Cholinesterase and Acid phosphatase in the Rabbit’s Retinae following Severance of the Optic nerve

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ABSTRACT

Group totalling 55 young rabbits (both sexes), whose right optic nerves had been severed intracranially, were fed for 1 week, 2 weeks, 4 weeks and 8 weeks respectively. The retina of the left eye was used as a control and that of the right eye for the experiment. The histochemical changes of cholinesterase, acid phosphatase and ribonucleic acid in the retina after to severance of the optic nerve were observed for 8 weeks after section.

In the retina of the young rabbit, whose visual connection to the central nervous system was blocked, there was a decreasing specific cholinesterase activity beginning at the 4th week after the section of it. By the 8th week, the enzyme activity in the perikaryon of the ganglion cell and the inner plexiform layer was considerably decreased.

Acid phosphatase activity in the young rabbit’s retina peaked at the 2nd week, but decreased below normal after the 4th week. This rapid decline of acid phosphatase activity was characteristic in the experimental retinae and was in contrast to the rather slow alteration of enzymatic activity in neurons undergoing wallerian degeneration.

Pyroninophilic granules contained in neural cytoplasm of the retina were affected by the surgical blocking of the visual connection with the central nervous system. By the 4th week the granules had partially disappeared from the perikaryon of the ganglion cell and from the inner nuclear layer.

Consequently, as the result of histochemical studies, firstly it is postulated that the gradual decline of specific cholinesterase activity in the rabbit’s retina was closely related to the intraorbital blocking of the optic nerve, and secondly, that the typical degeneration of the ganglion cell in the ganglion cell layer (which was associated with a partial disappearance of the ganglion cell) was related to the changes in the acid phosphatase activity and alteration of the pyroninophilic granules in the retina following optic nerve transection.

INTRODUCTION

Following the report of Loewi (1921) that a stimulating factor for vagal transmission was located at the synapse and that this factor was similar to acetylcholine in its physiological activity, Dale (1934) proposed that acetylcholine transmitted impulses at the neuromuscular junction.

Gerbitzoff (1955) postulated that transmission of neural impulses in the retinal synapses was probably associated with the acetylcholine-cholinesterase system. Anfinsen (1944) found high cholinesterase activity at the neural synaptic junction in the retina of the ox which strongly confirmed Dale’s theory that acetylcholine is the chemical transmitter of neural impulses at the synapses. In addition koelle and Friedenwald (1950), studying the rat’s retina, reported that high cholinesterase activity was conspicuous seen in the inner and outer plexiform
layers as well as adjacent to the inner nuclear layer.

With regard to the stimulation effect of natural light on retinal cholinesterase activity in the experimental animal, Liberman (1962) found that the specific cholinesterase activity of the control dog was greater than that found in the retina of the experimental dog which had been bred and lived in the dark since birth. Only a few histochemical studies of cholinesterase activity in animal retinas with emphasis on visual function have been reported.

The present study observes 1) the histochemical changes in cholinesterase activity in rabbit’s retinae, 2) the histochemical features of acid phosphatase activity in the retina and 3) the histochemical alteration of neuritic pyroninophilic granules and histological structures of the retinae following severance of the optic nerve.

**METHODS AND MATERIALS**

55 healthy young rabbits weighing 300 to 500 gm. were used in this study. Under ether anesthesia a horizontal incision was made in the skin of the upper right palpebra. The upper bony part of the orbital margin was removed to approach the posterior pole of the eye ball by widening the surgical field. The optic nerve was severed 3 to 4 mm. from the site where the optic nerve pierces the sclera. As much as possible. Vascular injury of the blood vessels which enter the eye ball through the posterior pole was avoided. Also, postoperative retinoscopy was done to eliminate unsuitable animals. The intact left retina of each animal was used as a control.

Following severance of the optic nerve, 15 rabbits were fed 1 week, 15 rabbits for 2 weeks, 13 rabbits for 4 weeks, and 12 rabbits for 8 weeks. Then each rabbit was sacrificed by injection of air through the ear veins.

