ATPase in human brain tumors

Edward R. Laws, Jr., M.D., and John S. O'Connor, M.D.
Division of Neurological Surgery, The Johns Hopkins Hospital, Baltimore, Maryland

The energy-dependent membrane transport ATPases have been quantitatively determined in 59 human brain tumors and control cerebral cortex. The values for total ATPase were significantly decreased in the 11 types of brain tumors tested, while in the glioma group there was a consistent further decrease in ATPase with increasingly malignant types. The findings suggest that a deficiency in ATPase is a characteristic of neoplasia in the central nervous system.

The energy-dependent membrane transport enzymes assayed as ATPase are responsible for the continuing supply of many of the substrates and ions necessary for brain function. The sodium-potassium-activated ATPase (Na⁺-K⁺-ATPase) described by Skou[10-19] is essential to the maintenance of cationic balance and osmotic stability in cerebral tissue,[10,19] and the optimum means of stimulation and inhibition of the enzyme have been investigated in detail.[14,7-9,20]


The basis for the work reported here was suggested by the findings of our studies in the slide histochemistry of brain tumors.[13,22] When appropriate stains[23] for the demonstration of ATPase in brain tumors were examined it was apparent that blood ves:el complexes with their surrounding glia often showed variable activity and that differences were related both to tumor type, and, in the case of gliomas, to the degree of malignancy. It was also evident that in most tumors, and even in normal brain, the distribution of the activity of the enzymes varied widely from one part of a section to another. These considerations and the limitations of histochemical techniques that use stains made it necessary to turn to quantitative techniques applied to small bits of carefully dissected and strictly controlled tissue.

The total activity of ATPase, stimulated by optimal concentrations of sodium and potassium, has been quantitatively determined in 59 human brain tumors. Values for basic Mg⁺⁺-ATPase and ouabain-inhibited ATPase were also determined in the majority of the tumors.

Materials and Methods
All pathological tissue was obtained directly at surgery. Blood was removed by a brief rinse in isotonic saline, and the tissue...
TABLE 1
Incubation medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Total ATPase (mM)</th>
<th>Mg(^{++})-ATPase (mM)</th>
<th>Ouabain-inhibited ATPase (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris buffer*</td>
<td>92</td>
<td>150</td>
<td>91</td>
</tr>
<tr>
<td>Tris-ATP</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Mg(^{++})</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>K(^+)</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Ouabain</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* The osmolality was adjusted to 286 mosmol/L by slight alterations of Tris buffer concentration, and the pH was adjusted to 7.4.

was cut in pieces of 2 × 2 × 5 mm and dropped into liquid nitrogen. It was then placed in a freeze-drying apparatus for 48 hours. Control tissue was obtained from "normal" human cortex removed to obtain exposure of aneurysms or deep seated tumors. Histological controls of immediately adjacent areas were obtained in each case.

Bits of freeze-dried tissue weighing from 75 to 150 µg were analyzed; at least four samples of each specimen were run for each reaction. The incubation media are shown in Table 1. Samples were incubated for 30 minutes at 38°C in 0.2 ml of medium, and inorganic phosphorus (P\(_i\)) was determined by the method of Lowry.\(^{12}\)

**Results**

The results are summarized in Table 2 and in Figs. 1 and 2. The values for each tumor are the average of at least four and usually eight simultaneous determinations. In general, the values for Mg\(^{++}\)-ATPase and ouabain-inhibited ATPase were proportional to those for total ATPase. The exception was in the acoustic neurinomas, where virtually no ouabain-inhibited Na\(^+-K^+\)-ATPase was present. The difference between values for total ATPase and Mg\(^{++}\)-ATPase does not always coincide with the values for ouabain-inhibited ATPase, suggesting, in some tumors, the presence of a Na\(^+-K^+\)-ATPase which is not sensitive to ouabain at the concentration used.

**Discussion**

The observed value for total ATPase in normal cerebral cortex is of the same order of magnitude as that described by Bonting, et al.\(^{2}\) Small discrepancies may result from the fact that we utilized bits of freeze-dried tumor rather than homogenates. It is evident that total ATPase is reduced in every tumor studied and suggests that this apparent deficiency may be a hallmark of neoplasia.

