Repression of glutaminase I in the rat retina by administration of sodium-L-glutamate

Jerome K. Freedman and Albert M. Potts

Newborn albino rats show the same loss of inner retinal layers and of b-wave on treatment with sodium L-glutamate as do mice. Values for four enzymes metabolizing glutamate or glutamine have been determined on normal and glutamate-treated rats. The retinas of normal rats contain almost twice as much glutaminase I per unit weight as those of glutamate-treated animals. There is no measurable "glutaminase II" activity in rat retina. There is no difference between retinas of normal and treated animals in content of glutamatergase synthetase and glutamotransferase. These findings are consonant with our hypothesis that glutamate action on the retina of the newborn rat is by repression of synthesis of an enzyme whose reaction product is glutamate itself. They do not eliminate the possibility that direct competitive inhibition by glutamate may play a role.

In an earlier report one of us (A. M. P.) confirmed the finding of Lucas and Newhouse that large doses of glutamate given to newborn mice resulted in failure to form or destruction of the ganglion cell layer and inner nuclear layer of the retina. Our report further described that such animals in which retinas consisted chiefly of receptor cells plus a narrow remnant of the inner layer showed a characteristically altered electroretinogram. The a-wave in these animals was intact and the b-wave was consistently abolished. We were impressed by the significant toxic effect of a normal body constituent and speculated in our previous publication that feedback repression of enzyme formation might be able to explain the phenomenon.

The present report represents further investigation of this hypothesis and describes the activity of a number of glutamate-metabolizing enzymes in treated and in untreated animals.

Methods

In order to have more retinal material for chemical study, a brief survey of larger experimental animals was performed with regard to maturity of the retina at birth and its susceptibility to the glutamate effect. It was found that, with an identical dose per unit weight, newborn rats showed the glutamate effect both in the histologic picture and in electrophysiologic behavior. All the data to be reported here were obtained with albino rats (Sprague-Dawley strain) which were treated from the second through the twelfth day of life with glutamate according to the dose schedule previously published. Experiments were performed between the third and fifth weeks of life, and, in each case, experimental retinas were compared with those of control animals of identical age.

Animals were sacrificed by cervical dislocation, eyes were enucleated, and the retina was quickly removed and weighed. Retinas were placed in ice cold distilled water and 5 per cent homogenates were made with the Elvejehm-Potter homogenizer.

Four enzyme activities were determined: glutaminase I, glutaminase II, glutamatergase synthetase, and glutamotransferase.

Glutaminase I. Glutaminase I, a phosphate-dependent enzyme with pH optimum between 7.2...
and 7.6, was determined by measuring the amount of ammonia liberated from a specific quantity of glutamine, as described by Richterich-van Baerle, Goldstein, and Dearborn. The reaction was run in 30 c.c. vials incubated in a shaker at 37° C. At the end of the reaction the ammonia formed was liberated by K₂CO₃ and allowed to diffuse to a drop of 10N sulfuric acid on an axial rod during rotation at room temperature. The collected ammonia was determined colorimetrically, as described by Seligson and Seligson, and modified by Richterich-van Baerle and co-workers.

Glutaminase II. This enzyme system which requires a keto acid for optimal activity and which is most active at pH 8.6 to pH 8.8 was assayed, as described by Goldstein, Richterich-van Baerle, and Dearborn. The ammonia formed was determined exactly as described above for glutaminase I.

Glutamosynthetase. Formation of glutamine from glutamic acid is the function of this enzyme which requires ATP and Mg²⁺. It can utilize primary amines as well as ammonia and synthesize N-γ-substituted glutamines. Thus, in the presence of hydroxylamine, glutamohydroxamic acid is formed and the color, developed by this product in the presence of ferric ions, was utilized for its determination, according to Rudnick and Waelsch.

Glutamotransferase. This enzyme catalyzes the transfer of the glutamyl radical from glutamine or glutamine peptides to other amine receptors. Here, too, hydroxylamine can be used, and the color of the resultant glutamohydroxamic acid can be utilized for determination, as described by Rudnick, Mela, and Waelsch.

It should be noted that, because of the weights of the retinas obtained, each homogenate was made with the pooled retinas of 5 to 8 animals.

Results

Glutaminase I. Enzyme activity in liver, brain, and retina is shown in Table I. The relatively high level in brain tissue compared to the two other tissues is worth noting. Krebs figures for higher activity in retina than brain tissue were obtained at pH 8.5. The difference between liver in treated and untreated animals is of questionable significance because of scatter of results in the two animals in each group. No such scatter applies to the decrease of activity in the glutamate-treated retina as compared to the control. There is nearly twice as much activity in the control retinas, and this is a consistent finding in six groups of animals sacrificed at varying periods after completion of treatment.