For histochemical demonstration of cholinesterase activity excised retinae were fixed for 24 hours in cold formalin-sucrose-ammonia fluid as recommended by Pearson (1963). After the retinae were rinsed briefly in distilled water, frozen sections, 10 microns in thickness, were made. The section was incubated for 2 hours at 37 degrees C. in the substrate containing acetylthiocholine iodide used by Gerebtzoff (1953) and mounted in histoclad. To differentiate the specificity of cholinesterase activity, a 10⁻⁴ M solution of di-isopropylfluorophosphate was used as a pre-incubating medium for specific cholinesterase and a 10⁻³ M solution of di-isopropylfluorophosphate or 10⁻³ M solution of eserine was used for total inhibition of cholinesterases. The pre-incubation in these inhibitors was carried out for 30 minutes.

For histochemical study of acid phosphatase activity excised retinae were fixed in cold neutral formol calcium solution for 24 hours. Frozen sections, about 10 microns in thickness, were prepared. Retinal sections were incubated for 20 minutes at 37 degrees C. in Gomori’s (1941) medium (in 0.1 M acetate buffer whose pH was 4.7) as modified by Eränkö (1952). This medium contains sodium beta-glycerophosphate as a main substrate. Sections were then mounted in histoclad. For a control testing of acid phosphatase activity, the section was similarly incubated in a medium lacking the main substrate of sodium beta-glycerophosphate. These controls all showed absence of acid phosphatase activity.

To demonstrate pyroninophilic granules in the retinal cells histochemically and show the histological structures of the retina as a whole, Rosa’s (1950) method of methyl green-pyronin was used.

**RESULTS**

A. Cholinesterase activity in the young rabbit’s retinae

1. Specific cholinesterase activity in the control retina(left) of the rabbit’s eye one week after severance of the right optic nerve:

In the intact left retina specific cholinesterase activity was demonstrated in the cytoplasm of the ganglion cells (in the ganglion cell layer) and in the inner plexiform layer. Also light enzymatic activity was frequently observed in the inner nuclear and outer plexiform layers. In this study definite sites of cholinesterase activity in both plexiform layers were not determined with the aid of light microscope,
2. Specific cholinesterase activity in the experimental retina (right) of the rabbit's eye one week after severance of the right optic nerve:

The right retinae showed enzyme activity of specific cholinesterase in the ganglion cell and inner plexiform layers which was comparable to that of the preceding group. Thus the specific cholinesterase activity, within the one week period, was not significantly altered by severance of the optic nerve.

3. Specific cholinesterase activity in the control retina (left) of the rabbit whose right optic nerve had been severed 2 weeks previously:

In this control retina specific cholinesterase activity was similar to that in the control retina of the preceding control group. Enzyme activity of the left retina was not affect 2 weeks after severance of the right optic nerve.

4. Specific cholinesterase activity in the experimental retina (right) of the rabbit whose right optic nerve was severed 2 weeks previously:

In the retina there was less specific cholinesterase activity in cytoplasm of the ganglion cell than in the control left one. However, the enzymatic activity showed little change in the inner plexiform and other retinal layers.

5. Specific cholinesterase activity in the control retina (left) of the rabbit's eye whose right optic nerve had been severed 4 weeks previously:

In this left retina specific cholinesterase activity was similar to that of the control retina of the preceding group. Enzyme activity in the retina underwent very little alteration in this 4 weeks experimental period.

6. Specific cholinesterase activity in the experimental retina (right) of the rabbit whose right optic nerve had been severed 4 weeks previously:

At this time the enzyme activity of specific cholinesterase could be demonstrated in each layer of the retina as in the previous group. However, its activity in the neural cytoplasm of the ganglion cell (in the ganglion cell layer) and the inner plexiform was reduced. This enzyme alteration clearly demonstrates a decline in enzyme activity in the experimental retina 4 weeks after optic nerve transection.

