Sodium-potassium–dependent ATPase

II. Cytochemical localization during the reversal of galactose cataracts in rat

Nalin J. Unakar and Jane Tsui

The importance of enzyme Na-K-ATPase in the development of galactose-induced cataractogenesis is now well realized. In our recent studies we reported decreased level of activity of this enzyme with an increased duration of galactose feeding and the induced alterations in rat ocular lens. Our approach was to determine the level of Na-K-ATPase activity by ultrastructural cytochemical analysis of lenses and spectrophotometric analysis of the incubating media used for cytochemical localization as described by Ernst. Using these approaches, we have determined the activity level of this enzyme during the reversal phase of the galactose-induced injury to the lens. Our findings are presented in this report and show that the activity of Na-K-ATPase recovers rapidly and attains the normal level when the animals were transferred to Rat Chow diet after the establishment of mature cataracts resulting from galactose feeding. This study supports the previous biochemical and morphological findings that partial reversal of galactose-induced cataractous lens occurs upon discontinuation of feeding of cataractogenic agent galactose.

Key words: Na-K-ATPase, galactose cataracts, cataract reversal, rat lens, lens cytochemistry

The role of Na-K-ATPase in sugar cataract development is well recognized. Recent studies from our laboratory with ultrastructural cytochemistry and spectrophotometric analysis demonstrated a decrease in the Na-K-ATPase level with the progression of galactose-induced alterations in rat ocular lens. Biochemical and morphological investigations have shown that galactose cataracts are partially reversed by returning the animal to a Rat Chow diet following the establishment of mature cataracts. In this report, we present data on the Na-K-ATPase activity during the regression of galactose-induced cataracts.

Materials and methods

Female Sprague-Dawley rats weighing 50 gm (Spartan Research Animals, Inc., Haslett, Mich.) were fed either 50% ground Purina Rat Chow plus 50% D-galactose or ground Purina Rat Chow alone (controls). Following 20 days of feeding, when mature cataracts were established, groups of animals receiving galactose-containing diet were transferred to Purina Rat Chow alone. Rats were sacrificed 6, 12, 20, 40, 60, and 100 days following the switch from galactose to Rat Chow. Eyes were enucleated, and lenses were removed. For ultrastructural cytochemistry, whole lenses were processed by the Ernst method for Na-K-ATPase (as modified and reported by our laboratory). In this study p-nitrophenyl-phosphate (NPP, disodium salt) was used as substrate. For controls, 10 to 20 mM ouabain were added to the preincu-
Fig. 1. Electron micrograph of a section of a lens from rats fed for 20 days on 50% galactose + 50% ground Rat Chow. This lens was fixed in 1.7% glutaraldehyde and incubated in experimental media containing 20 mM NPP (substrate for Na-K-ATPase). As compared to lenses from control animals (Fig. 2), note the decrease in reaction product of enzyme activity (arrows). In this and following figures, Ep = epithelium; C = capsule; F = fibers. (Unstained; x 13,500.)

For the spectrophotometric analysis of NPPase activity, the reaction was stopped at the end of incubation of the whole lenses in both experimental and control media (with ouabain) by the addition of 0.5 ml of 37.5% trichloroacetic acid. The pH of the media was adjusted with 1M Tris to obtain the required alkalinity for the development of the yellow color of nitrophenol. Optical density at 420 nm was measured, and the percentage differences of NPPase activity in lenses from both controls and galactose-fed animals were calculated.

