The effects of iodate and iodoacetate on the retinal adhesion

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Sodium iodate and sodium iodoacetate were employed as biological probes to evaluate component forces contributing to the strength of retinal adhesion to the retinal pigment epithelium (RPE). Iodate treatment produced a rapid reduction in the strength of adhesion, which was complete within 1 hr without evidence of concurrent structural change. The adhesive strength reduction produced by iodoacetate required 8 to 12 hr, corresponding to the appearance of early structural changes in the photoreceptor outer segments, although delayed metabolic disturbances in the RPE might also have been involved. The study suggests that metabolic activity in the RPE may account for a portion of the strength of adhesion and that structural changes in the photoreceptor outer segments may also contribute to the forces of adhesion.

Key words: sodium iodate, sodium iodoacetate, retinal pigment epithelium, retinal adhesion, photoreceptor outer segments, electroretinogram

A variety of hypotheses have been proposed to explain the forces of adhesion between retina and retinal pigment epithelium (RPE). It has been proposed that acid mucopolysaccharides in this region function as an extracellular cement. Hydrostatic pressure and flow conductivity as the result of active metabolic processes have been implicated. The demonstration that light adaptation modulates the force of retinal adhesion suggested that interdisc electrostatic forces in the photoreceptor outer segments may also be a component force.

This paper addresses the question of the contribution of active metabolic processes to the force of retinal adhesion. The effects of two potent visual poisons are compared: iodoacetate (considered primarily to block metabolic activity in the photoreceptors) and iodate (considered primarily to affect the retinal pigment epithelium).

Materials and methods

Male chinchilla rabbits, weighing 2.0 to 5.0 kg, were used in this investigation. Sodium iodate was injected into the marginal vein of the rabbit ear at a dose of 50 mg/kg for the 1 hr specimens or 30 mg/kg in two doses over 6 hr for the 30 hr and 5-day specimens. Sodium iodoacetate was given intravenously in a dose of 20 mg/kg of an intramuscular solution neutralized with 0.1N NaOH.

The time of the injection was recorded, and the enucleation was performed at the appropriate time interval after injection. All rabbits were anesthetized with 0.25 mg/kg pentobarbital prior to enucleation. No longer than 20 min passed between enucleation of the first eye and that of the second eye. The strength of the retinal adhesion was measured in grams on 5 mm square sections of retina separated by the exact pulling technique previously described by Owczarek et al. Four sections of retina were taken from each eye, with
Fig. 1. Five days following injection of iodate. The RPE is necrotic, and multiple pigment-laden mononuclear cells (arrows) are seen in the disrupted and disorganized photoreceptor outer segments. (Toluidine blue; X400.)

Table I. Strength of retinal adhesion after iodate injection

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>N</th>
<th>Retinal adhesion* (gm)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>2.53 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>19</td>
<td>1.79 ± 0.17</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>30 hr</td>
<td>20</td>
<td>1.67 ± 0.13</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>5 days</td>
<td>17</td>
<td>1.73 ± 0.12</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E.

Table II. Strength of retinal adhesion after iodoacetate injection

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>N</th>
<th>Retinal adhesion* (gm)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>2.53 ± 0.15</td>
<td>&lt;0.45</td>
</tr>
<tr>
<td>1 hr</td>
<td>7</td>
<td>2.48 ± 0.19</td>
<td>&lt;0.20</td>
</tr>
<tr>
<td>8 hr</td>
<td>10</td>
<td>2.35 ± 0.09</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>12 hr</td>
<td>10</td>
<td>1.86 ± 0.11</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>20 hr</td>
<td>10</td>
<td>1.53 ± 0.16</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E.

Results

Force measurements demonstrated a significant decrease in the strength of retinal adhesion within 1 hr after Na-iodate injection, with little subsequent change over the next 5 days (Table I). Iodoacetate poisoning affected retinal adhesion in a markedly different way; there appeared to be no significant decrease in retinal adhesion until between 8 and 12 hr following injection (Table II).

