HISTOPATHOLOGY AND ENZYMOPATHOLOGY OF THE CEREBELLUM DURING CHEMICAL CARCINOGENESIS IN RATS

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Malignant transformation of cerebellar tissue in rats, induced by the chemical carcinogen 9,10-dimethyl-1,2-benzanthracene, is accompanied by a statistically significant decrease in the activity of monoamine oxidase and 5-hydroxytryptophan decarboxylase. The degree of enzyme activity is directly proportional to the intensity of the morphological changes in the cerebellar tissue during carcinogenesis.

The reactions and enzymes of nitrogen metabolism in tumors have increasingly become subjects for research [2, 4, 8] aimed at identifying the specific disturbances in metabolism of amino acids and other nitrogeneous compounds. In conjunction with the morphological and functional changes, this information could shed light on the nature of malignant change and the growth of malignant tumors, and in conjunction with biochemical data it could help the search for effective methods of cancer treatment. Particular attention has been paid to the metabolism of 5-hydroxytryptophan and other biogenic amines in primary tumors in man [4, 8], in transplanted tumors [5, 6], and in tumors induced by chemical carcinogens [11]. These investigations have demonstrated either the total loss of the enzyme system or a marked decrease in activity of the enzymes catalyzing the decarboxylation of 5-hydroxytryptophan and oxidative deamination of several biogenic monoamines.

With these circumstances, and also the important role of biogenic amines in functions of the central nervous system [7, 12] in mind, it was decided to investigate morphological changes and the activity of two enzymes – 5-hydroxytryptophan decarboxylase (5-HTD) and monoamine oxidase (MAO) – in the rat cerebellum during chemical carcinogenesis.*

EXPERIMENTAL METHOD

In experiments on noninbred albino rats weighting 60-70 g, tablets of chemically pure 9, 10-dimethyl-1,2-benzanthracene (DMBA) weighing 1-2 mg were implanted in the region of the nuclei of the right cerebellar hemisphere. A tablet of paraffin wax of the same weight was implanted into the same region of the control animals. The method of preparing and implanting the tablets is described elsewhere [9]. Observations were kept on the animals for almost 1 year and they were sacrificed weekly until gliomas developed (240 days). Reactive changes appearing in the different stages of the experiment in all the structural elements of the cerebellum were investigated histologically.

*It is worth noting that the actual process of carcinogenesis in the cerebellum has received very little study, yet many brain tumors in children occur in the cerebellum.

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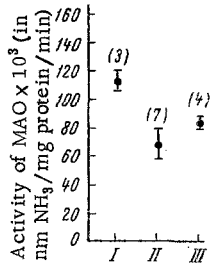

Fig. 1

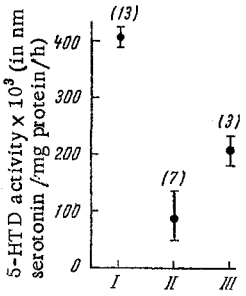

Fig. 2

Fig. 1. MAO activity in rat cerebellar homogenates under different experimental conditions: I) control (164-210 days after implantation of paraffin wax into cerebellum); II) primary tumor (strain 101/12); III) tumor induced by injection of DMBA (164-210 days after implantation). Results shown as mean values ± mean errors of arithmetical mean; number of experiments in parentheses.

Fig. 2. 5-HTD activity in cerebellar homogenate under different experimental conditions: III) tumor induced by implantation of DMBA (154-182 days after after implantation). Remainder of legend as in Fig. 1.

Monoamine oxidase activity was determined by a highly sensitive colorimetric method based on the use of P-nitrophenylethylamine as substrate for MOA [14], which gives a colored product on oxidative deamination [3]. The composition of the samples, experimental conditions, and method of calculating the results are described elsewhere [6].

The activity of 5-MTD was determined by the methods of Buzard [10] and Udenfriend [13], based on the spectrophotometric determination of the quantity of serotonin formed by decarboxylation of 5-hydroxytryptophan. The incubation mixture contained 0.5 ml of a 1:3 cerebellar tissue homogenate, 0.3 ml of 0.1 M phosphate buffer, 0.5 ml 5-hydroxytryptophan solution (10 μmoles), 0.5 ml phosphopyridoxal (25 μg), 0.1 ml octyl alcohol (MAO inhibitor), and distilled water to 3.5 ml. The control contained the same components of the mixture with boiled tissue homogenate. The samples were saturated with nitrogen and incubated in a Warburg's apparatus at 37°C for 1 h with constant shaking. After incubation, the proteins were precipitated by an equal volume of 10% TCA and removed by centrifugation. The supernatant was treated with sodium carbonate to pH 10.0, extracted 3 times in butanol from the alkaline solution, and the extinction of the formate solution containing serotonin was then determined in formate buffer, pH 4.0 with the SF-4A instrument at 275 nm.

Enzyme activity was expressed in μmoles serototin/mg protein per h incubation.

Protein in the samples was determined by Lowry's method, and the results were checked by Kjeldahl's method. Good agreement was obtained between the results in all cases.

EXPERIMENTAL RESULTS

Histological examination of the cerebellar tissues during chemical carcinogenesis * showed distinctive precancerous changes mainly affecting the connective-tissue structures, initially in the cells of the capsule surrounding the DMBA tablet, but later in the walls of the vessels close to the carcinogen. Against this background free gliomas developed: by the 28th day there was hyperplasia of the astrocytes, by the 49th day proliferation of glial elements, and by the 80th day foci of atypical proliferation identified as pregliomas. The process ended with the formation of a primary cerebellar gliobastoma on the 98th-105th day. Primary glioblastomas developed in 87.1% of cases after a mean latent period of 246 ± 37.5 days in the animals which survived.

The biochemical tests also were carried out at intervals during carcinogenesis, but attention was concentrated on the final stages of malignant transformation.

In the experiments of series I, MAO activity was studied in the cerebellar tissue. The results are given in Fig. 1. Appearance of a cerebellar tumor as the results of the action of the carcinogen DMBA was accompanied by a statistically significant decrease in MAO activity (Fig. 1, III) compared with its activity in the cerebellan tissue of control animals undergoing the mock operation.

In the next series of experiments, the 5-HTD activity was studied. 5-HTD activity fell sharply until the 154th-182nd days of carcinogenesis (Fig. 2), when the level of the enzyme was 2.3 times lower than its level in the control tissue. The activity of both enzymes in transplanted strains of cerebellar tumors induced by DMBA also was at a much lower level (Figs. 1 and 2) than in the primary induced tumor, probably because of significant changes in the primary strain of glioblastoma in the course of progression.

*The results of these changes have been described fully in a previous publication [1].

The results indicate that during malignant transformation of cerebellar tissue induced by the chemical carcinogen considerable disturbances arise in the metabolism of 5-hydroxytryptophan, the immediate precursor of serotonin, and also in the oxidative deamination of biogenic amines which perform a number of important functions in the body in general and in the central nervous system in particular. The biochemical changes in MAO and 5-HTD activity usually appear concurrently with the foci of atypical cell proliferation.

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