Results

The reaction product for Na-K-ATPase, as observed with the ultrastructural cytochemistry, was localized on the lateral and apical sides of the lens epithelial cell membranes (i.e., sides where adjacent epithelial cell membranes interdigitate and abut the underlying cortical fiber membranes, respectively). In our previous studies we demonstrated that there was a gradual and considerable reduction in observable reaction product for Na-K-ATPase with the continuation of feeding of a galactose-containing diet. At 20 days of galactose feeding (when mature cataracts were recognizable), lenses exhibited sparsely distributed reaction product (Fig. 1) vs. lenses in control animals, where continuous and heavy deposition of reaction product was visible (Fig. 2). The differences between the cataractous and control rat lenses gradually disappeared when rats were transferred to Rat Chow following the establishment of cataract. At 10 to 15 days following discontinuation of galactose, significant increase in the reaction product was observed at the above-stated reaction sites in the lens (Fig. 3). By 105 days on Rat Chow, the cytochemi-
Fig. 2. Electron micrograph of a section of a lens from Rat Chow-fed (control) rat. Lens was treated identically to that in Fig. 1. Reaction product of Na-K-ATPase action is observable on the lateral region of cell membranes of epithelial cells. (Uranyl acetate and lead citrate; X28,000.)

Table 1. Quantitative determination of NPPase in lenses

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of lenses</th>
<th>Incubation medium</th>
<th>NPPase activity (µM NPP/lens/hr)</th>
<th>Unaffected NPPase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days' recovery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control*</td>
<td>5</td>
<td>Standard</td>
<td>0.084 ± 0.0062</td>
<td>53.78</td>
</tr>
<tr>
<td>Galactose/Rat Chow 1:1</td>
<td>6</td>
<td>Ouoabain/standard</td>
<td>0.01960 ± 0.00217</td>
<td>26.35</td>
</tr>
<tr>
<td>13 days recovery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>Standard</td>
<td>0.03675 ± 0.00555</td>
<td>68.23</td>
</tr>
<tr>
<td>Galactose/Rat Chow 1:1</td>
<td>4</td>
<td>Ouabain/standard</td>
<td>0.03175 ± 0.00419</td>
<td>37.03</td>
</tr>
<tr>
<td>42 days' recovery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>Ouabain/standard</td>
<td>0.06217 ± 0.01075</td>
<td>74.39</td>
</tr>
<tr>
<td>Galactose/Rat Chow 1:1</td>
<td>4</td>
<td>Ouabain/standard</td>
<td>0.04625 ± 0.00411</td>
<td>74.39</td>
</tr>
<tr>
<td>66 days' recovery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>5</td>
<td>Standard</td>
<td>0.041 ± 0.00557</td>
<td>72.37</td>
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<tr>
<td>Galactose/Rat Chow 1:1</td>
<td>6</td>
<td>Standard</td>
<td>0.02677 ± 0.0032</td>
<td>45.93</td>
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<tr>
<td>105 days' recovery:</td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>4</td>
<td>Ouabain/standard</td>
<td>0.0680 ± 0.00557</td>
<td>72.37</td>
</tr>
<tr>
<td>Galactose/Rat Chow 1:1</td>
<td>6</td>
<td>Ouabain/standard</td>
<td>0.0680 ± 0.00543</td>
<td>100</td>
</tr>
<tr>
<td>Regular Rat Chow</td>
<td>17</td>
<td>Ouabain/standard</td>
<td>0.03259 ± 0.03150</td>
<td>74.22</td>
</tr>
</tbody>
</table>

*Controls were fed regular Rat Chow.
Figs. 3 to 5. Electron micrographs of sections of lenses from rats fed 50% galactose + 50% ground Rat Chow for 20 days (when mature cataracts were observable) and then transferred to Rat Chow alone for 13 days (Fig. 3) and 105 days (Figs. 4 and 5). The Na-K-ATPase activity appears to have recovered considerably by 13 days on Rat Chow diet, as seen in Fig. 3. This recovery of enzyme activity continues with increase in time on Rat Chow diet, and by 105 days, the cytochemical localization in both epithelium (Fig. 4) and anterior cortical fiber is similar to that observed in control animals. (Uranyl acetate and lead citrate; Fig. 3, x30,000; Fig. 4, x22,500; Fig. 5, x25,000.)

The assay of the incubation medium for the reaction product of membrane-bound NPPase activity, as determined by spectrophotometric analysis for the release of nitrophenol in the media, gave data which were in agreement with the results obtained...
Fig. 4. For legend see Fig. 3.

