Effect of iodoacetamide perfusion on outflow facility and metabolism of the trabecular meshwork

David L. Epstein, Joann M. Hashimoto, P. John Anderson, and W. Morton Grant

Freshly enucleated eyes were quantitatively perfused via the anterior chamber with varying dosages of iodoacetamide at constant pressure. Iodoacetamide caused a significant increase in facility of outflow in a dose-response manner in calf and monkey eyes. Almost complete inhibition of glycolysis in the calf trabecular meshwork was produced by a dosage of iodoacetamide that was too low to appreciably alter the facility. A similar response was produced by a higher dosage, which did significantly increase the facility. Our results, taken together with what is known of the properties of iodoacetamide, suggest that cellular sulfhydryl groups may be involved in a mechanism for aqueous flow through the trabecular meshwork and that iodoacetamide probably acts directly on cellular permeability rather than by inhibition of glycolysis or interference with the production of energy in the trabecular meshwork.

Key words: trabecular meshwork, facility of outflow, iodoacetamide, sulfhydryl groups, glaucoma, perfusion, cellular membrane permeability

The available evidence suggests that aqueous humor is formed by an energetic process in the ciliary body but passes through the trabecular meshwork and out of the eye by means of a passive mechanism that does not require energy from the cellular elements of the aqueous outflow system. It seems possible that cellular metabolic factors may modulate outflow through the trabecular meshwork, particularly under the influence of drugs, but this is still uncertain.

In 1953 in preliminary experiments with metabolic inhibitors, Bárány found that introduction of a neutralized solution of iodoacetic acid into the anterior chamber of enucleated cattle eyes caused a significant increase in the facility of outflow by affecting the hyaluronidase-insensitive component of outflow resistance. Other metabolic inhibitors, including cyanide, azide, fluoride, arsenite, and dinitrophenol, all at a 10 mM concentration, did not influence outflow. Although this discrepancy has not been explained, it is noteworthy that iodoacetate and arsenite were the only sulfhydryl reagents tested by Bárány. It is also of interest that most of the inhibitors Bárány chose were aerobic poisons. Our subsequent studies have demonstrated that anaerobic glycolysis is probably more important than aerobic mechanisms for producing energy in the trabecular meshwork.

In various tissues, sulfhydryl reagents such as iodoacetate have specific effects on passive cellular membrane permeability that
are independent of their metabolic effects. We have wondered whether such specific effects on sulfhydryl-dependent properties of trabecular tissue might explain Bárány's findings.

In this study we have investigated the effect of a related sulfhydryl inactivating agent, iodoacetamide, on the facility of outflow in both subprimate and primate eyes and have made comparisons between these effects and the degree of metabolic inhibition of the trabecular meshwork.

**Methods**

Eyes were taken from monkeys killed after completion of experiments not involving their eyes. The eyes were immediately enucleated after death of the animal, stored at 4°C in a moist chamber, and perfused within 48 hr.

Calf eyes were obtained from Joseph T. Trele- gan & Co., Cambridge, Mass. The animals were stunned electrically and killed by exsanguination. After enucleation the eyes were immediately chilled and then perfused within 24 hr.

Our standard constant-pressure perfusion technique was used. Briefly, a 5 mm central corneal trephine button was removed from each eye. Radial iridotomy was performed to prevent artificial deepening of the anterior chamber during perfusion. The anterior chamber was then gently irrigated with perfusion medium to remove pigment which might have been liberated by the iridotomy. A Grant stainless steel fitting was then placed into the cornea and connected by 23-gauge (i.d.) polyethylene tubing to the fluid reservoir of the perfusion apparatus.

The perfusion medium was Dulbecco's phosphate-buffered salt solution (PBS) (Grand Island Biological Co., Grand Island, N.Y.) with added 5.5 mM glucose. All solutions were filtered through a 0.22 μm Nuclepore filter.

