Reduction by indomethacin of the degeneration increase in outflow facility after superior cervical ganglionectomy in the rabbit. Brenda K. Colasanti and Ernst H. Barany.

The effects of indomethacin and aspirin on the increase in facility of aqueous outflow occurring 24 hr after removal of the superior cervical ganglion from rabbits have been studied. Both drugs were administered in the suppository form 1 hr prior to facility determinations. After pretreatment of rabbits with indomethacin in this manner, the degeneration increase in outflow facility was significantly reduced but not completely abolished. After pretreatment with aspirin, on the other hand, outflow facility of the denervated eyes, although showing a trend toward reduction from values for ganglionectomized eyes of untreated rabbits, was not changed statistically. The effect of indomethacin may be due to inhibition of prostaglandin or thromboxane synthesis. Because of the results with aspirin, however, a special action of indomethacin in reducing the elevated outflow facility must also be considered.

Within 24 hr after removal of the superior cervical ganglion from experimental animals, a wide variety of transient phenomena appear. With regard to ocular structures, one of the first effects to be described was a decrease in intraocular pressure in rabbits, which was subsequently shown to be due to a marked increase in facility of aqueous outflow. Other transient changes observed to follow sympathetic denervation include mydriasis and hyperemia of the iris vessels in rabbits.

A participation of norepinephrine leaking from degenerating nerve terminals in the mediation of some of the transient phenomena following ganglionectomy has been well documented. There has been growing evidence to suggest, however, that a compound or compounds other than norepinephrine are involved as well. An indication that the ganglionectomy increase in outflow facility may not be mediated totally by norepinephrine was initially provided by early studies in which this effect could not be completely prevented by a variety of drugs affecting the adrenergic system. Hendley and Crombie have subsequently shown that the alpha-receptor blocking agent phentolamine reduced this effect by only about 50% and the beta-blocker propranolol had no influence. Degeneration mydriasis likewise could not be completely eliminated by phenoxycinnamate, phentolamine, or reserpine pretreatment. The first two alpha-blockers, furthermore, exerted no influence at all on the degeneration hyperemia of the iris, and reserpine had only a minimal effect. More recently, Neufeld et al. have demonstrated that the hyperemia could be completely prevented by pretreatment with indomethacin, an inhibitor of prostaglandin synthetase, and a role of prostaglandins in the mediation of this phenomenon was thus suggested.

In the present experiments, the effect of indomethacin on the degeneration increase in facility of aqueous outflow occurring after sympathetic denervation has been studied in rabbits. Because this indene derivative was found to reduce the elevated outflow facility, the effect of a second prostaglandin synthetase inhibitor, aspirin, has also been examined.

**Methods.** Adult male albino rabbits weighing between 1.8 and 2.6 kg were used in these experiments. With the animals under anesthesia induced by intravenous sodium pentobarbital (40 to 50 mg/kg), the superior cervical ganglion together with small portions of the postganglionic fibers was unilaterally excised. Surgical removal was alternated between the right and left sides. Of the 39 animals contributing data, 20 underwent right and 19 underwent left ganglionectomies.

Outflow facility was measured either 1 or 24 hr after surgical denervation by the two-level constant pressure perfusion technique using mock aqueous humor. The rabbits were maintained under anesthesia induced by urethane, 1.5 gm/kg, given intravenously. Two needles were mechanically introduced into the anterior chamber of each eye, one for pressure recording and one for infusion. Immediately after anterior chamber cannulation, spontaneous intraocular pressure was recorded. Successive perfusions at two pressure levels, one 2 to 4 and the second 9 to 12 mm Hg above the initial pressure reading, were then begun.

Indomethacin (Indocid; Merck, Sharp & Dohme, Ltd), 100 mg, or aspirin, 500 mg, was given in the suppository form 23 hr after ganglionectomy, i.e., 1 hr prior to perfusion. The suppositories were inserted immediately after initiation of urethane anesthesia and were held in place with a specially designed acrylic "anus stopper." In a limited number of animals, the stopper only was inserted.

Because of needle clogging or mechanical failure, facility values were not always obtained for both eyes of the same animal. In all experiments undertaken 24 hr after ganglionectomy, therefore,
Table I. Effects of indomethacin and aspirin on the degeneration increase in outflow facility

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Outflow facility (μL/min/mm Hg ± S.E.M.) (N)</th>
<th>Ratio, ganglionectomized vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ganglionectomized</td>
</tr>
<tr>
<td>None</td>
<td>0.23 ± 0.02 (11)</td>
<td>0.54 ± 0.06 (10)c</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.23 ± 0.02 (9)</td>
<td>0.30 ± 0.01 (10)EF</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.27 ± 0.01 (7)</td>
<td>0.41 ± 0.05 (10)F</td>
</tr>
</tbody>
</table>

*Given 1 hr prior to perfusion.
Values include only data obtained from both eyes of the same animal.
c: Significantly different from corresponding control value; p < 0.001; b p < 0.01; p < 0.05.
EF: Significantly different from corresponding value for ganglionectomized group having no pretreatment; p < 0.001; b p < 0.05.

