DIURNAL RHYTHM OF TOTAL HISTONE CONTENT IN NORADRENALIN- AND SEROTONIN-SENSITIVE NEURONS AND THEIR GLIAL SATELLITE CELLS

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The content of total histones in the nucleus and body of neurons of the reticular formation and in the nucleus of their glial satellite cells was determined by thin-beam photoelectric spectrophotometry of sections stained with Fast Green FCF in rats at different times of the 24-hour period. In the noradrenalin-sensitive neurons of the lateral nucleus of the reticular formation a diurnal rhythm of changes in the histone content, characterized by two maxima and two minima, was found whereas only one period of fluctuation in the 24-h period was observed in the nuclei of these nerve cells. In serotonin-sensitive neurons of the paragigantocellular nucleus of the reticular formation, on the other hand, the histone content fluctuated once every 24 h in the cell bodies and twice every 24 h in the nuclei. The amplitude of the fluctuation in the histone content in cells of the perineuronal neuroglia was considerably lower than in the corresponding neurons.

Key words: neuron and neuroglia; histones; reticular formation; diurnal rhythm.

The writers showed previously [7] that neuron–neuroglia systems that differ in the type of their predominant neuromediator differ also in some properties of their intracellular histones.

The object of this investigation was to compare quantitative changes in total histones in these neuron–neuroglia systems under the influence of a natural combination of external and internal factors giving rise to the diurnal rhythm.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used. The animals were kept for 1 week in natural lighting in the period when the duration of daylight and night was 12 h for each. Every 4 h a group of 8 rats was decapitated, the medulla was fixed by Brodskii's method (formalin–ethanol–acetic acid, 9:3:1) and embedded in paraffin wax. Sections 6-9 μ thick were stained with Fast Green FCF [10]. The specificity of staining was verified by acid extraction of the histones with cold 0.25 N HCl solution for 1 h, after which treatment the sections no longer took up the Fast Green.

The lateral and paragigantocellular nuclei of the reticular formation, consisting predominantly of noradrenalin- and serotonin-sensitive neurons respectively [6, 12], were studied.

The total histone content in the whole body of the neurons and separately in their nuclei and also in the bodies (for practical purposes, the nuclei) of the satellite glial cells was determined by photoelectric cytospectrophotometry at 542 nm (at the limit of the absorption band) with a thin-beam cytospectrophotometer designed by Rozanov and Selivanova [8] in M. V. Arkhipov's modification. The numerical results were subjected to statistical analysis by the Minsk-22 electronic digital computer.

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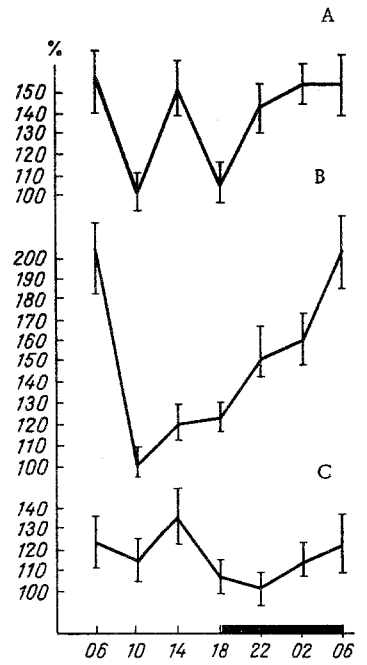

Fig. 1

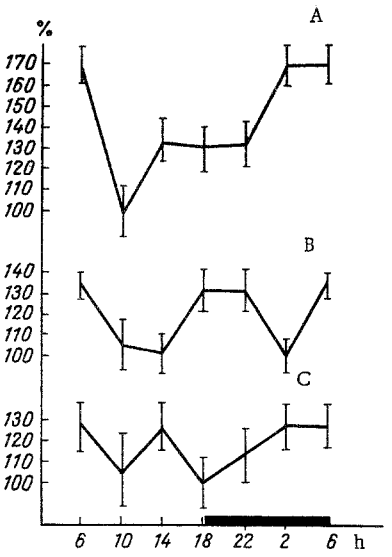

Fig. 2

Fig. 1. Diurnal rhythm of fluctuations in total histone content in nuclei (A) and bodies (B) of noradrenalin-sensitive neurons of lateral nucleus of reticular formation and also in nuclei (C) of their glial satellite cells. Abscissa, time on 24-h clock (black band corresponds to hours of darkness); ordinate, relative changes in histone content (calculated per cell) in per cent of corresponding minimal value. Vertical lines show standard error.