The initial flow value was determined after the eyes had been perfused for 1 hr, which allowed sufficient time to achieve adequate stability. Then the corneal fitting was removed, and the anterior chamber was gently emptied and refilled with fresh perfusion medium with added iodoacetamide (Sigma Chemical Co., St. Louis, Mo.). The corneal fitting was replaced, and the eye was perfused for an additional hour, at which time a second flow value was determined. (The flow value after this additional hour of perfusion was used to calculate the outflow facility). In some of the eyes a similar procedure was followed for each of two sequentially increasing dosages of iodoacetamide. For monkey eyes 0.2 ml of 38, 75, and 150 mM solutions was used to deliver respective dosages of 8, 15, and 30 μmol of iodoacetamide into the anterior chamber of a given eye at 1 hr intervals. For calf eyes 0.6 ml of 38, 75, and 150 mM solutions was used to deliver respective dosages of 22, 45, and 90 μmol of iodoacetamide into a given eye at 1 hr intervals. We estimate that during an hour of perfusion each iodoacetamide solution that was introduced into the anterior chamber should gradually have become diluted to 5% to 10% of its original concentration. Control eyes were treated similarly, except that an osmotically equivalent amount of sodium chloride was added to the perfusion medium in place of iodoacetamide. All perfusions were performed at 22°C. Calf eyes were perfused at 15 mm Hg, and monkey eyes at 20 mm Hg.

For the biochemical studies, 12 pairs of calf eyes were perfused in the standard manner, one member of each pair with iodoacetamide and the other as control. Afterward, the anterior chamber was filled with perfusion medium containing 1 mM dithiothreitol to inactivate the remaining iodoacetamide. The eyes were then perfused at 15 mm Hg for an additional 20 min. The trabecular meshwork was excised from each eye, and control
Table I. Relative dose-response effects of iodoacetamide on outflow facility in monkey eyes

<table>
<thead>
<tr>
<th></th>
<th>Sequential change in facility (%)</th>
<th>Overall change in facility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 µmol</td>
<td>15 µmol</td>
</tr>
<tr>
<td>Baboon:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>-3.2 ± 16.0</td>
<td>+8.0 ± 4.1</td>
</tr>
<tr>
<td>Iodoacetamide-treated</td>
<td>+22.6 ± 7.6</td>
<td>+67.8 ± 14.4</td>
</tr>
<tr>
<td>Nonbaboon:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>+7.8 ± 5.4</td>
<td>+6.4 ± 4.9</td>
</tr>
<tr>
<td>Iodoacetamide-treated</td>
<td>+13.4 ± 7.8</td>
<td>+42.8 ± 10.8</td>
</tr>
</tbody>
</table>

Table II. Effect of a single dose of 30 µmol of iodoacetamide on outflow facility in monkey eyes

<table>
<thead>
<tr>
<th>Facility (µl/min/mm Hg)</th>
<th>Baseline</th>
<th>After treatment</th>
<th>Significance of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.48 ± 0.11</td>
<td>0.47 ± 0.10</td>
<td>Not significant</td>
</tr>
<tr>
<td>Iodoacetamide-treated</td>
<td>0.36 ± 0.07</td>
<td>0.59 ± 0.14</td>
<td>p &lt; 0.05</td>
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</tbody>
</table>

Table III. Relative dose-response effects of iodoacetamide on outflow facility in calf eyes

<table>
<thead>
<tr>
<th></th>
<th>Sequential change in facility (%)</th>
<th>Experimental (iodoacetamide-treated)</th>
<th>Significance of experimental effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>Not significant</td>
</tr>
<tr>
<td>22 µmol</td>
<td>+13.4 ± 2.8</td>
<td>+17.7 ± 3.0</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>45 µmol</td>
<td>+4.8 ± 4.0</td>
<td>+20.6 ± 3.4</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>90 µmol</td>
<td>-2.2 ± 1.5</td>
<td>+13.0 ± 2.6</td>
<td>p &lt; 0.02</td>
</tr>
<tr>
<td>Overall change in facility</td>
<td>+16.7 ± 6.6</td>
<td>+61.8 ± 10.5</td>
<td></td>
</tr>
<tr>
<td>Baseline facility*</td>
<td>1.53 ± 0.18</td>
<td>1.52 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Final facility*</td>
<td>1.79 ± 0.22</td>
<td>2.40 ± 0.24</td>
<td></td>
</tr>
</tbody>
</table>