Table II. Effects of indomethacin and aspirin on the degeneration decrease in intraocular pressure

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Intraocular pressure (mm Hg ± S.E.M.) (N)</th>
<th>Δ intraocular pressure, control – ganglionectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ganglionectomized</td>
</tr>
<tr>
<td>None</td>
<td>15.02 ± 0.47 (11)</td>
<td>12.31 ± 0.67 (10)R</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>14.19 ± 0.56 (9)c</td>
<td>11.97 ± 0.31 (10)c</td>
</tr>
<tr>
<td>Aspirin</td>
<td>13.85 ± 0.49 (10)F</td>
<td>11.95 ± 0.49 (10)F</td>
</tr>
</tbody>
</table>

*Given 1 hr prior to measurement.
c: Significantly different from corresponding control value; p < 0.001; b p < 0.01; p < 0.05.
F: Significantly different from corresponding value for ganglionectomized group having no pretreatment; p < 0.05; b p < 0.01.

Table III. Intraocular pressure and outflow facility after interruption of the sympathetic impulse flow

<table>
<thead>
<tr>
<th>Time after ganglionectomy (hr)</th>
<th>Intraocular pressure (mm Hg ± S.E.M.) (N)</th>
<th>Outflow facility (μL/min/mm Hg ± S.E.M.) (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ganglionectomized</td>
</tr>
<tr>
<td>1</td>
<td>14.75 ± 1.12 (5)</td>
<td>15.0 ± 1.04 (4)</td>
</tr>
<tr>
<td>24</td>
<td>15.92 ± 0.47 (11)</td>
<td>12.31 ± 0.67 (10)A</td>
</tr>
</tbody>
</table>

An N of 10 facility values for ganglionectomized eyes of each group was preassigned to eliminate statistical bias. Statistical evaluations of the results for the control vs. experimental eyes were made with Student’s two-tailed t test.

Results. The increased outflow facility observed in seven denervated eyes of untreated rabbits 24 hr after ganglion removal was not significantly altered by anal insertion of the stopper (N = 3 eyes; p > 0.10). The values for all 10 eyes have therefore been treated as one group in Table I. After treatment of rabbits with indomethacin 1 hr prior to perfusion, in contrast, the increased facility occurring 24 hr after ganglionectomy was significantly reduced (Table I). Values for the denervated eyes after indomethacin, however, were still significantly elevated over those of the contralateral control eyes. Thus indomethacin did not completely prevent the degeneration increase in outflow facility. After treatment of rabbits with aspirin 1 hr prior to perfusion, outflow facility of the denervated eyes, although showing a trend toward reduction from the values for ganglionectomized eyes of untreated animals, was not changed statistically (Table I). Intraocular pressure of the control and denervated eyes, which was measured in the anesthetized animals immediately after anterior chamber cannulation, is shown in Table II. Ocular tension of the eyes on the operated side was significantly lower than that of the contralateral control eyes for all three experimental groups. Values for the ganglionectomized eyes of untreated animals, was not changed statistically (Table I).

Intraocular pressure of the control and denervated eyes, which was measured in the anesthetized animals immediately after anterior chamber cannulation, is shown in Table II. Ocular tension of the eyes on the operated side was significantly lower than that of the contralateral control eyes for all three experimental groups. Values for the ganglionectomized eyes of untreated animals, was not changed statistically (Table I).
with aspirin was likewise significantly lower than that for untreated animals. The corresponding value for animals pretreated with indomethacin, although somewhat lower than that for the untreated group, did not quite reach significance at the 0.05 level.

In order to ascertain that the increases in outflow facility measured 24 hr after ganglionectomy were not due to interruption of the sympathetic impulse flow per se, facility determinations were made in a small group of animals 1 hr after ganglion removal. As shown in Table III, neither intracocular pressure nor outflow facility of these denervated eyes differed significantly from the corresponding values obtained for the contralateral control eyes.

Discussion. Neufeld et al. earlier demonstrated that pretreatment of rabbits with indomethacin prevented the hyperemia occurring 20 hr after removal of the superior cervical ganglion. In the present experiments, indomethacin has likewise been found to prevent in part the increase in outflow facility occurring 24 hr after denervation. Results obtained after aspirin pretreatment, however, which were additionally evaluated in our study, were somewhat equivocal, with a reduction of the elevated outflow facility being seen in some, but not all, of the animals.

One of the main pharmacological actions of both indomethacin and aspirin is an inhibition of prostaglandin synthetase. If this were the mechanism whereby indomethacin exerted its antagonistic effect on the degeneration increase in outflow facility, then release of prostaglandins or related materials would presumably be responsible in part for mediation of the facility increase. Since ample evidence already exists to implicate norepinephrine in part in the production of the facility increase, an inability of indomethacin to block this effect completely was to be expected. The smaller effect of aspirin in lowering the increased outflow facility in comparison with indomethacin may be a reflection of the weaker ability of aspirin to inhibit ocular prostaglandin synthesis.

