PROLIFERATION OF PIRIFORM NEURONS OF THE RAT CEREBELLAR CORTEX DURING PRE- AND POSTNATAL DEVELOPMENT

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The method of histoautoradiography with thymidine-H^3 was used to establish the times of appearance of neuroblasts, later differentiating into piriform neurons of the cerebellar cortex, in the anlage of the cerebellum and the dynamics of proliferative activity of these cells was studied in the course of pre- and postnatal development of the experimental animals. It was concluded from the results that the last divisions of cells differentiating into the Purkinje cells of the cerebellar cortex cease by the 13th-15th day of embryonic development. Later no more incorporation of labeled precursor into the nuclear DNA of the differentiating piriform neurons takes place.

KEY WORDS: cerebellar Purkinje cells; DNA synthesis; embryonic and postnatal development.

The data on the possibility of incorporation of labeled thymidine into the DNA of the mature nerve cell are extremely contradictory. The results of histoautoradiographic studies [2, 5, 8] indicate that nerve cells lose their ability to replicate their DNA and to divide in the early period of neuronogenesis. Meanwhile, evidence has been obtained [1] that a particular labeled precursor can be incorporated into the DNA of the mature Purkinje cell.

The object of the present investigation was to determine the times of appearance of neuroblasts which will later differentiate into the piriform neurons of the cerebellar cortex in the anlage of the cerebellum, and then to study the dynamics of proliferative activity of these cells during pre- and postnatal development of the experimental animals.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 84 noninbred albino rats. In series I a single injection of thymidine-H^3 in a dose of 2 μCi/g body weight was given to 26 pregnant rats daily from the 10th to the 20th day of pregnancy and its incorporation and preservation were determined in the nuclear DNA of Purkinje cells in 30 rats aged 3, 5, 8, 10, 12, 14, 17, 21, and 30 days. The basis of this series of experiments was data in the literature [6, 7] according to which the appearance of neuroblasts from embryonic cells (medulloblasts, "matrix cells") can be established from the time of the last DNA synthesis, for in the course of this process the neuroblasts lose their ability to synthesize DNA. That is why the radioactive label, once incorporated by a neuroblast, persists there throughout the subsequent life of the nerve cell.

In the experiments of series II 28 rats of the same age groups received a single injection of thymidine-H^3 in a dose of 0.5 μCi/g body weight 1 h before sacrifice.

Since definite regional differences in the times of development are found in the cerebellar cortex [4], an area in the culmen (anterior vermis) bordering on the fissura prima was chosen for investigation.

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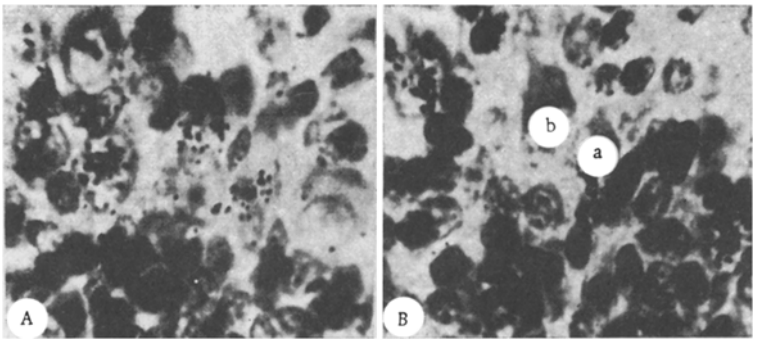

Fig. 1. Incorporation of thymidine-H^3 into DNA of piriform neurons of cerebellar cortex of rat aged 10 days (isotope injected on 14th day of embryonic development): A) concentrated label above nuclei of piriform neurons; B) labeled (a) and unlabeled (b) nuclei of piriform neurons. Hematoxylin–eosin, 630 ×.

The material was fixed in Carnoy's fluid and embedded in paraffin wax. Serial sagittal sections were cut to a thickness of 5–6 μ, coated with type M emulsion, and exposed at 4°C for 25 days. The index of labeled nuclei of the piriform neurons was determined in percent after 3000 cells had been counted from each animal. The results were subjected to statistical analysis. Differences were considered significant for which $P \leq 0.05$.

EXPERIMENTAL RESULTS

The appearance of neuroblasts subsequently differentiating into Purkinje cells in the anlage of the cerebellum was observed on the 13th day of embryonic development. The index of labeled nuclei of the piriform neurons in the cerebellar cortex of rats receiving thymidine-H^3 on the 13th day of intrauterine development was 83±3.2%. The intensity of incorporation of labeled precursor into DNA of the overwhelming majority of nuclei was low (5–8 grains of silver per nucleus), except for 9±1.3% of cells, above the nuclei of which the concentrated radioactive label was found (30–35 grains of silver per nucleus). This observation shows that a certain proportion of the precursors of the piriform neurons lose their ability to divide at this stage of development, and for that reason the radioactive label contained in them is not diluted.

By the 14th day of embryonic development neuroblasts were progressively leaving the population of DNA-synthesizing cells (Fig. 1), as shown by a sharp decrease in the index of labeled nuclei to 46±2.7%, and this was accompanied by a significant ($P \leq 0.05$) increase in the number of intensively labeled cells to 33±2.4%.

On the 15th days of embryonic development a further decrease in proliferative activity of the precursors of the piriform neurons was found, as shown by a decrease in the index of labeled nuclei to 10 ± 1.7%. The intensity of incorporation of thymidine-H^3 into the nuclei of all DNA-synthesizing cells was constantly high under these circumstances (30–35 grains of silver per nucleus) and no dilution of radioactive label took place in these cells during the subsequent stages of development; this indicates the metabolic stability of the DNA of the piriform neurons.

In animals receiving thymidine-H^3 after the 15th day of embryonic development, no incorporation of labeled precursor into the DNA of the cerebellar Purkinje cells took place.

The results thus showed that in the cerebellar anlage of albino rats, neuroblasts which subsequently differentiate into the piriform neurons of the cerebellar cortex arise from embryonic cells ("matrix cells") of the ependymal zone in the course of 13–15 days of embryonic development. This gives greater precision to data in the literature [3] according to which the last divisions of the precursors of these neurons cease by the 13th day of embryonic development.

Injection of thymidine-H^3 during postnatal development (experiments of series II) did not lead to incorporation of the labeled precursor into the DNA of the piriform neurons.

This investigation showed that the last divisions of cells from which the piriform neurons of the cerebellar cortex of albino rats differentiate cease by the 13th–15th day of embryonic development. Later, no further incorporation of labeled thymidine into the nuclear DNA of these neurons takes place.

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