CHANGES IN THE INTENSITY OF PROTEIN SYNTHESIS IN THE RETINA

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The intensity of protein synthesis in the mouse retina was shown to change substantially with time. The results were obtained by incubating retinas consecutively in media containing lysine-^{14}C and lysine-^{3}H.

KEY WORDS: *Retina; fluctuations in intensity of protein synthesis.*

Cytochemical and biochemical investigations of the mouse retina revealed approximately hourly changes in the dry weight of the ganglion cells and in incorporation of amino acids into proteins [1, 2]. This was demonstrated by the study of retinas from animals of the same age, the physiological activity of which was synchronized by prolonged dark adaptation to darkness or to flashes. These results suggested that the method of synchronization used brought the endogenous fluctuations in retinal proteins in the different animals into the same phase of the rhythm. It can tentatively be suggested that the dynamics of the retinal protein content in different animals reflects changes in the protein content in each individual retina, a fact connected with the periodic changes in the intensity of protein synthesis in each retina.

To detect fluctuations in the intensity of protein synthesis with time in the retina double-labeling experiments were carried out. The experiments consisted essentially of incubating the material consecutively for equal periods of time in media containing lysine labeled with ^{14}C and ^{3}H. In this way the dynamics of the intensity of incorporation of the precursors into retinal protein could be studied. Incorporation of the double label was characterized by the ratio between the intensity of incorporation of lysine labeled with one isotope and the intensity of its incorporation labeled with the other isotope.

EXPERIMENTAL METHOD

Animals with unsynchronized physiological activity of their retinas were used. The isolated retinas of CBA × C57BL/6 hybrid mice were placed in Eagle's medium without lysine for 2 h at 37°C. Half of the total number of retinas was then placed in the same medium containing lysine-^{14}C (75 μCi/ml, specific activity 180 μCi/ml, Czechoslovakia) for 15 min, washed for 2 min with Hanks's solution, and then placed in medium containing lysine-^{3}H (75 mCi/ml, specific activity 11.5 mCi/ml, Amersham, England) for 15 min, washed with Hanks's solution again, and fixed in 1 ml 5% TCA for 1 h. In the control group the retinas were kept for 15 min in medium containing both isotopes at once in the same dilutions as in the experimental series, washed for 2 min with Hanks's solution, and fixed in the same way as the experimental group. For each retina, after fixation for 1 h at 20°C the equivalent of the cell permeability was determined: the amino-acid reserves of precursors extracted with TCA. To analyze the protein fraction the retinas were freed from nucleic acid by hydrolysis for 20 min in an excess of 5% TCA at 90°C, washed with TCA, and dissolved in 1 M NaOH for 5 min at 90°C. Differential determination of the radioactivity of the amino-acid reserves and of the protein fraction was carried out for ^{14}C- and ^{3}H-channels on the S1-30 scintillator in Bray's solution. Altogether six experiments were carried out.

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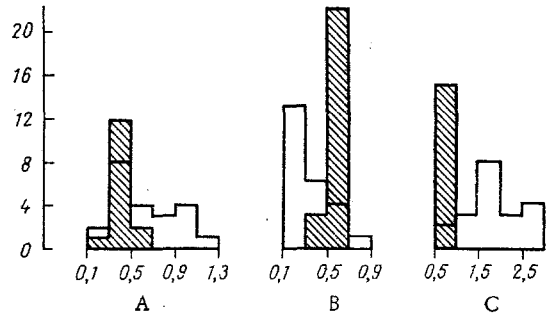

Fig. 1. Ordinate, number of retinas (for all histograms); abscissa: A) protein-^{14}C/protein-^{3}H ratio; B) lysine-^{14}C/lysine-^{3}H ratio in acid-soluble fraction; C) [protein/(protein + reserves)-^{14}C]/[protein/(protein + reserves)-^{3}H] ratio. Control distributions shaded.

EXPERIMENTAL RESULTS AND DISCUSSION

The existence of approximately hourly variations in the intensity of protein synthesis suggests that during the 30 min of incubation the intensity of synthesis in every retina must change. The use of "unsynchronized" animals in which fluctuations in the intensity of synthesis of retinal proteins at the time of the experiments were in different phases had the result that the ratio between ^{14}C-labeled and ^{3}H-labeled proteins calculated for each retina differed greatly individually. For retinas in which the intensity of protein synthesis increased during the experiments these ratios would be smaller than for retinas in the phase of diminishing intensity of protein synthesis during the experiments. In retinas in the extremal phases of fluctuations of synthesis these ratios would be intermediate in magnitude. For the control retinas differences in the phases of fluctuation of protein synthesis had no role to play in the calculation of the protein-^{14}C/protein-^{3}H ratios, for these retinas incorporated labeled precursors simultaneously. Consequently, the wider distribution of the ratios in the experimental and in the control series would confirm the hypothesis that changes in the intensity of protein synthesis in each retina varied with time.

This hypothesis was confirmed by the experimental results. The distribution of the protein-^{14}C/protein-^{3}H ratios from one of the experiments is shown in Fig. 1A. The coefficient of variation of the distribution was 45% in the experimental and 27% in the control series. The distributions of the lysine-^{14}C/lysine-^{3}H ratios in the amino-acid reserves for the experimental and control series are given in Fig. 1B; the coefficient of variation was 60% in the first case and 12% in the second. Experimental and control distributions of the protein-^{14}C/(protein + reserves)-^{14}C ratio divided by the protein-^{3}H/(protein + reserves)-^{3}H ratio are given in Fig. 1C. For these ratios the coefficient of variation was 33% in the experimental and 17% in the control series. The value of the protein/(protein + reserves) ratio for newly synthesized protein has been shown to reflect the intensity of protein synthesis at the moment of supply of the precursor and to be independent of any possible changes in the permeability of the cells to the precursor [3]. The fact that the coefficient of variation of the distribution of these ratios in the control series was only half its value in the experimental series shows that significant variations in protein synthesis exist and are independent of changes in the permeability of the retinal cells with time.

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