Because total outflow facility was measured in our study, the absolute contributions of true facility and pseudofacility to the values obtained are not known. Pseudofacility values are greater under conditions involving short-term pressure elevations, as in our study, rather than after long-standing increment of the pressure, and a pseudofacility component is accordingly inherent in the gross facility values obtained for both the control and ganglionectomized eyes. The decrease in total resistance occurring in the eyes of rabbits ganglionectomized 25 hr earlier has been shown to persist after death induced by interruption of the blood circulation. The increase in total facility of the ganglionectomized eyes thus appears to be due to an increase in true facility rather than pseudofacility.

In contrast to the results regarding outflow facility, neither indomethacin nor aspirin had any effect on the lowered intraocular pressure of the ganglionectomized eyes. Neufeld et al. likewise found no change in the time course and fall in intraocular pressure after ganglionectomy in conscious rabbits pretreated with indomethacin. Pressure differences between control and ganglionectomized eyes in our study, however, were somewhat less for the treated animals than those for the untreated controls. Lower pressure on the control side of the treated animals thus contributed to the change in pressure difference. This observation may indicate that indomethacin and aspirin have a slight tendency to lower normal secretion rate. If secretion is inhibited, increased facility on the ganglionectomized side would not produce much change in pressure.

In the study of Neufeld et al., prior treatment of rabbits with either indomethacin or the norepinephrine synthesis inhibitor α-methyl-p-tyrosine completely prevented the hyperemia occurring 20 hr after ganglionectomy. These findings suggested that the degeneration release of norepinephrine is at least one factor required for the synthesis and release of prostaglandins by the iris. Indirect evidence indicating that the degeneration release of norepinephrine occurs prior to release of either prostaglandins or other potential mediators has likewise been provided by the work of Treister and Bárány. In their study, degeneration mydriasis was completely reversibly by phentolamine administered within the first 2 to 3 hr after its appearance, whereas doses of this alpha-blocker administered later caused only partial reduction of the phenomenon.

In the present study, reduction of the facility increase by indomethacin amounted to 75%. Reduction of the facility increase by prior administration of phentolamine has likewise amounted to 50% to 75%. After pretreatment of rabbits with α-methyl-p-tyrosine, moreover, the facility increase was completely blocked. The results of the last-named study add support to the contention that the synthesis of prostaglandins after ganglionectomy is dependent upon the prior release of norepinephrine. Elucidation of the relative con-
tributions of norepinephrine and prostaglandins in the mediation of the increase in facility, however, must await further study.

In conclusion, the reduction by indomethacin of the increased outflow facility occurring after sympathectomy may point to a role of prostaglandins or thromboxanes in the mediation of this ganglionectomy effect. Because aspirin was not as effective in this regard, however, a special action of indomethacin in preventing the facility increase must also be considered.

From the Department of Medical Pharmacology, University of Uppsala, Uppsala, Sweden. This work was supported by grants 2 R01 EY00231, EY01217, and 1 K07 EY00075 from the U. S. Public Health Service, National Eye Institute. Submitted for publication Nov. 21, 1977. Reprint requests: Dr. Brenda K. Colasanti, Department of Pharmacology, West Virginia University Medical Center, Morgantown, W. Va. 26506.

Key words: outflow facility, cervical ganglionectomy, indomethacin, aspirin, prostaglandins, rabbit

REFERENCES

Abnormal retinogeniculate projections in a congenitally microphthalmic cat. T. L. Hickey and N. R. Cox.

The retinogeniculate projections from the normal eye of a unilaterally microphthalmic cat are abnormal in that optic tract fibers cross laminar borders and end, inappropriately, in geniculate layers that would normally receive input from the microphthalmic eye. This congenitally induced abnormal retinogeniculate projection is quite similar to that seen in cats with one eye surgically removed shortly after birth. Although most cells are shrunken in the laminae normally innervated by the microphthalmic eye, cells in the region of the abnormal projection appear normal. The normal pattern of geniculate lamination is also disrupted in that cell-free interlaminar regions are considerably more difficult to define in the microphthalmic cat.

Central visual system anomalies, including abnormal retinogeniculate projections, occur in a variety of microphthalmic animals such as rats,1, 9 and cats.10 Although, in some of these studies, teratologic agents were used to produce the microphthalmia, others have observed central nervous system disorders in congenitally microphthalmic animals. Recently, a congenitally microphthalmic cat became available to us through a breeding colony used to study feline GM2 gangliosidosis, a lysosome storage disease resulting from a β-hexosaminidase deficiency.1

Methods. The kitten reported on here was one of three offspring resulting from the mating of a 2-year-old heterozygote male and a 2-year-old heterozygote female. Of the three kittens, one was sacrificed immediately due to a large, subcutaneous, fluid-filled, fibrous sac over the dorsal thorax, a second died at 4 days of age, and the third, the microphthalmic kitten, was allowed to survive for 83 days. No other examples of microphthalmia or other visual system abnormalities have appeared in this colony in more than 100 births.