AUTORADIOGRAPHIC ANALYSIS OF URIDINE-H^3 INCORPORATION INTO HYPERCHROMIC MOTONEURONS OF THE MOUSE SPINAL CORD

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After local injection of a solution of uridine-H^3 the dynamics of incorporation of the isotope into RNA of the nuclei and cytoplasm of the perikarya of hyperchromic motoneurons was studied in the ventrolateral nucleus of the mouse spinal cord. An increase in the number of hyperchromic neurons among the populations studied was produced by making the mice swim for 4 h. The regression curves of the label were found to differ in character for hyperchromic motoneurons.

KEY WORDS: hyperchromic motoneurons; uridine-H^3.

The nature of the so-called hyperchromic (dark) cells has not yet been settled although there are clear indications that the morphological changes on the basis of which neurons of a certain type are classified as hyperchromic are functional in origin [1, 3, 4, 11, 12].

Several workers [1, 8, 10, 11] have carried out a cytological analysis of hyperchromic nerve cells. An essential effect of these investigations has been that mainly qualitative cytochemical methods were used.

The object of the present investigation was to make a comparative autoradiographic study of RNA metabolism in hyperchromic (HN) and normochromic (NN) neurons in the anterior horns of the mouse spinal cord.

EXPERIMENTAL METHOD

By means of a special device a solution of uridine-H^3 (1 μCi/μl) in a dose of 3 μCi was injected directly into the tissue of the spinal cord. Altogether 78 mice weighing 25-30 g were used in four series of experiments. Series A consisted of control animals, the mice of series B were made to swim for 4 h in water at 38°C, and the mice of series C and D rested for 3 and 6 h after swimming for 4 h. The animals were killed 1, 3, 6, 12, and 24 h after the injection. A segment of spinal cord at the level L_4 was fixed in Carnoy's fluid and treated by the usual histological method. Serial sections 7 μ in thickness were mounted on slides, coated with type M nuclear emulsion, and exposed for 1 month. After development of the autoradiographs the sections were counterstained with methylene blue. The autoradiographs were analyzed with the Opton-01 cytophotometer by densitometry of the perikaryon and nucleus at 460 nm (the minimum of absorption of the dye). Using a probe describing the perikaryon (or nucleus), 30 to 40 motoneurons were examined with the cytophotometer from each of the 3 or 4 animals in the group. The intensity of the label over the nucleus was determined from the optical density of the autoradiograph of the nucleus, and its intensity above the cytoplasm was determined from the difference between the optical density of the autoradiograph of the perikaryon (corrected for the relative areas of the probes) and the optical density of the nuclear autoradiograph.

It is generally considered that the intensity of incorporation of uridine-H^3 reflects the level of RNA synthesis, although the end result is influenced also by such factors as the size of the intracellular pool of

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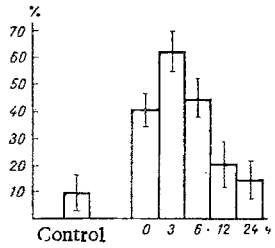

Fig. 1. Changes in number of HN in ventro-lateral nucleus of spinal cord after swimming for 4 h. Abscissa, time after swimming (in h); ordinate, number of HN (in % of total number of cells.

the corresponding precursors and the difference in density of the karyoplasm and cytoplasm, determining the intensity of the self-absorption effect. It is in fact desirable to estimate the effect of these factors in order to obtain a full picture of the intensity of RNA synthesis, but it is not always possible to do so for technical reasons. Accordingly, in the present investigation, the main parameter by which the neurons were compared was the regression curves of labeling.

EXPERIMENTAL RESULTS

Counting the number of HN in serial sections of the spinal cord showed that their number was maximal in the motoneuron population of the ventro-lateral nucleus of animals resting for 3 h after swimming. After a longer rest a progressive decrease in the number of these cells was found. In the control, HN accounted for only a very small part of the motoneuron pool (Fig. 1).

