LABELING OF DIFFERENTIATED RAT PURKINJE CELLS AFTER [^{3}H]THYMIDINE INJECTION

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Neuroblasts of the mammalian CNS cease to enter the mitotic cycle once the axon has begun to grow. Many attempts to reveal DNA replication in Purkinje cells after their migration to their definitive site in the cerebellum likewise have been unsuccessful. However, two observations require an explanation. First, single (usually fractions of 1%) Purkinje cells are found with twice (4c) the normal DNA content have been found. Second, Purkinje cells with a DNA content intermediate between di- and tetrapolid (H2c cells) are rather more numerous [6]. The excess of DNA in H2c cells is known to be localized in the nucleolar zone. If the increase in DNA takes place in Purkinje cells *in situ*, in the case of complete duplication of the genome uniform labeling of the nucleus can be detected after injection of [^{3}H]-thymidine into the animal. If DNA is reproduced in the nucleolar region alone, local labeling must be expected.

More than 10 years ago, after injection of [^{3}H]thymidine into rats aged 9-13 days, single intensively and uniformly labeled Purkinje cell nuclei were found in one 11-day-old animal [1]. The authors concerned did not draw attention to local labeling. Soon after, in a study of Purkinje cells from mice aged 7-12 days, saturated repeatedly with [^{3}H]thymidine, a few local inclusions were observed in the zone of the nucleoli, and no uniformly labeled nuclei were found [8]. It must be noted that the first information on hyperdiploidy of Purkinje cells was not published until a little later [2]. Cameron et al. [7] recently described uniform labeling of mouse Purkinje cell nuclei after injection of [^{3}H]thymidine. However, the background on the radioautograph in this investigation was of such intensity that not even comparing total label with that remaining after treatment with DNase could furnish a basis for reliable conclusions on DNA synthesis. On examining autoradiographs with a low background level we found both single uniformly labeled nuclei and also local nucleolar labeling in cerebellar Purkinje cells after repeated injections of labeled thymidine into 15-day-old rats [9].

The aim of this investigation was to study ability of Purkinje cells of animals of different ages to incorporate labeled thymidine and to make a more detailed study of the localization of the label.

EXPERIMENTAL METHOD

Two similar experiments were carried out with labeling by a single injection of [^{3}H]-thymidine (USSR origin, specific activity 4 Ci/mmole in the first experiment and 27 Ci/mmole in the second, dose 3 μCi/g in the first experiment and 5 μCi/g in the second). The cerebellum was isolated 1-1.5 h after intraperitoneal injection of the isotope. Pieces of cerebellum were fixed in formalin—alcohol—acetic acid (9:3:1) mixture and embedded in paraffin wax. Sections 5 μ thick were coated with type M emulsion (Photographic Chemical Research Institute) and exposed for 4 months in the first experiment and 2 months in the second. Radioautographs were stained with methyl green and pyronine or cresyl violet.

In the first experiment the cerebellum of rats aged 6, 8, 10, 12, and 15 days was studied; three animals were examined at each time and 2000 Purkinje cells studied in each rat. In the second experiment similar numbers of Purkinje cells were studied in the cerebellum of 14- and 30-day rats.

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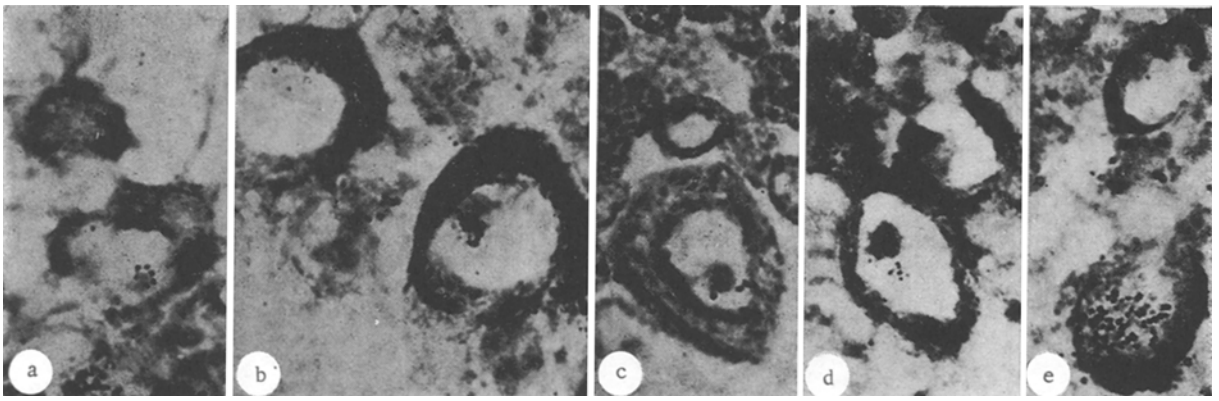

Fig. 1. Examples of different types of localization of label in Purkinje cells of rat cerebellum after a single injection of [^{3}H]thymidine. a-c) Local labeling in zone of nucleolus: a) rat aged 6 days, b) 15 days, c) 1 month; d) local labeling outside nucleolus, rat aged 15 days; e) intensively and uniformly labeled nucleus, animal aged 12 days, 1000 ×. Methyl green—pyronine.