7. Specific cholinesterase activity in the control retina (left) of the rabbit whose right optic nerve had been severed 8 weeks previously:

In this control retina specific cholinesterase activity was similar to that of the control retinae of each of the previous groups.

8. Specific cholinesterase activity in the experimental retina (right) of the rabbit whose right optic nerve had been severed 8 weeks previously:

Enzyme activity of specific cholinesterase was only slightly demonstrable in the neural cytoplasm of the ganglion cell and in the inner plexiform layer.

In summary, 8 weeks after severance of the optic nerve and cutting or blocking the connection to the central nervous system in the rabbit there was a fairly demonstrable decrease in the ipsilateral retinal specific cholinesterase activity.

B. Acid phosphatase activity in the young rabbit's retinae

1. Acid phosphatase activity in the control retina (left) of the rabbit whose right optic nerve had been severed 1 week previously:

In the left side of the control retina small granular activity of acid phosphatase was observed in the perikaryon of the ganglion cell (in the ganglion cell layer) and in the neural cytoplasm of the inner nuclear layer.

2. Acid phosphatase activity in the experimental retina (right) of the rabbit whose right optic nerve had been severed 1 week previously:

In the experimental retina (right side) the enzyme activity of acid phosphatase was fairly similar to that of the control as above.

3. Acid phosphatase activity in control retina (left) of the rabbit whose right optic nerve had been severed 2 weeks previously:

Enzyme activity of acid phosphatase was similar to that of the control retina in the previous group.

4. Acid phosphatase activity in the experimental retina (right) of the rabbit whose right optic nerve had been severed 2 weeks previously:

The enzyme activity of acid phosphatase was
Cholinesterase and acid phosphatase following severance of the optic nerve

Table 1. Specific cholinesterase activity in rabbit's retinal layers of the control and the experimental group

<table>
<thead>
<tr>
<th>Group</th>
<th>1st Week</th>
<th>2nd Week</th>
<th>4th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retina</td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Ganglion cell layer</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Inner plexiform layer</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Inner nuclear layer</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Outer plexiform layer</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Outer nuclear layer</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

Degree of enzyme reaction: (-) no activity
(+ ) slight activity
(++) moderate activity
(+++) strong activity

Table 2. Acid phosphatase activity in rabbit's retinal layers of the control and experimental group

<table>
<thead>
<tr>
<th>Group</th>
<th>1st week</th>
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<th>8th week</th>
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<td>Outer nuclear layer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Degree of enzyme reaction: (-) no activity
(- ) slight activity
(++) moderate activity
(+++) strong activity

Generally increased in the perikaryon of the ganglion cell and in the neural cytoplasm of the inner nuclear layer. Also small granules, probably contained in lysosomes, which had been demonstrated previously in the positive reaction of acid phosphatase, had enlarged (and converted to probable cytosolm). This result may be comparable with the acid phosphatase activity observed in the neurons, which undergoes pathological degeneration.

5. Acid phosphatase activity in the control retina (left) of the rabbit whose right optic nerve had been severed 4 weeks previously:

The enzyme activity was generally similar to that of the control retina of the preceding group. However, enzymatic activity was stronger in the neural cytoplasm of the inner nuclear layer than that noted in the control retina.

6. Acid phosphatase activity in the experimental retina (right) of the rabbit whose right optic nerve had been severed 4 weeks previously:

The enzyme activity in the neural cytoplasm of the ganglion cell and the inner nuclear layer was remarkably decreased. The decline in enzyme activity seen so soon after severance of the optic nerve is comparable to the changes in enzyme activity in other neurons which have undergone a Wallerian degeneration.

7. Acid phosphatase activity in the control retina (left) of the rabbit whose right optic nerve had been severed 8 weeks previously:

Enzyme activity was similar to that of the other controls.