The dynamics of the changes in intensity of labeling of the nuclei and cytoplasm of the perikarya of NN and HN differed (Fig. 2). Maximal intensity of labeling of the nuclei in the case of NN was observed 3 h after injection of the solution of the precursor. This was followed by a gradual decrease, coinciding with the increase in level of labeling of the cytoplasm.

Differences in the character of the change in intensity of labeling of HN were as follows.

1. The maximum of the intensity of labeling was less marked and lower in value in the HN nuclei than in the control immediately after the mice had finished swimming.

2. The regression curve of label detected above the nuclei of these HN was more sloping.

3. The shape of the curve of accumulation of label in the cytoplasm of HN repeated that in the control, but in the region of higher values.

4. As the time from exposure to the factor inducing an increase in the number of HN increased, the shape of the regression curves of the label for HN and NN became more similar in its basic features.

The marked increase in number of HN in the motoneuron population of the spinal cord observed immediately after intensive physical exertion implies that the appearance of such cells is determined by functional considerations. This conclusion is confirmed by results showing differences between the dynamics of intensity of labeling of HN and NN.

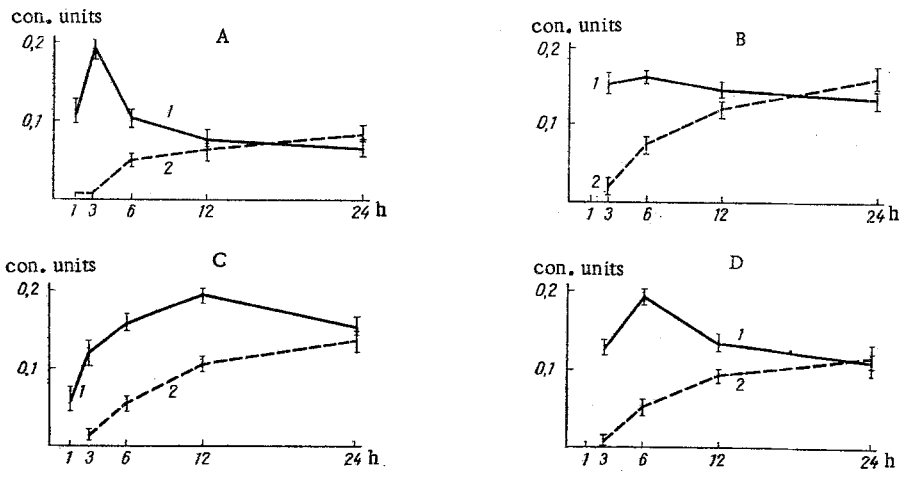

Fig. 2. Changes in intensity of labeling of nucleus (1) and cytoplasm (2) of NN and HN in ventro-lateral nucleus of mouse spinal cord at various times after injection of isotope: A) NN; B) HN of mice swimming for 4 h; C, D) HN of mice resting for 3 and 6 h respectively after swimming for 4 h. Abscissa, time after injection of uridine-H^3 (in h); ordinate, intensity of labeling (in conventional units).

Comparison of HN and NN of the rabbit brain showed reduced incorporation of uridine-H^3 into HN [8]. Some of the results of these experiments are in formal agreement with those cited above (Fig. 2B). Meanwhile these results cannot be reconciled with the conclusion drawn by the authors cited above that RNA synthesis is reduced in HN. The truth is that comparison of the character of the curves reflecting the dynamics of labeling the nucleus and cytoplasm of NN and HN at different times after injection of the isotope indicate that the latter liberate newly formed RNA more rapidly into the cytoplasm.

The results of some special investigations suggest that the appearance of HN in material for analysis is a fixation artifact [2, 5-7, 9]. The results now obtained contradict this hypothesis and suggest that fixation by emersion evidently simply detects and converts into a morphologically identifiable form those differences in the state of neurons forming a functionally united population that exist at the time of collection of the material.

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