EXPERIMENTAL BIOLOGY

Chronobiological Regularities of Protein Content in Rat Retinal Ganglion Cells during Exposure to Products of Astrakhan Gas-Processing Plant

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Biorhythms of protein content in rat retinal ganglion cells during pregnancy are sensitive to exposure to natural gas of the Astrakhan gas condensate plant. Diurnal rhythm of sensitivity to hydrogen sulfide was revealed.

Key Words: *rat; retina, ganglion cells; hydrogen sulfide*

The conjunctiva is the first organ reacting to gaseous products of the Astrakhan gas fields (AGF). An essential increase in H_2S concentration leads to irritation of the conjunctiva (gas eye). There are good grounds to believe that AGF products produce toxic effects on the retina as well. Elucidation of the effects of AGF products on animal eye during different periods of antenatal development seemed to be an important problem.

The state of the retinal ganglion cells can be evaluated by their protein content, especially if these parameters are studied using a chronobiological approach.

MATERIALS AND METHODS

We investigated chronobiological regularities of protein content in the retinal ganglion cells of rats exposed to AGF products (H_2S concentration 300 mg/m^3) on days 7, 14, and 21 of pregnancy. The aftereffects of gas exposure on the retina were studied in 1, 14, and 28-day-old rats. Protein in retinal cells were visualized cytochemically by staining with 0.1% fast green in 1% acetic acid (pH 2.2). The cells were studied by cytophotometry on a scanning microscope photometer. Protein content was measured in 100 cells for each case and expressed in arbitrary units.

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For evaluation of chronobiological regularities in protein content in retinal ganglion cells, the animals were sacrificed every 4 h for 3 days (5 animals per point). Cosinor analysis with Cosinor diagrams plotting and the graphic parametrical method were used. Mesor, acrophase, absolute amplitude (AA), relative amplitude (RA), synchronization coefficient (SC), length of active and passive phases (AP and PP, respectively), AP position over 24 h, AP median, and percentage of protein content for AP were determined using graphic and parametrical methods. Cosinor analysis and the graphic parametrical method were used for evaluation of the parameters of circadian rhythms of protein content on each day of the experiment and their mean values over 3 days were estimated. The parameters of circadian rhythms determined by two methods were compared.

RESULTS

Circadian rhythms of protein content in ganglion cells of the retina were revealed in 14- and 28-day-old rats.

In control animals the acrophase during the three consecutive days was not later than 4 h and occurred mainly in the middle of the dark hours or at the end of the dark period. The shifts in the time of AP median did not surpass 2 h, the start and end of AP rhythm in

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different days of experiment differed by no more than 3 h (Fig. 1).

According to Cosinor analysis and graphic parametrical method, the mesor value changed little from day to day. On the other hand, it tended to change in 28-day-old control animals in comparison with 14-day-old ones.

AA values were different in different days of experiment, but both methods demonstrated that this value decreased with age. The mean values of acrophase, mesor, and AA determined by the two methods differed during the entire period of observation; a trend to a decrease of the mesor and acrophase with age was observed. RA, SC, and the percentage of protein values observed during AP differed little in different days of experiment in control animals of both age groups.

Hence, AA and acrophase position proved to be the parameters most liable to changes of all the studied parameters of circadian rhythms of protein content in the retinal ganglion cells of control rats; we consider that these changes were adaptive. The changes were compensated for by slight fluctuations of AP in different days of experiment and, to a certain measure, of the protein content falling on AP. This seemed to maintain the constant mesor as a result of activity of the system contributing to the maintenance of protein content in the retinal ganglion cells during a unit of time (in our case day).

The mean mesor and AA were notably decreased in animals exposed to AGF gas on day 7 of embryonic development and examined on days 14 and 28 of postnatal development, these decreases being more pronounced in 14-day-old rats. The mean acrophase clearly shifted to the right, particularly in 28-day-old rats, in which the shift was as great as 8-9 h (Fig. 1). The acrophase and median of AP in 14-day-old experimental rats were observed at the end of the dark period and in 28-day-old animals during the first $^2/_3$ of the light time of the day. Differences in the time of acrophase and median of AP in different days were no more than 4 h. In 14-day rats AP was observed during the second half of the dark and first half of the light periods of the days, while in 28-day animals it was observed mainly at the end of the dark period and during almost the entire light period. In 14-day-old animals the acrophase and median of AP were not shifted at all or shifted negligibly, while in 28-day-old animals almost complete inversion of these parameters was observed. As a result of these changes, AP in 28-day-old animals occupied almost the entire light period or the second half of the light and first part of the dark period of the day (Fig. 1).

Similar changes in the rhythmic parameters were observed in rats exposed to gas on day 14 of embryonic development and examined on days 14 and 28 of postnatal development. Differences in the mesor and AA values in comparison with the control were more pronounced in 14-day-old rats than in 28-day-old ones. In contrast to animals exposed on day 7 of embryonic development, the phase shift was absent in 14-day-old rats exposed to gas on day 14 of gestation, while in 28-day-old animals it led to inversion of the rhythmic phase structure.