The consideration of the values obtained for cerebral cortex as true controls is probably only valid for the gliomas. Studies on normal meninges and Schwann cells have not yet been completed. One can interpret the progression of ATPase values in the gliomas in the light of Hydén's\(^{3}\) findings that the membranes of neuroglia are rich in both types of ATPase. In the Grade II astrocytomas, the relatively large amount of ATPase may simply represent a greater concentration
ATPase in human brain tumors

### TABLE 2
ATPase in brain tumors

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total ATPase</th>
<th>Mg**+-ATPase</th>
<th>Na**+-K**+-ATPase* (Ouabain-inhibited)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µ mole P/i/mg dry weight/30 min</td>
<td>mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N)</td>
<td></td>
</tr>
<tr>
<td>Normal cortex</td>
<td>1.61 ± 0.108 (4)</td>
<td>0.764 ± 0.072 (4)</td>
<td>0.822 ± 0.088 (4)</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>0.097 ± 0.014 (15)</td>
<td>0.060 ± 0.018 (15)</td>
<td>0.028 ± 0.018 (10)</td>
</tr>
<tr>
<td>Astrocytoma Grade II</td>
<td>0.339 ± 0.072 (10)</td>
<td>0.298 ± 0.068 (10)</td>
<td>0.036 ± 0.010 (6)</td>
</tr>
<tr>
<td>Cerebellar astrocytoma</td>
<td>0.338 ± 0.085 (5)</td>
<td>0.201 ± 0.102 (5)</td>
<td>0.087 (2)</td>
</tr>
<tr>
<td>Oligodendrogloma</td>
<td>0.160 ± 0.081 (2)</td>
<td>0.120 ± 0.085 (2)</td>
<td>0.040 (1)</td>
</tr>
<tr>
<td>Mixed glioma</td>
<td>0.166 (1)</td>
<td>0.082 (1)</td>
<td>—</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>0.173 ± 0.158 (2)</td>
<td>0.112 ± 0.124 (2)</td>
<td>0.014 (1)</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>0.158 ± 0.152 (3)</td>
<td>0.138 ± 0.155 (3)</td>
<td>0.020 (2)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>0.347 ± 0.062 (10)</td>
<td>0.263 ± 0.082 (10)</td>
<td>0.055 ± 0.012 (9)</td>
</tr>
<tr>
<td>Acoustic neurinoma</td>
<td>0.054 ± 0.020 (3)</td>
<td>0.032 ± 0.024 (3)</td>
<td>0.004 (3)</td>
</tr>
<tr>
<td>Chromophobe adenoma</td>
<td>0.148 (1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>0.127 ± 0.069 (3)</td>
<td>0.057 ± 0.075 (3)</td>
<td>—</td>
</tr>
</tbody>
</table>

* Represents that amount of total ATPase inhibited by 0.1 mM ouabain.

of gliotic cells per unit area of tissue. The decline in amount of ATPase in glioblastomas may be the result of malignant change, with the cellular elements of the tumor no longer behaving biochemically like the glia from which they presumably developed.

Alterations in ATPase content might also be considered as reflections of differing degrees of vascularity, since normal cerebral vessels contain large amounts of ATPase. While this may be the explanation for the high values in the meningiomas, it does not hold for the gliomas, as glioblastomas are most invariably more vascular than low-grade astrocytomas. It is noteworthy, however, that the vessels in glioblastomas are abnormal.14

Two explanations may be offered for the general lack of ATPase activity in the tumors studied and for the very low amounts of measurable ATPase in the malignant tumors. It may be presumed that a variety of intrinsic metabolic deficiencies commonly occurs in neoplastic cells, and that lack of ATPase is one such deficiency. A less speculative observation may be that cellular respi-
ration is depressed in neoplasms and that oxidative phosphorylation is uncoupled. As malignancy progresses, the supply of ATP is probably severely limited both by a lack of efficient oxidative phosphorylation and by the necessity to maintain the ADP/ATP ratio at an optimum value. Lehninger has shown that this ratio provides the key to efficient energy metabolism in respiratory systems, and ATPase has been suggested as a regulator of cellular respiration. In the competition that exists for the small amounts of ATP produced in tumor cells, ATP-utilizing systems of cationic transport (Na"-K"-ATPase) may hold a low priority. This may be the reason for the common finding of cerebral edema surrounding malignant intracranial tumors.

The membrane ATPases undoubtedly play a role in the dynamics of the blood-brain barrier. The breakdown of this barrier in brain tumors, which is the rationale for the effectiveness of radioisotopic brain scanning techniques, may at least in part be due to alterations in the energy-dependent ATPase activity.

The metabolic changes in brain tumors demonstrated by this work may serve as the basis for a new therapeutic approach, either by an osmotic attack on the tumor cell, which can no longer maintain its internal milieu in the face of stress, or by toxic cations for which the tumor no longer has an enzymatic barrier.

Acknowledgment

The authors are grateful to Mrs. Mabel O. Smith for her expert technical assistance.

References

22. UDVARHELYI, G. B., O'CONNOR, J. S., WALKER, A. E., LAWS, E. R., Jr., and KRAININ,
ATPase in human brain tumors


Received for publication November 13, 1969.

Presented at the Congress of Neurological Surgeons, Boston, Massachusetts, September 18, 1969.

Supported by the National Institute of Health, Grant NB 06144.

Address requests for reprints to: Edward R. Laws, Jr., M.D., The Johns Hopkins Hospital, 601 North Broadway, Baltimore, Maryland 21205.