If grains of silver were found in a Purkinje cell nucleus, they were counted and their localization noted: uniformly throughout the nucleus, only in the zone of the nucleolus, or, again, locally but outside the nucleolus. The background of the radioautograph was then determined. As near to the labeled cell as possible (in the cytoplasm of the same or of neighboring cells, in the molecular layer) the number of grains of silver was counted in 10 areas of the same size as the nucleus, or in about 20 areas of the same size as the nucleolus. Nonspecific labeling determined in this way proved to be so weak (in the first experiment 0.9 grain per nucleus, in the second 0.2 grain, on average) that it could be disregarded even in the case of labeling with two-three grains.

EXPERIMENTAL RESULTS

Examples of uniform and local labeling in Purkinje cell nuclei after a single injection of [^{3}H]thymidine into rats are given in Fig. 1. Local labeling was found either in the whole of the nucleolar zone (nucleolus and perinucleolar chromatin) or in a ring around the nucleolus, or on one side, or in similar groups but at a distance from the stained nucleolus.

Analysis of local labeling revealed several different values (Fig. 2). Most frequently three grains were found both in the nucleolus and outside it. This labeling differs considerably from the background. Whatever the case — threshold labeling with three grains or labeling with five grains (in the latter case the grains almost cover the nucleolus) — the conclusions are the same: 1) In all 15 animals in the first experiments and in six rats in the second, local intranuclear labeling was found; 2) the frequency of labeled nucleoli was usually higher than that of extranucleolar local labeling; 3) the frequency of nuclei with local labeling was appreciably higher in rats aged 12-30 days than in rats aged 6-10 days; 4) uniformly labeled nuclei were found exceptionally rarely: Intensive labeling (35-50 grains) was found in only three of 30,000 nuclei studied in the first experiment, and 20-25 grains were found in another three nuclei; all intensively labeled nuclei were found in two of the 15 rats studied. In one experiment only one intensively labeled nucleus was found in 12,000 nuclei studied in six rats.

It was pointed out above that, with the exceptionally low background level, as few as two grains in the nucleus in our radioautographs constituted a significant value. However, we by no means exclude the possibility of random occurrence of grains above the nucleus. That most of our data truly reflect incorporation of label is confirmed by the fact that all conclusions could be drawn both after "complete" counting of labeled nuclei and after substantial restriction of the counting level. The number of labeled nuclei in the latter case was sharply reduced, sometimes by several times, but locally and uniformly labeled nuclei still remained in all age groups studied. After restrictions of this kind it also became clear why intensive, uniform labeling in Purkinje cell nuclei was found previously in only one investigation: In order to detect such a nucleus (30-50 grains) it is necessary to study at least 10,000 Purkinje cells in about 10 animals.

We do not give absolute values for the labeling index. They differed considerably depending on the threshold level: minimally three or five grains in the case of local labeling.

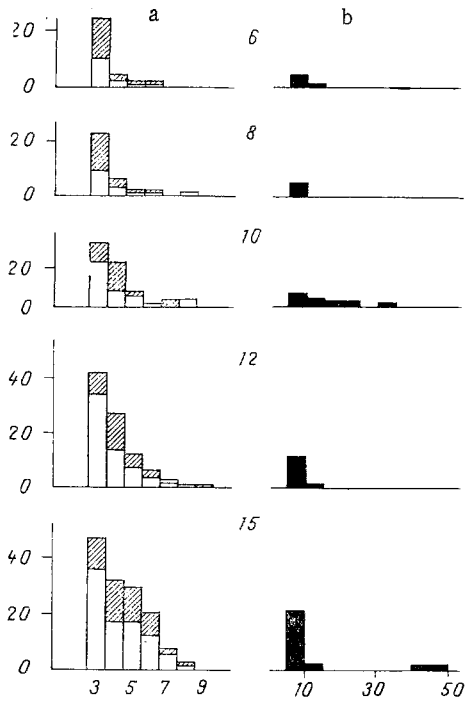

Fig. 2. Number of labeled Purkinje cell nuclei after a single injection of [^{3}H]thymidine into rats. a) Local labeling in zone of nucleolus (unshaded columns) and outside nucleolus (obliquely shaded columns); b) uniform labeling throughout nucleus. Numbers indicate age of rats (in days). Abscissa, intensity of labeling (number of grains of silver); ordinate, number of Purkinje cells.

