CYCLIC NUCLEOTIDES OF HUMAN AND ANIMAL GLIAL BRAIN TUMORS

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Evidence of a connection between tumor growth and the cyclic nucleotide level has been published [5, 12]. The concentration of cyclic AMP as a rule is low in tumor tissues of different origin [6, 7]. An artificial increase in the concentration of this compound inhibits tumor growth [4, 9, 11]. Another cyclic nucleotide, 3,5-guanosine monophosphate (cyclic GMP), on the other hand, acts as a trigger of cell proliferation [8].

In view of the considerable heterogeneity of brain tissue, most studies of cyclic nucleotide metabolism in the brain have been carried out on tissue culture. The results of these investigations show that the principles governing changes in the cyclic AMP concentration in the brain tissue are the same as in other tissues: During malignant change the cyclic AMP level in the brain cells falls, and after exogenous addition of cyclic AMP derivatives or phosphodiesterase inhibitors to the culture malignant growth is retarded and the cell morphology is changed: more mature forms are produced [10].

The data on cyclic nucleotide metabolism in human brain tumors are extremely few, yet these tumors account for about 40% of cerebral neoplasms. The study of changes in the cyclic nucleotide level in these tumors thus remains an urgent problem, the solution of which may open up new approaches to the chemotherapy of glial tumors. The choice of an adequate experimental model is essential for this purpose.

The aim of this investigation was to compare the cyclic AMP and cyclic GMP concentrations in human and animal glial tumors.

TABLE 1. Concentration of Cyclic Nucleotides in Human Brain (in picomoles/mg protein)

Tissue	Cyclic AMP	Cyclic GMP	Cyclic AMP/ Cyclic GMP
Gray matter of cerebral cortex	39,4	8,1	4,9
The same	35,4	8,4	4,2
" "	31,2	6,5	5,0
" "	48,5	11,2	4,3
Cerebellar cortex	18,0	3,9	4,6
" "	48,5	11,2	4,3
M	33,2	7,4	4,5
$\pm m$	4,3	1,0	0,04
$\pm\sigma$	10,5	2,4	0,11

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TABLE 2. Concentrations of Cyclic Nucleotides (in picomoles/mg protein) in Human Glial Tumors

Tumor	Cyclic AMP	Cyclic GMP	Cyclic AMP/ Cyclic GMP
Astrocytoma	9,9	3,6	2,8
Astrocytoma with signs of dedifferentiation	11,3	3,3	3,4
Fibrillary astrocytoma	3,9	1,6	2,4
Astrocytoma	32,2	13,7	2,4
Fibrillary astrocytoma	1,5	0,6	2,7
Oligoastrocytoma	21,8	6,4	3,4
Fibrillary astrocytoma	17,7	5,6	3,2
Astrocytoma	7,8	2,6	3,0
Dedifferentiated astrocytoma	18,8	8,2	2,3
Glioblastoma multiforme	1,4	0,7	2,0
Glioblastoma multiforme	10,2	3,2	3,1
M	11,6	4,5	2,8
$\pm m$	2,6	1,2	0,04
$\pm\sigma$	8,5	3,8	0,14
t	4,3	1,9	27,3
P	<0,001	0,05	0,001

Legend. Values of significance obtained by comparing corresponding data for brain tissue and glial tumors.

EXPERIMENTAL METHOD

Tissue of human glial tumors and brain was obtained during neurosurgical operations and quickly transferred to liquid nitrogen. The concentration of cyclic nucleotides was determined by a radioisotope method using kits from the Radiochemical Centre, Amersham, England.

Two transplantable strains of experimental gliomas of laboratory animals (Laboratory of Histopathology of the Central Nervous System, Institute of Human Morphology, Academy of Medical Sciences of the USSR), induced by local injection of chemical carcinogens into the brain [2, 3], also were used. A dedifferentiated rabbit astrocytoma, strain No. 103, was obtained in October, 1961, after introduction of a methylcholanthrene pellet into a rabbit's brain. By the time of the investigation the tumor had completed 65-67 intracerebral passages. The duration of survival of an animal with a transplanted tumor was 30-45 days. The rabbits were killed on the 35th-37th day after transplantation of the tumor.

