Reports

Effect of Cholinergic Drugs on Outflow Facility After Ciliary Ganglionectomy

Kristine A. Erickson-Lamy* and Paul L. Kaufman†

In cynomolgus monkeys, resting total outflow facility was unaltered 1 and 6 or more months after ciliary ganglionectomy (CG) or postganglionic ciliary neurectomy (PCN). Intraocular pressure (IOP) was decreased in the denervated eye 1 week and 1 month after surgery, but returned to normal after 6 or more months. Although baseline facilities were comparable in CG/PCN and fellow control eyes 6 or more months after surgery, even maximal intracameral doses of pilocarpine did not increase outflow facility in previously denervated eyes, while a normal facility increase occurred in fellow control eyes. However, both previously denervated and fellow control eyes exhibited a large facility increase to both submaximal and greater than maximal intracameral doses of eserine.

Previous investigations on the effects of ciliary ganglionectomy (CG) or postganglionic ciliary neurectomy (PCN) in the cynomolgus monkey eye have demonstrated that 1 month after surgery, the ciliary muscle is completely parasympathetically denervated, but that within 6 months nearly complete reinnervation occurs.1,2

Continuing our studies of the effects of parasympathetic denervation on ocular physiology, we determined the effect of CG/PCN and subsequent reinnervation on outflow facility. Although the reinnervated ciliary muscle mediated a normal accommodative response to both pilocarpine and eserine,1,2 we report here the surprising finding that even greater than maximal doses of pilocarpine had no effect on outflow facility (a response also normally mediated by ciliary muscle3) in reinnervated eyes, whereas large facility increases occurred with even submaximal doses of eserine.

Materials and Methods. Animals and Eyes: Twenty-two young adult cynomolgus monkeys (Macaca fascicularis) underwent unilateral ciliary ganglionectomy and posterior ciliary neurectomy (CG) or posterior ciliary neurectomy and subtotal ciliary ganglionectomy (PCN) via a lateral orbitotomy as previously described.1

Measurement of Total Outflow Facility and Intraocular Pressure: Total outflow facility was measured by two-level constant pressure perfusion of the anterior chamber with Bárány's solution.4 After determination of baseline facility, (C₀), a cumulative dose-response relationship to eserine or pilocarpine was determined. Details of drug injection have been described previously.3,4 On separate occasions, intraocular pressure (IOP) was measured using a minified Goldmann applanating prism.5

Drugs and Dosages: Eserine sulfate (ES) was administered as a 10 μl bolus of 0.4% and 2% solutions, while pilocarpine HCl (PILO) was administered as a 10 μl bolus of 0.05%, 0.2%, and 1% solutions; both drugs were dissolved in Barany's solution.4 The ES dosages are submaximal and greater than maximal (Kristine Erickson-Lamy, PhD, and Paul Kaufman, MD, unpublished data) while the PILO dosages are slightly above threshold, submaximal and greater than maximal6 in normal cynomolgus monkey eyes.

Anesthesia: Anesthesia for IOP measurement was ketamine HCl (10 mg/kg i.m.). Perfusions were conducted under methohexital Na (15 mg/kg i.m.) followed by pentobarbital Na (35 mg/kg i.m.).

These investigations conform to the ARVO Resolution on the Use of Animals in Research.

Results. IOP 1 Week, 1 Month, and 6 or More Months after CG or PCN: IOP was significantly lower in denervated eyes 1 week and 1 month after CG/PCN. In all cases, the IOP (measured 6–17 months after CG/PCN) returned to normal (Table 1).

Baseline Facility 1 Month and 6 or More Months after Ciliary Ganglionectomy: One month after CG/PCN, C₀ was similar in denervated and fellow control eyes, suggesting that denervation of the anterior ocular segment per se had little effect on C₀ (Table 1).

C₀ in the previously denervated but now reinnervated1,2 eye 6 or more months after CG/PCN was similar to that in the contralateral control eye (Table 1).

Effect of Cholinergic Drugs on Outflow Facility in Eyes with Reinnervated Ciliary Muscles: Six or more months after CG/PCN, the outflow facility-increasing effect of a greater than maximal dose of PILO was minimal in reinnervated eyes. The small apparent facility increase that did occur with the 100 μg dose
Table 1. IOP and $C_0$ after CG or PCN

<table>
<thead>
<tr>
<th>Eye</th>
<th>$n$</th>
<th>$C_0$</th>
<th>$C_0$</th>
<th>$C_{100}$</th>
<th>$C_{100}/C_0$</th>
<th>$C_{100}/C_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG/PCN</td>
<td>14</td>
<td>10.1 ± 1.0</td>
<td>15.3 ± 0.8</td>
<td>0.67 ± 0.05</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>7.9 ± 1.3</td>
<td>15.0 ± 1.5</td>
<td>0.68 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>15.6 ± 1.6</td>
<td>15.7 ± 1.5</td>
<td>0.99 ± 0.02</td>
<td></td>
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</tr>
<tr>
<td>$C_0$</td>
<td></td>
<td>0.301 ± 0.041</td>
<td>0.338 ± 0.099</td>
<td>1.08 ± 0.25</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>0.218 ± 0.040</td>
<td>0.288 ± 0.032</td>
<td>0.79 ± 0.12</td>
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</tr>
</tbody>
</table>

Table 2. Outflow facility response to intracameral pilocarpine and eserine 6 or more months after ciliary ganglionectomy (CG) or posterior ciliary neurectomy (PCN)