Histopathologic examination of light-adapted control specimens consistently showed a complex separation plane, primarily through the apical processes of the RPE. Iodate-
Iodate, iodoacetate effects on retinal adhesion

Fig. 2. Plane of retinal separation is variable in sections 5 days after iodate injection. A, Along the outer segments (arrow). B, Along the plane of the apical process of RPE (arrows). (Toluidine blue; both ×400.)

treated unseparated specimens at 1 hr revealed no morphologic change to light microscopy (as in previous investigations) even though the strength of the adhesion was reduced significantly by this time. The separated specimens exhibited a plane most often through the outer segments; however, there were multiple areas revealing separation through the apical processes of the RPE. By 30 hr the unseparated iodate-treated specimens showed disruption of the RPE as well as the outer segments. At 5 days after iodate injection there was much RPE degeneration as well as necrosis (Fig. 1). The outer segments also revealed fragmentation and disorganization. The separation of the retina in these instances occurred through the apical processes of the RPE, the necrotic epithelium above the basal lamina, and the outer segments (Fig. 2, A and B).

Iodoacetate-treated specimens revealed significant coagulative necrosis and vacuolization of the outer segments only in those specimens obtained 20 hr following iodoacetate injection. The RPE revealed mild alterations in the apical processes, with loss of pigment granules and vacuolization (Fig. 3, A and B). Separation of the retina in these samples showed a complex plane through the apical processes and outer segments, but the predominant plane of separation was through the processes of the RPE (Fig. 4).

Discussion

Sodium iodate produces a rapid decrease in the strength of the retinal adhesion within 1 hr of injection. The alteration in the strength of adhesion is essentially complete by this time and does not change significantly during 5 days of observation. No significant structural changes are seen in the 1 hr specimens; structural changes appear only in the 30 hr and 5-day specimens. The development of these structural changes is not accompanied by any marked change in the strength of adhesion. These observations suggest that
Fig. 3. A, Unseparated retina 20 hr following injection of iodoacetate exhibits necrosis and vacuolization of photoreceptors. There are some retinal pigment epithelial cells showing vacuolization of cytoplasm (arrows). (Toluidine blue; ×400.) B, Electron micrograph of outer segments of retina (20 hr after iodoacetate injection) exhibits coagulative necrosis of photoreceptor outer segments (arrows). The necrosis is characterized by loss of lamellar architecture of the discs and electron-dense change. (×12,000.)
Fig. 4. Twenty hours following injection of iodoacetate, the plane of separation is along the apical process of RPE. The phagocytized outer segments show coagulative necrosis (arrow). (×8,000.) Inset, light micrograph of toluidine blue-stained section shows a representative section with separation plane through the RPE. (×250.)

the poisoning of active metabolic processes in the RPE accounts for the adhesive force alterations.

Sodium iodoacetate requires 8 to 12 hours for an alteration in the strength of the adhesion to occur. This corresponds to the time when significant structural changes begin to appear in the photoreceptor outer segments. These observations suggest that structural characteristics of the photoreceptor outer segments may contribute to the retinal adhesive forces, although the possibility of delayed metabolic disturbances in the RPE might also be important.

The effects of sodium iodate and sodium iodoacetic acid on the RPE and photoreceptors have been extensively documented. Both appear to act by blocking triose phosphate dehydrogenase and metabolic pathways dependent on this enzyme. The specific tissue toxicity appears to be related to the uptake of the different poisons by each tissue.

Sodium iodate shows selective action on the RPE, with degenerative changes developing much earlier in that tissue than degeneration of the outer segments or the choroid. The c-wave of the electroretinogram (ERG) is abolished much earlier than the a- and b-waves, which decrease several hours after the c-wave is lost.

Sodium iodoacetic acid acts primarily on the visual cells. Degeneration is demonstrated within a few hours following injection, with complete destruction by 4 to 6 days. The photoreceptor response of the ERG is eliminated within minutes of the injection. The electrical phenomena occur prior to the appearance of the histopathologic changes in both iodate- and iodoacetic acid-treated animals.
In our experiments using both iodate- and iodoacetate-treated specimens, the plane of separation between retina and RPE is highly complex. We have been unable to quantify satisfactorily the proportion of the cleavage plane which passes through the outer segments compared to the apical processes of the RPE because of the many artifacts inherent in the preparation.

Clearly, active metabolic processes contribute to the force of retinal adhesion but certainly do not account for the entire force of adhesion. The temporal correlation between adhesive force changes and structural alterations in the photoreceptor outer segments taken in conjunction with the previously reported light-induced changes in the strength of retinal adhesion suggest that photoreceptor outer segment structure is also a component in the force of retinal adhesion.

Our measurements of the alterations in the strength of retinal adhesion induced by iodate and by iodoacetate (as well as by light in previous work) suggest that the components affected by these experimental manipulations account for only a portion of the total strength of retinal adhesion. Other mechanisms must make a considerable contribution to the adhesion between sensory retina and RPE.

REFERENCES