8. Acid phosphatase activity in the experimental retina (right) of the rabbit whose right optic nerve had been severed 8 weeks previously:

This experimental retina showed a marked decline of enzyme activity in the neural cytoplasm of the ganglion cell and inner nuclear layer as compared with the 4 weeks experimental group.

In summary, the enzyme activity of acid phosphatase in the retina after severance of the optic nerve once increased and then decreased. This phenomenon
was similar to that seen in other neurons which have undergone a Wallerian degeneration. However, the decline of acid phosphatase activity in the neural cytoplasm of the ganglion cell layer and the inner nuclear layer came earlier than has been reported by others.

C. Pyroninophilic granules in the retina of the young rabbit

There are a moderate number of pyroninophilic granules in the cytoplasm of the ganglion cell and inner nuclear layers. 4 weeks after severance of the optic nerve, the granules in the cytoplasm of the ganglion cell had decreased. There are less of a decrease in the granules in the cytoplasm of the inner nuclear layer.

DISCUSSION

Anfinsen (1944), studying the distribution of cholinesterase in the bovine retina chemically, presented that the results indicate a predominantly synaptic localization of cholinesterase. Koelle and Friedenwald (1950) postulated that histochemical cholinesterase activity of the rabbit's retina appeared to be greatest in the inner nuclear layer and the immediately adjacent portions of the plexiform layers. Koelle et al. (1952) found that specific cholinesterase activity of the cat's retina was represented in the thicker histochemical sections chiefly by two dark bands, one in the innermost regions of the inner nuclear layer and one in the inner part of the inner plexiform layer extending into the outer part of the ganglion cell layer, and very faint staining was noted in the outer plexiform layer.

Leplat and Gerebtzoff (1956) found that acetylcholinesterase activity was positive in the inner plexiform layer of the cat's and rabbit's retinas. Eichner (1956, 1957, 1958) and Viale and Apponi (1961) found that positive enzyme activity of acetylcholinesterase was localized in the outer plexiform layer of the human retina in addition to the positive enzyme results shown in the ganglion cell of the retina, and in the inner plexiform layer. Eränkö et al. (1961) postulated that a positive reaction of specific cholinesterase in various experimental animals was only seen in the outer plexiform layer after prolonged incubation.

In this study the authors found that specific cholinesterase activity was demonstrated in the perikaryon of the ganglion cell of the ganglion cell layer and in the inner plexiform layer associated with a light enzyme activity in the inner nuclear layer and in the outer plexiform layer. With regard to the histochemical localization of specific cholinesterase activity in each retinal layer, the authors found very similar results obtained in the intact rabbit's retina with those secured in the human retina by Viale and Apponi (1961) and observed high enzyme activity of specific cholinesterase in the synaptic layers of the retina, especially in the inner plexiform layer but less in the outer plexiform layer in which negative enzyme activity of specific cholinesterase was postulated by Leplat and Gerebtzoff (1956) and others.

About the relationship between the light deprivation and cholinesterase activity in the retina of the experimental animals: Eränkö et al. (1961) found that keeping the animals in the dark for 1 to 2 days did not affect the distribution of the activity of acetylcholinesterase in the retina. Liberman (1962), studying biochemical activity of retinal cholinesterase of the rat, found that rats raised in the dark from birth to 17 weeks of age have significantly lower acetylcholinesterase activity in the retina than the control rats. Also Liberman (1962) added a possible interpretation of relationships among the tissue level of acetylcholine, the activity of acetylcholinesterase, and enzyme synthesis of acetylcholinesterase under such experimental conditions while reviewing the presentation of Chang et al. (1941) and of Burkharter et al. (1957). Pak and Choi (1964) studied histochemically retinal cholinesterases in the albino rabbit of different early postnatal ages and found that the retina of neonatal rabbits whose eyes were not yet opened, showed generally less cholinesterase enzyme activity than that of young rabbits which were able to see.