<table>
<thead>
<tr>
<th>Eye</th>
<th>$n$</th>
<th>$C_0$</th>
<th>$C_0$</th>
<th>$C_{100}$</th>
<th>$C_{100}/C_0$</th>
<th>$C_{100}/C_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG/PCN</td>
<td>7</td>
<td>0.217 ± 0.049</td>
<td>0.342 ± 0.090</td>
<td>0.614 ± 0.146</td>
<td>1.72 ± 0.34</td>
<td>3.01 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.278 ± 0.040</td>
<td>0.539 ± 0.187</td>
<td>0.604 ± 0.131</td>
<td>1.89 ± 0.43</td>
<td>2.30 ± 0.36</td>
</tr>
<tr>
<td>(CG/PCN)/N</td>
<td>7</td>
<td>0.93 ± 0.23</td>
<td>0.74 ± 0.16</td>
<td>1.26 ± 0.38</td>
<td>1.22 ± 0.42</td>
<td>1.69 ± 0.49</td>
</tr>
</tbody>
</table>

Contrary to results with PILO, reinnervated eyes showed a large facility increase in response to submaximal and greater than maximal doses of ES, comparable to responses in the fellow control eyes (Table 2).

Discussion. In this study we show that CG or PCN results acutely in a lowering of IOP. IOP returns to normal when measured 6–17 months after surgery, at which time the ciliary muscle appears to be reinnervated.  

The IOP reduction after CG or PCN was apparently not due to denervation-induced changes in outflow facility, since total outflow facility was the same in denervated and contralateral control eyes 1 month after denervation. Ciliary ganglionectomy in the cat, which appears to involve exclusively parasympathetic denervation, also results in a transient IOP decrease, suggesting that the lowered IOP in our study was indeed mediated by parasympathetic denervation rather than disruption of sensory and/or sympathetic nerves (also disrupted during CG/PCN). It is possible that the surgical procedure of lateral orbitotomy alone or in combination with CG/PCN may disrupt the blood supply to the ciliary body, thereby causing a reduction in aqueous humor formation and a consequent reduction in IOP. However, we found aqueous humor flow to be unchanged 1 month after lateral orbitotomy, and, in the four animals so studied, 1 to 3 months after ciliary ganglionectomy (Erickson-Lamy, KA and Kaufman, PL, unpublished data).

Although resting outflow facility did not change either acutely or 6 or more months after CG/PCN, an outflow facility-increasing effect of PILO could not be demonstrated in reinnervated eyes. Yet, substantial outflow facility increases occurred in response to submaximal and maximal doses of ES.

The relative receptor affinities of acetylcholine compared with PILO in this system are unknown.
However, there is no evidence that the paradoxical finding with respect to the outflow facility response can be explained by a greater efficacy of acetylcholine compared with PILO, since the magnitude of the facility increase following maximal doses of PILO and ES in control eyes was approximately equal, and the number of $^3$H-quinuclidinyl benzilate (QNB) binding sites in the ciliary muscle at this point in time was similar in denervated and fellow control eyes. It is possible that there are multiple receptor subtypes in the primate ciliary muscle for which PILO exhibits some selectivity. PILO is known to be somewhat selective for the M$_3$ subtype in other systems. Assuming that longitudinal and circular fibers of the ciliary muscle can contract independently of one another, a unifying hypothesis which would explain our findings is that the longitudinal fibers of the ciliary muscle contain multiple receptor subpopulations. There is a transient decrease in specific ciliary muscle QNB binding sites following denervation, with recovery accompanying reinnervation. Therefore, it is possible that during the process of denervation the PILO-selective receptors disappear and/or are replaced by a subtype for which PILO has poor affinity.

Another possible explanation for our results is that the ES-induced facility increase was mediated by a mechanism other than cholinesterase inhibition. In earlier investigations, Bárány found that ES was still capable of increasing outflow facility in monkey eyes after complete ganglionic blockade by hexamethonium. Furthermore, ES has recently been shown to interact directly with the nicotinic acetylcholine receptor. Collectively, these findings suggest the possibility that ES may have stimulated neurotransmitter release from noncholinergic nerves in the ciliary muscle (eg, VIP) or from adrenergic, parasympathetic, or sensory fibers in the trabecular meshwork. Therefore, it may be that in the normal eye ES increases outflow facility by both the expected acetylcholine-mediated ciliary muscle contraction mechanism (due to anticholinesterase activity) as well as through a direct, as yet to be determined mechanism perhaps involving the innervation of the trabecular meshwork. The direct mechanism may only be apparent when the cholinesterase-mediated mechanism is no longer operative (eg, parasympathetic denervation, damage to the ciliary muscle, ciliary muscle disinsertion).

The finding of a disparity in the outflow facility responses to eserine and pilocarpine is especially intriguing in light of the fact that both drugs cause a near normal accommodative response in reinervated eyes. This suggests a greater receptor, innervational and/or muscle contraction "reserve" in the ciliary's muscle's accommodative function. Further study in an in vitro system designed to functionally classify muscarinic receptors in the various portions of the ciliary muscle may provide a more complete understanding of these surprising findings.

**Key words:** ciliary ganglionectomy, outflow facility, Macaca fascicularis, parasympathetic denervation, pilocarpine, eserine, cholinergic drugs

**Acknowledgments.** We are grateful to Richard K. Dortzbach, MD for performing the orbitotomies, to B'Ann True-Gabelt and Kathryn Crawford for running the perfusions and assisting at surgery, and to Pat Fitzgerald for manuscript preparation.

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**References**

Five patients with dominant retinitis pigmentosa who were monocularly entrained to a 14 hr light: 10 hr dark cycle showed an abnormal diurnal rhythm in the rod electroretinogram of the entrained eye. These patients as a group showed larger-than-normal reductions in b-wave sensitivity 1.5 hr and 8 hr after light onset relative to other times of day. The findings raise the possibility that these patients have an abnormality in rod photoreceptor function associated with the process of outer segment renewal. Invest Ophthalmol Vis Sci 29:494-498, 1988.