Fig. 2. Diurnal rhythm of fluctuations in total histone content in nuclei (A) and bodies (B) of serotonin-sensitive neurons of paragigantocellular nucleus of reticular formation and also in nuclei (C) of their glial satellite cells. Legend as in Fig. 1.

EXPERIMENTAL RESULTS

To judge from the intensity of staining with Fast Green, the histone concentration in the cytoplasm of the neurons was a little lower than in the nuclei of the neurons and of the perineuronal glial cells. The total histone content calculated per cell, however, was determined also by the sizes of the actual types of cells. No significant differences were found between nuclei of neurons of the two nuclei of the reticular formation or between the glial cells surrounding the noradrenalin- and serotonin-sensitive neurons. The mean content of total histones in the bodies of the serotonin-sensitive neurons of the paragigantocellular nucleus, on the other hand, was a little higher than in the noradrenalin-sensitive neurons of the lateral nucleus of the reticular formation.

During the 24-hour period the total histone contents in all the types of cells studied fluctuated. The amplitude of the diurnal fluctuation in the glial cells of both nuclei of the reticular formation was rather lower than that in the corresponding neurons (Figs. 1 and 2). The diurnal rhythm of the histone content in the noradrenalin-sensitive neurons was characterized by two maxima and two minima in the cell bodies but only one maximum and one minimum in the cell nuclei (Fig. 1). The histone content in the serotonin-sensitive neurons, on the other hand, fluctuated twice in the 24-h period in the nuclei and only once in the same period in the cell bodies (Fig. 2). The amplitude of the fluctuations in the bodies of the serotonin-sensitive neurons was distinctly higher, but in the noradrenalin-sensitive neurons a little lower than in the nuclei of these cells (Figs. 1 and 2).

An important role is ascribed to histones as regulators of intracellular RNA and protein metabolism [2, 4, 9, 11], including in the cells of nerve tissue [1, 15]. The histone content in the cell nuclei of the nervous system may be presumed to change depending on the level of function, and to give rise in turn to changes in the metabolism of macromolecules in the neurons. The results of the present experiments evidently

confirm the presence of such changes in the histone content during the 24-h period. The changes discovered took place not only in the nuclei of the neurons (and also in the nuclei of the glial cells) but also in the cytoplasm of the nerve cells (Figs. 1 and 2). Besides nuclear histones, neurons also contain cytoplasmic basic (histone-like) proteins, the content of which may change during experimentally induced changes in the functional state of the nervous system [3, 5, 14]. On the other hand, the staining method for basic proteins with Fast Green used in the present investigation has not yet been subjected to strict analysis with respect to individual protein fractions revealed by this histochemical reaction. Although verification of specificity (preliminary extraction of histones from the section) gave encouraging results (see "Experimental Method"), the possibility cannot be ruled out that besides the amino groups of basic amino acids (arginine and lysine), Fast Green also stains other functional groups of proteins in the sections. Nor is it known whether different fractions of basic proteins, differing in their relative proportion of diamino acids, have identical staining properties.

The writers showed previously [7] that qualitative characteristics of the basic proteins (the ratio between arginine- and lysine-rich histones) are similar in neurons with different neuromediator sensitivity but differ significantly in their glial satellite cells. The quantitative characteristics (total histone content calculated per cell), however, varied in the course of the 24-h period predominantly in the neuron (Figs. 1 and 2). This confirms the conclusion drawn from investigation of RNA in the neuron–neuroglia system [5], that adequate stimulation for the nervous system affects metabolism of the neurons to a greater degree than the metabolism of their glial satellite cells.

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