Comparison of absolute values for our tissues with those obtained by others is of some interest. Although we noted modest variation in the values found in liver, all values for the three tissues were within the same order of magnitude and for any given tissue results were highly consistent. This contrasts markedly with glutaminase I values reported by others. Five different reports on enzyme activity of normal rat kidney, when converted to our units, vary from 0.83 μM to 150 μM per 100 mg. wet weight per 30 minutes.

Glutaminase II. We found no glutaminase activity in the retinas studied that specifically required pyruvate. Since it has been shown that glutaminase II activity represents the action of at least two enzymes, i.e., a transaminase followed by a deaminase, only one of the two might be missing. Work in progress shows a very active transaminase in the retina, so the likelihood is that the specific deaminase is lacking.

Glutamosynthetase. Results of this determination (Table II) were consistent and showed no differences between experimental and control retinas.

Glutamotransferase. Here again there

### Table I. Glutaminase I activity in rat tissues (as μM ammonia liberated/100 mg. wet tissue/30 min. incubation)

<table>
<thead>
<tr>
<th></th>
<th>Retina</th>
<th>Brain</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>20.5</td>
<td>76.9</td>
<td>10.3</td>
</tr>
<tr>
<td>Treated</td>
<td>12.3</td>
<td>76.1</td>
<td>14.3</td>
</tr>
</tbody>
</table>

### Table II. Glutamosynthetase and glutamotransferase activity of rat retinal homogenates (as μM of glutamohydroxamic acid produced/100 mg. wet tissue/30 min. incubation)

<table>
<thead>
<tr>
<th></th>
<th>Glutamosynthetase</th>
<th>Glutamotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>7.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Treated</td>
<td>7.0</td>
<td>12.0</td>
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</table>
was amply measurable transferase activity but no difference between glutamate-treated and control animals (Table II). Thus, the only clear-cut effect on the enzymes studied was the marked decrease of glutaminase I in the glutamate-treated animals.

Discussion

We suggested in our previous publication that the mechanism by means of which the effect of glutamate on the retina can be explained is that of repression of enzyme synthesis. Gorini and Maas\textsuperscript{13} have amply documented the fact that repression of enzyme formation by the products of the specific reaction can occur. Within the last few years specific instances have been found among glutamate and glutamine enzymes in vertebrate tissues. De Mars\textsuperscript{14} reported on repression of glutamyltransferase formation in cultures of human cells by glutamine; and, while our own work was in progress, Goldstein\textsuperscript{15} reported that sodium glutamate would suppress the increase of glutaminase I in the rat called forth by ammonium chloride.

It should be emphasized that our assumption about the effect of glutamate involves one further postulate, namely, that repression of the specific enzyme in question results in cell death or failure of cell development. The retinas with which we are working lack these cells which, according to our supposition, normally contain glutaminase I and possess only cells (that is, chiefly receptor cells) in which this enzyme does not play a major role. It is not easy to demonstrate that, in fact, the inner layers of the retina do contain relatively larger amounts of glutaminase, but experiments on this subject are now in progress. Determination of glutaminase activity in retinas where receptor cells have degenerated after iodoacetate treatment, and histochemical studies of glutaminase in retinas at various stages of development are being undertaken with this end in view.

Even if such a demonstration proves feasible, it does not eliminate the possibility that direct competitive inhibition of glutamic acid for glutaminase, as described by Krebs\textsuperscript{8} is responsible for our observations. It is not likely that concentrations capable of competitive inhibition are maintained for a significant period of time by a single daily dose of glutamate. However, one cannot rule out the possibility that a brief daily insult caused by direct inhibition of glutaminase is adequate to explain our findings. It is hoped that histochemical studies of glutaminase in developing retinas at increasing time intervals after the administration of glutamate will throw light on the matter. These results will be correlated when necessary with retinal content of radioactively labeled glutamate.

The question has been raised in the past on just how much injected glutamate reaches the retina after each dose. We note the work of Himwich, Petersen, and Allen\textsuperscript{26} who described ready access of glutamate to the brain at birth and establishment of a barrier to this compound by the tenth day. This finding makes us confident that glutamate reaches the eye of our newborn animals in high concentration. However, we shall again make direct measurements on the eye with labeled compound to confirm or deny the fact.

Until the studies projected above are complete, we can only state that the present results on glutaminase I are not incompatible with our original postulate that repression of enzyme formation is the means by which sodium glutamate exerts its retinotoxic effect.

REFERENCES

4. Seligson, D., and Seligson, H.: A micro-

5. Goldstein, L., Richterich-van Baerle, R., and Dearborn, E. H.: Kidney glutaminases. II.