A protoplasmic astrocytoma with signs of dedifferentiation was found in a rat at the site of introduction of a pellet containing 9,10-dimethyl-1,2-benzanthracene into the cerebellum in February, 1967. Material from the 29th and 36–39th passages was used in this investigation. The survival period of the animals with a tumor was 10-12 days. Passage of the tumor was carried out by transplantation of fragments of tumor into the brain of a young animal by means of a needle and trocar. Passage alternated with preservation of the material under deep refrigeration (at −78°C). Rabbits were lightly anesthetized with ether, the skull was trephined, and pieces of tumor tissue, brain tissue from the affected hemisphere, and tissue from the intact hemisphere were removed, thereby copying the conditions under which material was obtained from the human brain. The rats were decapitated and the tissues for testing were removed from them as quickly as possible (30 sec).

EXPERIMENTAL RESULTS

The results of investigations of cyclic nucleotides in the human brain and gliomas are given in Tables 1 and 2.

As the results show, the cyclic AMP concentration in the tissues of tumors of the astrocyte series was statistically significantly lower than in brain tissue. The marked fluctuations in cyclic AMP level in both brain and tumor tissue will be noted. This may be due to several factors, including the patient's age, differences in illumination of the operative field at the time of taking the material, and so on [1, 13]. Statistically significant differences could not be found in the cyclic AMP concentration depending on the degree of anaplasia of the tumor, although the possibility cannot be ruled out that this was because of an insufficient amount of material.

TABLE 3. Concentrations of Cyclic Nucleotides (in picomoles/mg protein) in Normal Brain and Glial Tumors of Rabbits

Animal No.	Tumor			Affected hemisphere			Intact hemisphere		
	cyclic AMP	cyclic GMP	cyclicAMP/ cyclic GMP	cyclic AMP	cyclic GMP	cyclicAMP/ cyclic GMP	cyclic AMP	cyclic GMP	cyclic AMP/ cyclic GMP
1	5,4	2,2	2,4	18,2	6,4	2,8	15,6	5,3	2,9
2	8,9	3,6	2,5	24,6	8,4	2,9	19,4	5,1	3,8
3	5,6	2,8	2,0	21,8	7,8	2,8	18,1	7,1	2,5
4	8,5	3,4	2,5	20,4	7,8	2,6	17,6	5,9	2,9
5	8,2	3,6	2,3	18,6	4,8	3,9	15,4	4,4	3,5
M	7,3	3,1	2,3	20,7	7,0	3,0	17,2	5,5	3,1
$\pm m$	1,7	0,6	0,2	2,6	1,3	0,5	1,7	1,0	0,4
$\pm\sigma$	0,75	0,27	0,09	1,19	0,57	0,22	0,76	0,45	0,17
t	9,58	4,49	17,2	0,07	2,79	4,72	2,54	0,72	5,05
P	<0,001	0,001	0,001	0,5	0,01	0,001	0,02	0,2	0,001

Legend. Values of significance obtained by comparison with corresponding data for control animals.

The cyclic GMP concentration in the tumors also was lower than in brain tissue ($P < 0.05$), but the difference was smaller than for cyclic AMP. Consequently, the cyclic AMP/cyclic GMP was reduced. It was shown for the first time that this ratio is the most stable indicator of cyclic nucleotide metabolism both in normal tissue (4.5 ± 0.45) and in glial tumors (2.8 ± 0.04) of the human brain.

Similar results were obtained during a study of cyclic nucleotides and the ratio between them in rabbits with a dedifferentiated astrocytoma (Table 3).

