CHANGES IN ARGINASE ACTIVITY DURING GROWTH OF A NEURINOMA

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Arginase is a cytostatic enzyme, for by breaking down arginine it inhibits protein synthesis and disturbs transition of cells prepared for division from the G_1 phase into the S phase of the mitotic cycle [1, 7]. Investigations have shown [5, 9] that growth of a cell culture is inhibited by arginase and activated by the addition of arginine. Activity of the enzyme in tumor cells varies within wide limits. In some cases correlation is observed between activity of the enzyme and the rate of growth of certain tumors [5, 7, 9]. Data on changes in arginase activity in brain tumors could not be found in the literature.

The aim of this investigation was to study arginase activity in the rat brain and other tissues at various stages of growth of a neurinoma of the gasserian ganglion of the trigeminal nerve.

EXPERIMENTAL METHOD

Female rats of the Wag line were used. In the region of the right hemisphere tissue of a transplantable neurinoma of the gasserian ganglion of the trigeminal nerve (strain 10-13-3; 9th passage) was transplanted into the rats. For this purpose, the skull was trephined with a dental drill in the region of the right hemispheres and minced tumor tissue, in a volume of 20 μl, was injected through the hole thus made into the brain tissue to a depth of 3-5 mm.

Growth of the neoplasms in the brain tissue was verified morphologically after decapitation, parallel with biochemical testing.

A mock operation included all stages of injury to the right hemisphere (trephining, puncture with a needle) but without injecting the tumor into the brain tissue. The left hemisphere remained intact in all cases.

To determine arginase activity homogenates of brain, skin, liver, and kidney tissue were prepared in 0.01 M Tris-HCl buffer, pH 7.2, containing 25 mM $MnCl_2$, and they were centrifuged for 20 min at 1500 g. Arginase activity in the supernatant was determined by the method described previously [3] and protein by Lowry's method [6].

EXPERIMENTAL RESULTS

During growth of the neurinoma, arginase activity changed in the brain and in the skin of the head and thigh, whereas a mock passage had no significant effect on activity of the enzyme in these tissues. As Fig. 1a shows, after transplantation of the tumor arginase activity rose significantly in both hemispheres as early as on the 1st-2nd day compared with its level after mock passage. On the 4th day arginase activity in the affected hemisphere was six times higher than in the control, but by the 7th-8th day, arginase activity fell to normal. In the intact hemisphere during this period the trend was similar, but the increase in enzyme activity was smaller. The neoplasm could be differentiated visually by the 9th day after injection of the graft, but not until the 16th day was arginase activity increased many times over in the tumor tissue. Meanwhile in the intact and affected hemispheres arginase activity remained within normal limits. Arginase activity also increased

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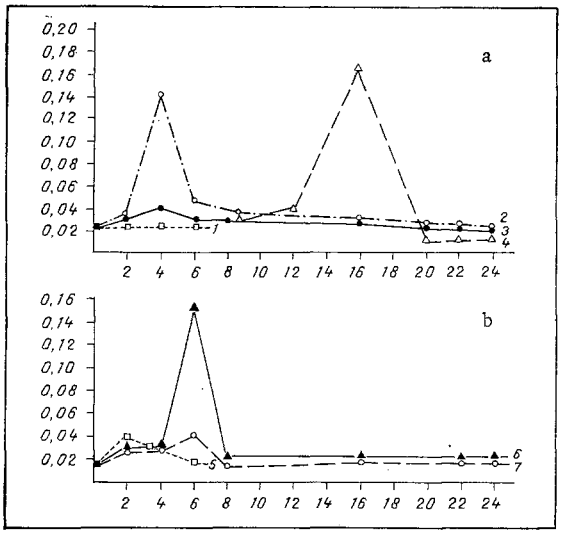

Fig. 1. Arginase activity during growth of transplantable neurinoma. Abscissa, time (in days); ordinate, enzyme activity (in relative units). a) Brain and tumor tissue: 1) control (mock operation), 2) affected hemisphere, 3) intact hemisphere, 4) tumor; b) skin, 5) mock operation, 6) skin of head, 7) skin of thigh.

in the skin of the head and thigh. The most marked changes were observed in arginase activity in the skin of the head (Fig. 1b), where two peaks were recorded: on the 1st-2nd day after the operation and also on the 6th day after implantation. In the latter case arginase activity in the skin of the head was 9-10 times higher than normally. Meanwhile in other tissues (kidneys, liver) activity of the enzyme was unchanged during growth of the neurinoma.