*µl/min/mm Hg.

and experimental tissue were separately pooled and processed as previously described. In this process the tissue was blotted on cold dry Whatman No. 1 filter paper and dropped into a preweighed tube containing 5 ml of Dulbecco's PBS. After centrifugation at low speed, the saline was discarded, and the tissue was combined at a ratio of 1 gm/5 ml with a buffer containing 140 mM KCl, 5 mM potassium phosphate (pH 7), 2.5 mM MgSO₄, 2 mM NH₄Cl, 1 mM EDTA, and 1 mM dithiothreitol, then homogenized in a Tissumizer (Model SDT 100 N; Tekmar, Cincinnati, Ohio). The extract was centrifuged at 27,000 × g, and the supernatant was incubated with and without glucose to determine the rate of glycolysis. Samples for lactate assay were deproteinized with perchloric acid (final concentration 6%), which was subsequently removed with KOH. The lactate content of the deproteinized solution was determined by the method of Gutman and Wahlefeld.

Results are expressed as mean values ± S.E., except for the lactate studies (Fig. 2) for which the S.D. is shown.

Results

Ten pairs of monkey eyes (five baboon, four cynomolgus, one rhesus) received sequentially increasing dosages of iodoacetamide at 1 hr intervals. Baboon eyes received sequentially into the anterior chamber 8, 15, and 30 µmol of iodoacetamide; in the other monkey eyes only the latter two dosages were used. An 8 µmol amount of iodoacetamide did not significantly change outflow facility. However, both 15 and 30 µmol of
Figs. 2A and 2B. Lactate production by extract of calf trabecular meshwork from eyes perfused with and without iodoacetamide. Points are means of two values ± S.D.

Fig. 2A. Control extract: o—o, incubated with glucose; o—o, incubated without glucose. Experimental extract (iodoacetamide dose 22 μmol): •—•, incubated with glucose; •—•, incubated without glucose.

Table IV. Effect of a single dose of 90 μmol of iodoacetamide on outflow facility in calf eyes

<table>
<thead>
<tr>
<th>Facility (μl/min/mm Hg)</th>
<th>Controls</th>
<th>Experimental (iodoacetamide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.48 ± 0.14</td>
<td>1.42 ± 0.10</td>
</tr>
<tr>
<td>After treatment</td>
<td>1.42 ± 0.09</td>
<td>1.85 ± 0.17</td>
</tr>
<tr>
<td>Significance of change</td>
<td>Not significant</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

iodoacetamide did significantly increase the facility of outflow (p < 0.05 and p < 0.01, respectively) (Table I, Fig. 1).

Nine other pairs of primate eyes (four rhesus, three cynomolgus, two baboon) received a single dose of 30 μmol of iodoacetamide, which resulted in a significant increase in facility of outflow (Table II). However, this effect was not as great as the apparently cumulative effect in those eyes that had previously received sequential fillings of lower dosages prior to the 30 μmol dosage (Table I).

In nine pairs of calf eyes the experimental eyes received sequentially 22, 45, and 90 μmol of iodoacetamide. The lowest dosage did not significantly change outflow facility (Table III). Each of the subsequent incremental dosages did significantly increase the facility of outflow (Table III).

In separate experiments on 14 pairs of calf eyes, the experimental eyes received a single dose of 90 μmol of iodoacetamide, which caused the facility of outflow to increase significantly (Table IV). However, this increase was not as great as that observed in the eyes that had previously been exposed to the lower dosages (Table III).