As Table 3 shows, the concentrations of cyclic AMP and cyclic GMP in glial tumors of rabbits also were lower ($P < 0.001$) than in the brain tissue of control animals, where the mean cyclic AMP concentration was 20.8 picomoles/mg protein and that of cyclic GMP, 5.1 picomoles/mg protein. Here also, however, the decrease in the cyclic AMP level was greater than that in the cyclic GMP concentration, so that, just as in human glial tumors, the cyclic AMP/cyclic GMP ratio was lowered (2.3 ± 0.09 compared with 4.9 ± 0.6 in normal brain tissue; $P < 0.001$).

The cyclic AMP and cyclic GMP levels in the affected and intact hemispheres did not differ significantly from their concentrations in the brain of the control animals. However, the cyclic AMP/cyclic GMP ratio was lowered in these tissues also. The results thus indicate a disturbance of cyclic nucleotide metabolism not only in the affected hemisphere, but also in the opposite hemisphere. This means that the ability of transplanted brain tumors to survive can be revealed by punch biopsy of any part of the brain, determining the cyclic nucleotide concentrations in the sample, and calculating the ratio between them.

During the study of cyclic nucleotides in the rat brain and glial tumors higher levels of both cyclic AMP and cyclic GMP were discovered. However, in animals of this species also, a decrease in the concentrations of both cyclic nucleotides in the tumors and in the ratio between them was observed (11.7 in normal tissue, 5.3 in tumors).

When the cyclic nucleotide levels in human and animal glial tumors are compared it will be seen that they are more stable in the latter, evidently because of the histological homogeneity of transplanted tumors compared with primary human gliomas. The cyclic AMP/cyclic GMP ratio was the most stable and characteristic indicator for the group of tumors studied (it is significantly lower in tumor tissue both in man and in animals).

The rat brain tumor differed from the rabbit and human gliomas by a greater degree than the two latter tumors differed from each other. The higher level of both nucleotides and the higher ratio between them are characteristic features of rat brain tumors. Accordingly, it can be recommended that rabbits are used as experimental models for brain tumors when a study of their cyclic nucleotides is contemplated.

Despite certain differences in the concentrations of cyclic nucleotides and the ratio between them, no fundamental differences were found between these indicators in the tissues of human and animal tumors.

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COMPARATIVE STUDY OF DNA BREAKS IN HUMAN LEUKEMIC AND NORMAL BLOOD LEUKOCYTES

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The action of ionizing radiation, carcinogens, and oncogenic viruses is known to be accompanied by fragmentation of the DNA of the normal cell. Diseases due to genetic defects of the DNA repair system, such as xeroderma pigmentosum, are characterized by an increase in the frequency of tumor development. Lymphocytes of patients with primary and secondary aplastic anemia are known to have more single-stranded DNA breaks than healthy human lymphocytes [5].

With these facts in mind, the level of single-strand DNA breaks was compared in peripheral blood leukocytes from healthy subjects and from patients with chronic lymphatic and myeloid leukemia.

EXPERIMENTAL METHOD

Lymphocytes and granulocytes from the peripheral blood of healthy donors served as the control. Lymphocytes were isolated by Böyum's method [3] using a mixture of Ficoll and Urotrast with a specific gravity of 1.08. To isolate granulocytes, the Ficoll–Urotrast mixture was layered above an equal volume of polyvinol (mol. wt. 20,000) with a specific gravity of 1.12. The isolated lymphocytes or granulocytes were washed three times with cold physiological saline and the cells were separated by centrifugation for 10 min at 400 g. Single-strand DNA breaks were determined by the method of Fedorov and Borisov [1, 2]. A cell suspension (2×10^6) was lysed by addition of 4 volumes of a solution of the following composition: 2.5M NaCl, 0.025M EDTA-Na_3, 5 g/liter Na laurylsarcosinate; pH 9.0. The lysate was passed through a BS filter (from Millipore) by means of a peristaltic pump and washed with 5 ml 0.002M EDTA solution, pH 10.0. DNA was eluted with 0.1M NaOH at 40°C at the rate of 0.1 ml/min and four fractions each of 2.5 ml were collected;

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