The important point is that in every case the frequency of labeled Purkinje cells was about twice as high in rats aged 12-30 days as in those aged 6-10 days. Values for 12-day rats in the first experiment also coincided in every case with those for 14-day rats in the second experiment. These values did not differ from the labeling index of nuclei in 15-day rats after repeated injection of thymidine [9].

We thus found that Purkinje cells in the cerebellum of rats of different ages incorporate labeled thymidine. Local labeling of the nuclei also has been demonstrated for cerebral cortical neurons of adult rats [10], and in these cells also labeling was localized to the nucleolar zone in half of the cases.

To judge thymidine incorporation into DNA in such an unusual place as the nucleus of a differentiated neuron, repeated studies of labeled cells after treatment with DNase are necessary. Interpretation of local labeling is most difficult. Specific incorporation is supported by the discovery of the label in many animals, and evidence against a random photographic state is given by the fact that silver grains are found often in a fairly large group (five to eight grains) not only immediately above the nucleolus, but also outside the stained nucleolus. Such groups or, indeed, smaller groups (three to four grains) were never observed above the cytoplasm of Purkinje cells.

Labeling of nuclei may be a reflection of reparative DNA synthesis, of which nerve cells have been shown to be capable [3]. In the study of cortical neurons cited above the results obtained are discussed from this standpoint; the authors tentatively regard nucleolar labeling as proof of the greater sensitivity of nucleolar DNA to the action of injurious agents. However, incorporation of labeled thymidine may also be an indication of synthesis of "hyperdiploid" DNA, which has been found in Purkinje cell nuclei by cytophotometeric measurements. Local labeling of the nuclei is in good agreement with the view that excess of DNA in H2c cells is located in the perinuclear zone and that uniform, intensive labeling of nuclei may lead to complete duplication of the genome. This possibility is also supported by the frequency of discovery of locally and intensively labeled nuclei, which agrees sufficiently closely with the frequencies of H2c-nuclei of Purkinje cells. The increase in the number of Purkinje cells incorporating thymidine after the 12th day of postnatal development is in good

agreement with data obtained by Italian workers [5], who showed that the number of H2c Purkinje cells begins to increase between the 9th and 12th passages after birth.

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MORPHOLOGICAL DATA ON INCREASED FUNCTIONAL ACTIVITY OF MAST CELLS IN SPONTANEOUSLY HYPERTENSIVE RATS

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The system of mast cells (MC), capable of secreting biologically active substances (histamine, serotonin, heparin, etc.) which are deposited in their cytoplasm, is a highly important close-range peripheral regulator of microcirculatory permeability [1, 4]. Through their influence on the tissue blood flow, MC in chronic arterial hypertension ought evidently to reflect changes in circulatory conditions.

The object of this investigation was to study the general morphological characteristics of MC in spontaneously hypertensive rats (SHR) before the development of hypertension and during the period of stable elevation of the arterial pressure. The test object was the dura mater, which is a convenient object with which to study the state of the connective tissue MC system.

EXPERIMENTAL METHOD

Female Kyoto—Wistar rats aged 2-22 weeks, with blood pressure of 170-190 mm Hg were used. The control consisted of inbred female NKWR (Kyoto—Wistar) rats with blood pressure of 70-120 mm Hg (Table 1). Allowing for the circadian rhythm of MC activity in the dura [2] the rats were decapitated at 10-11 a.m. The dura was stretched on a slide, dried, and treated by the method in [3]. Preliminary tests showed that luminescence microscopy revealed all MC but does not allow their functional state (stage of the secretory process or type of MC) to be identified. To determine these parameters sections were stained with 0.1% toluidine blue solution and the number of MC in the different stages was counted and expressed as a percentage. The ratio between these values gave the secretory formula for the whole MC population of the dura. For these counts and determination of the total number of MC in the dura the whole area of the latter was "scanned" by means of a square ocular diaphragm, corresponding to one field of vision of the preparation with an area of 1 mm^2. The total number of MC counted in each specimen of dura ranged from 800 to 1000 in 500 fields of vision, with a ×25 objective. The following criteria were used for guidance when determining the secretory formula of MC. MC with a mean diameter of 10.8 ± 0.3 μ and with marked metachromasia, with readily distinguishable separate granules and outline of nucleus, and also very dark MC whose cytoplasm was

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