Fig. 5. For legend see Fig. 3.
Fig. 6. Percentage of unaffected NPPase activity in the lens following the transfer of rats from galactose to Rat Chow diet is plotted. NPPase activity recovers to a considerable extent, as evident from the data presented for lenses incubated in experimental media containing the substrate (o). With the presence of ouabain (x) the level of NPPase activity is significantly inhibited. (Number of lenses used for each data point is provided in Table I.)

from ultrastructural cytochemistry. The data obtained from this quantitative determination for both experimental and ouabain-containing media are presented in Table I. As previously reported for rats fed galactose for 20 days,5 the percent of unaffected NPPase activity was reduced to 25% of the level observed in control animals. However, upon removal of the rats from galactose diet there was a significant increase in unaffected enzyme activity within 6 to 10 days. This impressive initial recovery of the enzyme activity was followed by gradual recovery and attained the enzyme activity level found in the control group by 105 days following transfer to Rat Chow diet. The data obtained on percentage of NPPase activity at different periods following the transfer of animal to Rat Chow diet are presented in Fig. 6. In this figure, for comparison, the percent of unaffected enzyme activity during the period of galactose feeding and the recovery (following transfer to Rat Chow) are plotted together.

The presence of ouabain in the incubating media reduced the Na-K-ATPase activity, and this inhibitory effect of ouabain was observable with both ultrastructural cytochemical and spectrophotometric analyses.

Discussion

It is known that Na-K-ATPase plays an important role in the maintenance of the clarity of the lens through its action on preserving the cationic balance within the lens in relation to its environment. The effect of alterations in this enzyme action on the process of
cataractogenesis, particularly in sugar cataracts, is well documented.\textsuperscript{1-4} Recent ultrastructural cytochemical and spectrophotometric studies from our laboratory demonstrated gradual reduction in the activity of this enzyme during galactose-induced cataract development.\textsuperscript{5} We suggested that the reduction in the enzyme activity during galactose-induced cataractogenesis could be due either to reduction in the number of viable cells or to a direct and specific effect of galactose on the enzyme itself. It is known that following the discontinuation of cataractogenic agent galactose and prior to the establishment of mature cataract, the opacity could be completely reversed.\textsuperscript{10,11} The biochemical studies from Reddy et al.\textsuperscript{6} and our morphological investigations\textsuperscript{7} demonstrated that lens transparency could be re-established to a considerable extent even after the development of mature cataracts if galactose was removed from the diet. Both biochemical and morphological parameters used in these studies showed changes leading to near-normal values and exhibited close correlation with the establishment of lens transparency.

It has been demonstrated by von Sallmann\textsuperscript{12} and Grimes and von Sallmann\textsuperscript{13} that in the earlier stages of galactose feeding there is a stimulation of epithelial cell proliferation. If galactose feeding is discontinued, then the new fibers formed may replace the damaged fibers and cells, and/or the existing damaged fibers in the lens will undergo repair. The same mechanism for the re-establishment of partial lens transparency in advanced cataract is not ruled out. Our previous studies\textsuperscript{7} support this mechanism of cataract regression. However, the possibility of the repair of damaged fibers and the extent of contribution of this mechanism are difficult to determine. In the studies presented here, even the enzyme activity has been shown to reach almost normal values at an extended stage of reversal. Although the morphology, biochemistry, and enzyme activity recover significantly, a small nuclear opacity is always observable, indicating incomplete reversal. Since all other aspects of lens (transparency, biochemistry, and morphology) are normal, it is tempting to conclude that all damaged fibers are concentrated in the opaque nuclear region and that therefore most of the regression is due to formation of new fibers. The current studies on Na-K-ATPase activity during reversal of galactose cataract support the possibility that the above-stated mechanism of the reversal of galactose cataract and reinstatement of lens transparency exists in this tissue. In order to determine the role, if any, played by a repair mechanism, we have undertaken cytochemical studies which will exhibit the activity of hydrolytic enzymes during induction and reversal of the galactose cataracts. It is now well recognized that hydrolytic (lysosomal) enzymes play an important role in tissue repair, and our previous studies have shown a similar role of these enzymes in the lens.\textsuperscript{14,15}

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**REFERENCES**


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