RHYTHM OF LYSINE-H^3 INCORPORATION INTO PROTEINS OF THE MOUSE RETINA AS AN EXAMPLE OF A COMPLEX CELL SYSTEM

V. Ya. Brodskii and A. M. Aref'eva

UDC 612.398.6-087.45

Scintillation analysis after pulse labeling showed that the incorporation of lysine-H^3 into proteins of the whole mouse retina is rhythmic in character. The period of the rhythm is about 30 min. Rhythmic changes in the label were found in the retina both of active animals and in animals in a state of dark adaptation.

KEY WORDS: proteins of the retina; rhythm of incorporation of isotope; lysine-H^3.

Previous communications from the writers' laboratory [1, 2] described rhythms of changes in the dry weight and incorporation of amino acids into proteins of the ganglionic cells of the retina and acinar cells of the parotid salivary gland. In all cases cells or suspensions of homogeneous cells were studied. Are the changes in the parameters of protein synthesis in other cells of the same organs synchronized? Is there a definite global pattern of protein synthesis in a complex system containing many types of cells with differing functions? The investigation described below was carried out to find the answers to these questions.

EXPERIMENTAL METHOD

Experiments were carried out on male hybrid CBA × C57BL/6 mice. On each occasion a large group of animals was taken and kept for 2 h in darkness. One mouse was sacrificed every 10 min in darkness and during subsequent exposure to intermittent light (2 Hz). Altogether 130 animals were used. The retina was quickly detached, weighed, and placed in an incubation mixture consisting of medium No. 199 and lysine-H^3 in concentrations of 20 or 35 μCi/ml (specific activity 20 and 13 Ci/mmole, respectively). Incubation continued for 10 min at 37°C. The retina was then rinsed several times with cold medium containing an excess of unlabeled lysine and then placed in cold 5% TCA solution. The whole material was then treated simultaneously with hot TCA for 20 min, with alcohol for 1 h, and hiamine for 12 h, and then flooded with toluene scintillator.

The activity of the samples was determined with the FL-30 scintillation counter, allowing for the activity of an external standard in order to correct possible errors during analysis.

EXPERIMENTAL RESULTS

To estimate the reality of the changes in labeling, their reproducibility was specially tested. For this purpose the retina of 8 mice was taken after exposure to photic stimulation for the same time (20 min). These experiments were repeated twice. The error of the arithmetic mean was 16%. The reproducibility of the measurements on dark-adapted animals was 7%. The results of the investigation are presented as curves in Fig. 1. Inflections were placed on the curves to allow for the significance of the differences between the points. It follows from Fig. 1A that lysine-H^3 was incorporated periodically into the proteins of the mouse retina, when exposed to flashes after 2 h of dark adaptation. The radioactivity

Institute of Biology of Development, Academy of Sciences of the USSR. (Presented by Academician P. K. Anokhin [deceased]). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 9, pp. 95-97, September, 1974. Original article submitted November 23, 1973.

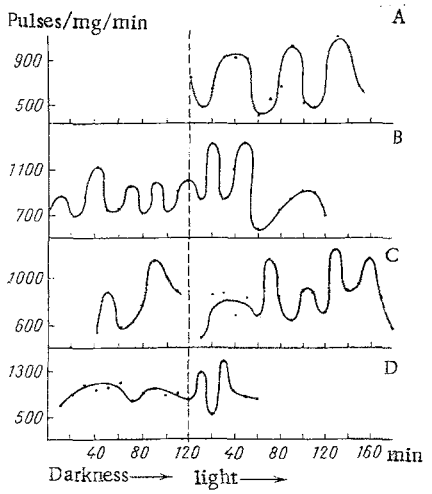

Fig. 1. Incorporation of lysine-H^3 into mouse retina in darkness and during exposure to flashes. A, B, C, D) Separate experiments. Abscissa, time (in min); ordinate, activity of incorporation (pulses/mg/min).

of the retinal proteins also fluctuated in animals kept in darkness for different times (the first reading was obtained 2 h after the mice had been placed in a dark room). In this case the amplitudes of the fluctuations in radioactivity in the dark-adapted retina was lower than in animals stimulated by light (Fig. 1B). The mean period of the fluctuations was similar to results of the previous experiments (Fig. 1A) – about 30 min.

Further experiments (Fig. 1C, D) confirmed the fluctuations in the radioactivity in the retina of the dark-adapted animals. The amplitude of the fluctuations in radioactivity in darkness was not always lower than that in light. The period was always of the same order (20-40 min), and in most cases it was 30 min.

Rhythmic incorporation of lysine-H^3 into the mouse retina was thus found. These observations by themselves do not prove the rhythmic character of protein synthesis. However, since the incorporation of the amino acid was pulsed (10 min) and since cophased changes in dry weight were described previously for one type of retinal cells (ganglionic cells) [3], the hypothesis that protein synthesis in the retina is rhythmic in character seems very likely to be correct.

One conclusion that follows from this observed rhythmic incorporation of the amino acid into the whole retina must be that the course of metabolism of the different forms of retinal cells is relatively well synchronized. This conclusion applies whether these cells synthesized protein synchronously or, for example, whether their permeability to precursors fluctuates synchronously.

Another conclusion from this investigation that appears important is the rhythmic incorporation of lysine-H^3 into proteins of the comparatively inactive retina. Rhythmic protein synthesis has hitherto been associated with stimulation of cell function. The observation of changes in radioactivity in relatively inactive cells can be discussed from the standpoint of the endogenous nature of hourly rhythms of protein synthesis.

The authors are grateful to V. I. Fateeva for her help with the organization and conduct of these experiments.

LITERATURE CITED

1. V. Ya. Brodskii, Zh. Vyssh. Nerv. Deyat., No. 2, 323 (1973).
2. V. Ya. Brodskii, Abstracts of Proceedings of the 5th All-Union Conference on Neurochemistry [in Russian], Tbilisi (1968), p. 15.
3. V. Ya. Brodskii and A. M. Aref'eva, Tsitologiya, 10, No. 3, 329 (1968).