DYNAMICS OF THE AMMONIA–GLUTAMINE SYSTEM OF THE BRAIN IN RATS AFTER SURGICAL REMOVAL OR CHEMOTHERAPY OF TUMORS

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Changes in the metabolism of the brain caused by growth of a tumor (sarcoma C-45) disappear after removal of the tumor. Regression of the sarcoma under the influence of thio-TEPA takes place parallel with changes in metabolism in the rat brain. In the early periods after treatment, processes in the rat brain associated with ammonia formation are activated, and after 1 month the components of the ammonia–glutamine system of the brain are restored to normal.

The object of this investigation was to study the state of the ammonia–glutamate–glutamine system in the brain of rats with sarcoma C-45 following treatment of the tumor by surgery and chemotherapy.

EXPERIMENTAL METHOD

Male rats weighing 100-150 g were inoculated subcutaneously in the thigh with a suspension of sarcoma C-45 cells. The transplanted cells developed well for 14 days, and the tumor was then removed surgically. The wound was sutured and the animals kept under normal conditions in the vivarium. The brain was examined 7-10 days, 3 weeks, and 3 months after operation. Another group of rats started to receive thio-TEPA treatment when the subcutaneously inoculated tumor had reached the size of a pea (about 1 g). The antitumor compound was dissolved in physiological saline and injected daily into the animals in one therapeutic dose (2 mg/kg) intraperitoneally. The course for the rats consisted of 14 injections (28 mg/kg). Investigations of ammonia, glutamic acid, and glutamine in the brain were carried out on the day after the course of treatment ended, and 10 days,and 1 month later. Healthy rats and rats receiving thio-TEPA by the same scheme served as controls. The rats were frozen in liquid air and all investigations were carried out on frozen brain tissue. Ammonia and glutamine were determined by Silakova's method [4], and glutamic acid by electrophoresis on paper by Dose's method [5].

EXPERIMENTAL RESULTS

The state of the animals returned to normal a few days after the operation. The concentrations of ammonia, glutamine, and glutamic acid in the brain of the control animals were 0.48 ± 0.06, 4.7 ± 0.37, and 148.5 ± 14.5 mg%, respectively, in agreement with published data [1,2]. When the ammonia concentration in the brain was investigated 1 week after the operation, a high concentration of ammonia and glutamine was found (Table 1). A similar result was found previously when the brain of rats was investigated during the 3rd-4th week of growth of sarcoma C-45 [3].

The results obtained indicate either persistence of changes in the chemical properties of the brain caused by growth of the tumor or an effect of the operation. It may be considered that systems participating in intensive ammonia formation during growth of the tumor still remained disturbed at this period. Later (toward 3 months), however, the metabolism of the ammonia–glutamine system in the brain returned completely to normal. The relative constancy of the glutamic acid concentration suggests that ammonia is removed not by combination with it, but in some other way. The brain metabolism, when modified by growth of a malignant tumor, thus returns to normal after removal of the tumor.

When thio-TEPA was given to rats with an inoculated sarcoma, at first arrest of growth of the tumor was observed, and this was followed by a gradual decrease in its size or its complete disappearance. At the

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TABLE 1. Concentration of Ammonia, Glutamine, and Glutamic Acid in Brain of Rats after Removal of Tumor (in mg%)

Number of animals	Time of investigation	Ammonia		Glutamine		Glutamic acid	
		$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
10	7—10 days	1,3±0,1	<0,001	6,7±0,4	<0,001	161,5±7,7	<0,5
7	1 month	0,66±0,2	>0,5	6,3±0,7	>0,1	129,4±10,5	<0,2
7	3 months	0,56±0,01	>0,2	5,2±0,3	<0,2	126,3±4,6	<0,1

TABLE 2. Concentrations of Ammonia, Glutamine, and Glutamic Acid in the Brain after End of a Course of Thio-TEPA Treatment (in mg%)

Group of animals	No. of animals	Time of investigation (in days)	Ammonia		Glutamine		Glutamic acid	
			$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
Control	20		0,48±0,06	—	4,69±0,37	—	148,5±14,5	—
Healthy rats	5	1	0,98±0,12	<0,001	6,93±0,6	<0,01	147,7±4,8	<0,5
receiving	5	10	1,22±0,11	<0,001	7,11±0,5	<0,001	119,1±6,2	<0,1
compound	8	30	0,42±0,05	>0,5	4,97±0,5	>0,5	143,9±8,6	<0,5
Rats with tumor	9	1	1,09±0,14	<0,001	3,32±0,33	<0,01	121,3±7,6	<0,5
receiving	10	10	1,32±0,11	<0,001	5,84±0,12	<0,01	127,9±10,5	<0,2
compound	10	30	0,48±0,12	—	5,43±0,37	<0,2	126,9±6,5	<0,2

site of the tumor nodule, scar tissue was formed. In animals with an inoculated tumor but not treated with thio-TEPA, the tumor grew rapidly in size and the animals died by the 28th-32nd day.

Thio-TEPA treatment caused changes in the dynamics of nitrogen metabolism of the brain of the control and experimental animals (Table 2).

A sharp increase in the concentration of ammonia and glutamine was found in the brain of the healthy animals after a course of treatment. The glutamic acid level remained unchanged. It can be concluded that the antitumor compound acts as a stimulator of the nervous system. The increased concentration of ammonia evidently indicated, not a disturbance of nitrogen metabolism, as has been considered, but an adaptive response of the body to the action of thio-TEPA at that particular moment. The concentrations of the components of the ammonia–glutamine system in the brain of the healthy animals returned to normal only after 1 month.

An increase in the ammonia concentration was also found in the brain of animals with tumors 1 day after the end of the course of thio- TEPA injections, and this increase was greater than that during growth of sarcoma C-45 [3]. The ammonia concentration in the brain was increased 10 days after the end of treatment. Evidently the effect of the tumor on the ammonia concentration in the brain was reinforced by the action of thio-TEPA. This substance activates brain metabolism of healthy animals and of animals with tumors, influencing growth of the tumor. The results obtained suggest that in animals with tumors glutamine is a source of ammonia only in the early period after treatment: its concentration in the brain was sharply reduced (3.32 mg%), although later its concentration increased. The relatively constant concentration of glutamic acid in the brain at this period indicates the many ways in which it participates in metabolism. One month after treatment the concentration of the components of the ammonia–glutamine system had returned to normal.

It may thus be concluded that thio-TEPA not only has a direct specific action on the tumor, causing its absorption, but also an indirect action on the metabolism and reactivity of the body, which may play a role in the therapeutic effect. The results obtained indicate one of the ways in which the brain participates in the metabolism of the tumor-bearing animal.

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