In the surgical blocking of the visual pathway (or visual impulse) by intraorbital severance of the optic nerve the authors found histochemical activity
of specific cholinesterase in the retina, whose optic nerve was previously severed, was stepwise decreased. In comparison, the enzyme activity of the control retina was not changed during this experiment. The decrement of retinal specific cholinesterase activity following severance of the optic nerve may be associated with subnormal enzyme synthesis occurring in the ganglion cell. For instance, partial there may be a lack within the ganglion cell and a reduction of the RNA content.

Studying histochemical activity of acid phosphatase in the nerve cell under various physiological and pathological conditions, Becker and Barron (1961) found that acid phosphatase activity was increased in neuronal lysosomes of the rat brain and in Purkinje cell of the rat cerebellum during postmortem autolysis and in anoxic and anoxic-ischemic encephalopathy and that enlargement and clumping of the lysosomes (cytolysosome formation) in such neurons occurred. Barron and Sklar (1961), studying a neural alteration of acid phosphatase activity of the cat spinal anterior horn and hypoglossal nucleus in a Wallerian degeneration, found increased enzyme activity in the spinal cord moer than 6 months after the initial injury of axon sections.

Following severance of the optic nerve, the characteristic feature of histological structure of the retina was partially missing in the ganglion cell. There were degenerative changes of the ganglion cell in the ganglion cell layer associated with a light cellular derangement of the inner nuclear layer and a visible reduction of RNA granules in the ganglion cell which might cause less enzyme synthesis of specific cholinesterase.

It is clear that the optic nerve section causes reduction of specific cholinesterase activity in the retina and partial missing and degenerative changes of the retinal ganglion cell.

REFERENCES

Becker, N. H. and Barron, K. D.: Am. J. Path. 38:161,

1961.

Fig. 1. Young rabbit's control retina. Upper margin of the retina corresponds to the internal limiting membrane. Rosa's method, X 100

Fig. 2. High magnification of Fig. 1. Pyroninophilic granules were found in the perikaryon of the ganglion cell and the nerve cell of the inner nuclear layer. Rosa's method, X 450

Fig. 3. Young rabbit’s experimental retina 2 weeks after the section of the optic nerve. Rosa’s method, X 100

Fig. 4. High magnification of Fig. 3. Partial disappearance of the ganglion and decrease of pyroninophilic granules in the ganglion cell are visible. Rosa’s method, X 450

Fig. 5. Young rabbit’s experimental retina 8 weeks after the section of the optic nerve. Rosa’s method, X 100

Fig. 6. High magnification of Fig. 5. Partial disappearance of the ganglion cell and a cellular derangement of the inner nuclear layer are remarkable. Rosa’s method, X 450
Fig. 7. Young rabbit’s control retina. Upper margin of the retina corresponds to the internal limiting membrane. Gerbtzoff’s method, X 450

Fig. 8. Young rabbit’s experimental retina 2 weeks after the section of the optic nerve. Decrease of specific cholinesterase activity in the ganglion cell and inner plexiform layer is remarkable. Gerbtzoff’s method, X 450

Fig. 9. Young rabbit’s experimental retina 4 weeks after the section of the optic nerve. Decrease of specific cholinesterase activity in the ganglion cell and inner plexiform layer is remarkable. Gerbtzoff’s method, X 450

Fig. 10. Young rabbit’s control retina. Granular acid phosphatase activity in the ganglion cell and in the nerve cell of the inner nuclear layer is visible. Eränkö’s method, X 1000

Fig. 11. Young rabbit’s experimental retina 2 weeks after the section of the optic nerve. Enlarged granular acid phosphatase activity is in the ganglion cell and inner nuclear layer. Eränkö’s modified method, X 1000

Fig. 12. Young rabbit’s experimental retina 8 weeks after the section of the optic nerve. Granular acid phosphatase activity is mostly seen in the nerve cell of the inner nuclear layer. Eränkö’s modified method, X 450