The role of arginase in the metabolism of nerve tissue has not been fully elucidated. The key role of the enzyme in synthesis of several biologically active substances and, in particular, of glutamic and γ-aminobutyric acids, of monosubstituted guanidines, and also of proline and polyamines have been demonstrated [7]. Accordingly interest in the study of the role of this enzyme in lesions of the nervous system has increased.

The causes of the changes in arginase activity discovered in the rat brain during growth of the neurinoma still remain unknown, but they have nothing to do with mechanical trauma. The marked increase in arginase activity in the affected hemisphere may be one manifestation of the protective reaction of glial tissue of introduction of the tumor cells at the molecular level. A similar effect has been described morphologically in the literature [4]. This hypothesis is confirmed by the fact that arginase activity was uniformly increased in the tissues of both the affected and the intact hemisphere. Meanwhile, morphological investigation of the brain tissue revealed the presence of only single tumor cells in the region of the wound canal, and considerable growth of the neurinoma evidently was not yet observed at this time. This may perhaps be attributable to an increase in arginase activity and a corresponding decrease in the reserves of arginine, an essential amino acid for fast-growing tissue.

Although the half-life of arginase from different sources varies from 24 to 96 h [8], in this case as early as 48 h after the maximal rise, arginase activity in the affected hemisphere fell by several times, whereas in the healthy hemisphere it returned to normal. This indicates that the change in arginase activity took place, not through increased synthesis of the enzyme de novo, but through changes in the ratio of arginase activator to arginase inhibitor, with an increase in the fraction of activator. The increase in arginase activity in the skin of the head and thigh on the 6th day after tumor transplantation (against the background of a decrease in arginase activity in nerve tissue) points to possible release and blood-borne spread of an activator from the brain into other tissues. The writers discovered previously [2] a similarity of the arginase of the skin with one of the arginase isozymes in the brain as regards charge and action of certain inhibitors. Conversely, no changes were recorded in total arginase activity of the liver and kidneys, in agreement with data in the literature on heterogeneity of the many different forms of arginase, isolated from different tissues, and with different pathways of control of their activity. It must be pointed out that the effect of activation of arginase in

brain and skin tissue was revealed for the first time during the latent period after injection of neurinoma cells. It can be tentatively suggested that after removal of the activator and a reduction in activity of the enzyme, favorable conditions were created for growth of the graft. In fact, on the 8th-9th day the tumor nodule in the brain tissue could be detected morphologically and visually. The increase in arginase activity on the 16th day in the neoplasm was an independent phenomenon, for no change in arginase activity could be found in other parts of the brain or skin. This fact can evidently be explained by the characteristics of metabolism of this tumor. A similar change in arginine activity has been observed in hepatomas induced by azo compounds, and associated with the need for rapidly growing tissues to synthesize additional polyamines [7]. The possibility cannot be ruled out that the transient increase in arginase activity in the neurinoma was due to the same cause. The results of these experiments, indicating sporadic changes in arginase activity during the period of growth of the neurinoma, are in agreement with the concept of nonuniformity of tumor growth.

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DIFFERENCES IN MICROSOMAL MONO-OXYGENASE ACTIVITY IN CELLS OF ASCITES AND SOLID TUMORS

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A serious obstacle to the detailed study of microsomal mono-oxygenases (MMO) in tumor cells, especially during long-term passage, is their extremely low activity. Nevertheless, this is a problem of great practical importance, because MMO metabolize not only important endogenous substrates (hormones, cholesterol), but also most hydrophobic xenobiotics, for they activate and detoxicate many antitumor agents. The most important aspect of the problem is the study of factors determining MMO activity. In normal cells activity of this very important system of adaptive enzymes is determined not only by the character of the substrates to be metabolized (inducers, inhibitors), and by sex, age, linear, and tissue differences [3, 12], but also by the position of the cell in the complex structure of the organ [2].

The aim of this investigation was to determine how activity of aryl hydroxylase (AH), one of the most important characteristics of MMO, depends on the type of organization of the tumor cells. In particular, activity and inducibility of AH were compared in ascites and solid forms of three transplantable tumors.

EXPERIMENTAL METHOD

Male mice weighing 23-25 g were used, eight to 10 animals in each experimental group. Sarcoma MCh-11 [8] was transplanted into C57BL/6j mice, hepatoma 22a [15] into C3HS mice, and Ehrlich's tumor (from the

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