Monophasic circadian rhythm of protein content of the retinal ganglion cells was preserved in 14- and 28-day-old rats exposed to gas on day 21 of gestation. The decrease in the mean value of mesor was less pronounced than in the two former groups of animals and was observed only in 14-day-old rats. The same is true for changes in the mean AA. Mesor changes in

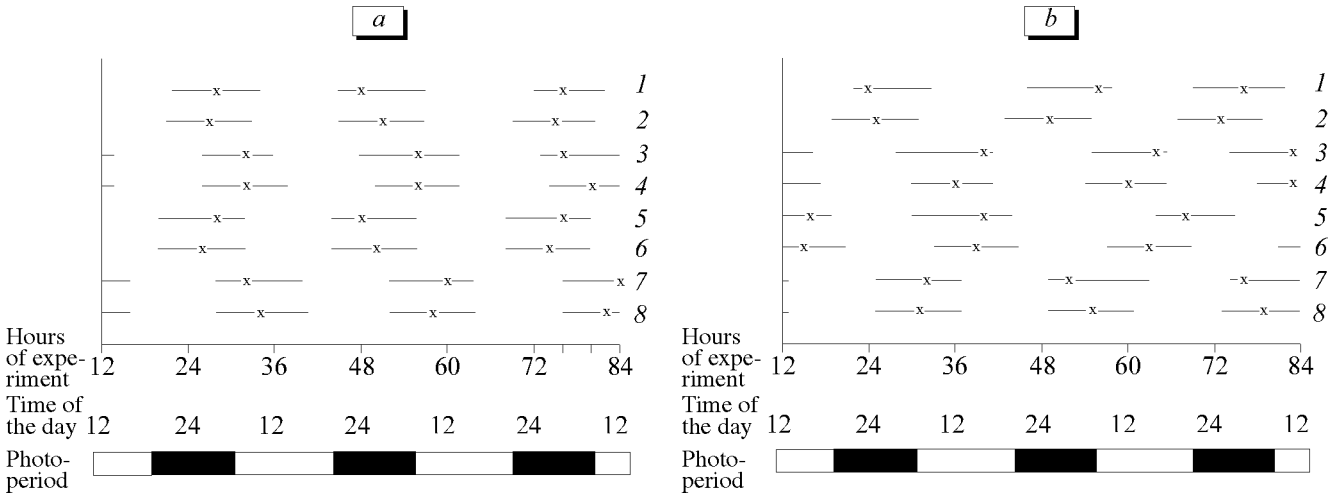

Fig. 1. Phasograms of circadian rhythm of protein content in retinal ganglion cells in 14- (*a*) and 28-day-old rats (*b*) after exposure to natural gas with H_2S concentration of 300 mg/m^3 in different periods of gestation. *1*, *2*) control; *3*, *4*) exposure on day 7; *5*, *6*) on day 14; *7*, *8*) on day 21 of gestation. *1*, *3*, *5*, *7*) Cosinor analysis; *2*, *4*, *6*, *8*) graphic parametrical method.

different days were more expressed in this group of animals than in two former groups. Day-by-day changes in AA and its decrease in comparison with the control were more pronounced in 14-day-old animals than in 28-day ones. Changes in acrophase and AP median from day to day were slight in both age groups, but in 28-day-old animals the phase shift to the right was either absent or was 2.7-5.5 h, while in 14-day-old rats it was more pronounced and reached 7.3-8.2 h on different days of experiment (Fig. 1). Due to these phasic changes, AP in 14-day-old rats was observed at the end of the dark and first $^2/_3$ of the light periods, while in 28-day-old rats it occurred in the second half of the dark and first half of the light period (Fig. 1).

Hence, the mesor of circadian rhythm of retinal ganglion cells protein content of rats is the most sensitive to AGF natural gas on day 7 of embryonic development and the least sensitive on day 21 of gestation. AA also decreased under conditions of gas exposure. In this case, too, rats were the most sensitive to the exposure on day 7 of embryonic development. A decrease in AA in animals exposed to gas on days 14 and 28 of embryogenesis was more expressed in 14-day-old rats than in 28-day-old ones.

AGF natural gas induced characteristic phase shifts in the circadian rhythm of protein content in the retinal ganglion cells. Both methods used in our study showed that phasic shifts (sometimes inversion) were more pronounced in 28-day-old rats. In addition, they were more manifest in 14-day-old animals exposed to gas on day 21 of embryogenesis and 28-day-old rats exposed to gas on day 7 and, more so, on day 14 of embryogenesis.

Hence, it seems that time organization of protein content in rat retinal ganglion cells is sensitive to natural gas over the entire course of gestation, but the changes are to a certain extent compensated with age.

It is noteworthy that under conditions of exposure to natural AGF gas with H_2S concentration of 300 mg/m^3 the percentage of protein in ganglion cells during AP virtually did not differ from the control, despite fluctuations in the values on different days. This can be regarded as the absence of deep changes in the distribution of protein content in the rhythmic phases under these conditions.

Apart from circadian rhythms of protein content in rat retinal ganglion cells, we revealed circadian rhythm of sensitivity of this parameter to gas at H_2S concentration of 300 mg/m^3. The highest sensitivity was observed in the night hours in all experimental groups. However in 14- and 28-day-old rats the amplitude characteristics of the rhythm differed, depending on the period when the embryos were exposed to gas.

The results indicate that the parameters of circadian rhythm of protein content in rat retinal ganglion cells obtained using Cosinor analysis and the graphic parametrical method are similar.

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