C57BL/6 hybrid mice aged 2 months were kept before isolation of the retina in darkness or were stimulated with flashes (intensity 90 lx, frequency 2 Hz). Immediately after isolation, the retina was placed in medium No. 199 with lysine-H^3 or leucine-H^3 for 10 min at 37°C. It was then washed with medium containing excess of the unlabeled amino acids and treated with 5% $HClO_4$ for 1 h. The radioactivity of the free H^3-amino acid was determined in the acid-soluble fraction. The tissue was then treated with 5% $HClO_4$ at 90°C for 20 min, hydrolyzed with 1 ml 1 N NaOH, and kept for 5 min at 90°C. From each sample, 0.5 ml was taken for scintillation analysis and 0.4 ml for determination of the protein concentration by Lowry's method. Radioactivity was measured with the SL-30 counter. The stock of amino acid and its incorporation into protein were investigated regularly every 10 min in the course of dark adaptation, using the retinas of one or two animals at each time. The dynamics of quantitative changes in retinal proteins also was studied during photic stimulation of the animals.

EXPERIMENTAL RESULTS

The principle of calculation of the rate of incorporation is explained in Fig. 1. Incorporation of the precursor into the substance synthesized evidently depends not only on the actual rate of its incorporation, but also on the rate at which the labeled precursor is supplied to the cell. This latter value, equal to the combined radioactivity of proteins and of the amino acid stock, characterizes the permeability of the cell for the amino acid. It is usually taken as equal to the stock only, but this is valid only if the radioactivity of the stock is at least an order of magnitude higher than the radioactivity of the proteins. In the present case (Fig. 1), this method of calculation is unacceptable. The true rate of incorporation was calculated by introducing a correction for total uptake of amino acid by the cells. The significance of changes in the rate

Institute of Biology of Development, Academy of Sciences of the USSR, Moscow. (Presented by Academician S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 10, pp. 104-106, October, 1975. Original article submitted February 7, 1975.

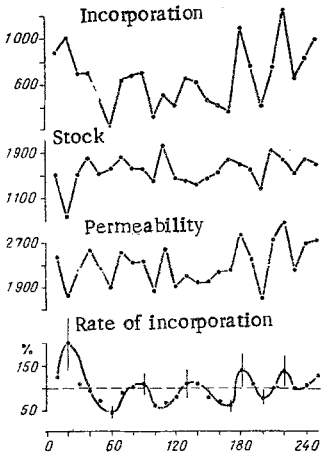

Fig. 1

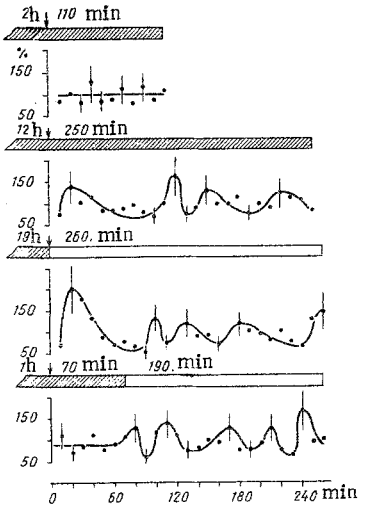

Fig. 2

Fig. 1. Example of calculation of rate of incorporation. From top to bottom: incorporation of lysine-H^3 into retinal proteins, stock of free lysine-H^3, combined radioactivity – permeability of the retina for lysine-H^3, rate of incorporation – incorporation with correction for permeability. Rate of incorporation expressed as percentage of mean level, reliability of measurements estimated by two standards of distribution. Each point relates to one or two retinas. Abscissa – time from beginning of sampling (in min); ordinate, measured or calculated values (in counts/min/mg wet weight).

Fig. 2. Dynamics of rate of lysine-H^3 incorporation into retinal proteins of mice kept for various times in darkness (see strip above curve; arrow shows time of taking first sample), then in darkness or in light (the shaded area of the strip represents darkness). Rate of incorporation expressed as percentage of mean level, reliability of inflections assessed by two standards of distribution.

TABLE 1. Comparison of Rhythms of Rate of Incorporation of Amino Acids into Retinal Proteins of Mice after Prolonged Dark Adaption and after Stimulation by Flashes

State of animals	No. of periods studied	Duration of periods (in min)		Intensity of changes (in %/min)
		limits of fluctations	mean	
Dark adaptation	11	30—120	63	2,8
Photic stimulation	8	40—120	62	2,7

of uptake was assessed by two standards of distribution. This value, corresponding to 95% of the total variability, was determined by studying 10-13 retinas at one time, i.e., when their functional state was the same.

The results summarized in Figs. 1 and 2 show that the rate of incorporation of H^3-amino acids into retinal proteins fluctuates regularly. The mean period of the fluctuations was close to 1 h. The rhythm was found after the mice had been kept for 12 and 24 h in darkness and also during exposure to photic stimulation under various conditions. During the 1st and 2nd hours of dark adaptation the changes in the rate of incorporation were not significant.

Fluctuations in the rate of incorporation of amino acids into retinal proteins are not initiated by external stimuli. Throughout the experiment with animals kept for long periods in darkness, their retina was not subjected to external stimulation. The time from decapitation of the mice to measurement of radioactivity was the same for each point of time of the experiment (Figs. 1 and 2). All procedures were similar each time. The only difference was the time from the beginning of keeping the animals in darkness. If this factor had been sufficient to bring the cells out of their stable state, the rhythm would have been more marked in mice kept for short times in darkness. However, this was not so: After dark adaptation for 2 h no fluctuations whatsoever were found, but after 24 h their amplitude was not less than after

12 h. The more acceptable hypothesis is that of constant but unsynchronized fluctuations in the rate of incorporation. Synchronization of the states of the retinas after random activity of the animals, when they were taken from the animal house, requires several hours of dark adaptation. Photic stimulation synchronizes the state of the retinal cells faster.

Increased activity of the cells during photic stimulation of the retina did not affect the character of fluctuations in the rate of incorporation (Fig. 2, Table 1). Possibly more specific forms of stimulation of the retina than flashes should have been used in order to detect the changes. Probable differences between individual cell types within the organ cannot be ruled out.

Fluctuations in the rate of incorporation of amino acids were most probably connected with the rhythm of protein synthesis. Roughly hourly fluctuations in the rate of incorporation, independent of changes in permeability, have been demonstrated in cells of the rat parotid gland and in cleaving sea urchin eggs [2, 4]. In the latter object, the stability of the endogenous stock of amino acids was determined. It is not yet clear whether the intensity of translation itself fluctuates or whether fluctuations are observed at a different stage of protein synthesis. However, the rhythms of protein metabolism already discovered – periodic changes in the dry weight of the cells, in the rate of incorporation of amino acids into proteins, and in some cells in the secretion and activity of certain enzymes – are evidence that the protein rhythm plays an important role in cell physiology, more especially because adaptive and ontogenetic changes in some of these rhythms have already been found [1, 4].

LITERATURE CITED

1. V. Ya. Brodskii and A. M. Aref'eva, Byull. Éksperim. Biol. Med., No. 9, 95 (1974).
2. N. V. Nechaeva and V. I. Fateeva, Tsitologiya, 16, 1033 (1974).
3. R. Klevecz, J. Cell Biol., 43, 207 (1969).
4. J. Mano, Develop. Biol., 22, 433 (1970).