Perfusion of calf eyes with both 22 and
90 μmol of iodoacetamide caused almost complete inhibition of glycolysis in the trabecular meshwork (Fig. 2).

**Discussion**

Our studies indicate that iodoacetamide causes an increase in facility of outflow in a dose-response manner in both enucleated calf and monkey eyes. This presumably corresponds to Bárány's original observation of the effect of iodoacetate on cattle eyes. The concentration of iodoacetamide required to produce this effect is somewhat higher in our studies, most likely because we did not deliver the agent into the anterior chamber in a closed system but in an open system with the corneal fitting removed and the eye at zero pressure. Differences in cellular penetration between iodoacetic acid and iodoacetamide also must be considered.

Iodoacetate and iodoacetamide act primarily by combining with protein sulphydryl groups. Iodoacetate is reported to inhibit more than 240 enzymes, including 21 enzymes involved in glycolysis, 26 involved in electron transport, 14 in the tricarboxylic acid cycle, 21 in the pentose shunt and related pathways, 31 in lipid metabolism, 62 in amino acid metabolism, 26 proteolytic, and 53 miscellaneous. In low concentrations iodoacetate may preferentially inhibit anaerobic glycolysis at the level of glyceraldehyde 3-phosphate dehydrogenase.

The increase in outflow facility induced by iodoacetamide could be attributed to an inhibitory effect on glycolysis and on production of energy in the trabecular meshwork. However, this conclusion is not supported by our observation that a dosage which raised facility as well as a dosage which was too small to affect facility both induced almost complete inhibition of glycolysis in calf trabecular meshwork (Tables III and IV and Fig. 2). It is possible, however, that there may be a very critical, small level of glycolysis in the trabecular meshwork (or only in certain cells of it) that is necessary for the maintenance of normal outflow resistance, and that may have been unaffected by the lowest dosages used. It is reassuring that trabecular meshwork excised from our perfused control eyes demonstrated a rate of...
glycolysis similar to that of fresh, nonperfused tissue.\(^3\)

The fact that the lowest dosage of iodoacetamide severely inhibited glycolysis without significantly affecting outflow facility and that the higher dosages increased rather than decreased outflow facility (Tables III and IV and Fig. 2) seems to argue against the presence of a large active transport process that is dependent on cellular energy production which is involved in the egress of aqueous humor from the eye through the trabecular meshwork. Paradoxically, from the effects of iodoacetamide that we have described, it could be argued that if the trabecular meshwork is capable of actively pumping aqueous humor, such pumping, when uninhibited, would seem to be moving fluid back into the eye rather than out of it.

A more attractive possibility is that iodoacetate and iodoacetamide may have a specific sulfhydryl effect\(^4\) on cellular permeability in the trabecular meshwork that occurs independently of its metabolic inhibitory effect. Membrane sulfhydryl groups in the trabecular endothelium may be directly involved in the process of aqueous outflow. Bárány’s observation\(^2\) that the other metabolic poisons which he tested did not alter outflow facility is consistent with this hypothesis of a specific sulfhydryl effect, except in the case of arsenite. Arsenite has been reported to react with sulfhydryl groups\(^16\) yet did not affect aqueous outflow in Bárány’s experiments. However, there may well be a difference in the type of sulfhydryl groups with which iodoacetate, iodoacetamide, and arsenite react.

Further studies with other sulfhydryl agents and nonsulfhydryl anaerobic poisons clearly need to be performed. By correlation of flow studies with the biochemistry of the perfused meshwork tissue, it should be possible to understand better the influence of metabolic factors in the trabecular meshwork on the aqueous outflow processes of the eye. Morphological studies with particular comparison to the effects of cytochalasin B\(^18\)–\(^20\) and EDTA\(^21\) also need to be performed.

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REFERENCES


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