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Reports

Effect of Cholinergic Drugs on Outflow Facility After Ciliary Ganglionectomy

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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. Invest Ophthalmol Vis Sci 29:491–494, 1988

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 muscle[3]) 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, (C_0), 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 Bárány'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 maximal[6] 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, C_0 was similar in denervated and fellow control eyes, suggesting that denervation of the anterior ocular segment per se had little effect on C_0 (Table 1).

C_0 in the previously denervated but now reinnervated[1,2] 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

n	*CG or PCN*	*N*	*(CG or PCN)/(N)*
	A. IOP		
	1 week		
14	10.1 ± 1.0	15.3 ± 0.8	0.67 ± 0.05[b]
	1 month		
7	9.8 ± 1.3	15.0 ± 1.5	0.68 ± 0.10[a]
	6+ months		
9	15.6 ± 1.6	15.7 ± 1.5	0.99 ± 0.02
	B. C_0		
	1 month		
4	0.301 ± 0.041	0.338 ± 0.099	1.08 ± 0.25
	6+ months		
7	0.218 ± 0.040	0.288 ± 0.032	0.79 ± 0.12

A. IOP was measured by applanation tonometry at 1 week, 1 month, and 6–17 months after unilateral ciliary ganglionectomy (CG) or posterior ciliary neurectomy (PCN); (N) = contralateral control eye; n = number of monkeys.

B. On separate occasions, total outflow facility (C_0) was measured in several monkeys by two-level constant pressure perfusion approximately 1 month and 6 or more months after CG or PCN. Data are mean ± SEM IOP (mm Hg) or C_0 (μl/min/mm Hg) for n paired eyes. (CG or PCN)/(N) significantly different from 1.0 by the two-tailed paired t-test [a]$P < 0.02$, [b]$P < 0.001$.

(+38%) represents, when corrected for "washout" (the increase over baseline facility induced by perfusion itself), at most a 17% increase in facility, in contrast to the 156% corrected facility increase in the fellow control eye (Table 2).

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.[1,2]

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,[7] 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,[8] 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.

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

A. Eserine

Eye	*n*	C_0	C_{40}	C_{200}	C_{40}/C_0	C_{200}/C_0
CG/PCN	7	0.217 ± 0.049	0.342 ± 0.090	0.614 ± 0.146	1.72 ± 0.34[a]	3.01 ± 0.46[c]
N	7	0.278 ± 0.040	0.539 ± 0.187	0.604 ± 0.131	1.89 ± 0.43[a]	2.30 ± 0.36[b]
(CG/PCN)/N	7	0.93 ± 0.23	0.74 ± 0.16	1.26 ± 0.38	1.22 ± 0.42	1.69 ± 0.49

B. Pilocarpine

Eye	*n*	C_0	C_5	C_{20}	C_{100}	C_5/C_0	C_{20}/C_0	C_{100}/C_0
CG/PCN	6	0.248 ± 0.048	0.218 ± 0.036	0.271 ± 0.060	0.298 ± 0.075	0.95 ± 0.10	1.17 ± 0.21	1.15 ± 0.15
N	6	0.289 ± 0.029	0.504 ± 0.082	0.639 ± 0.191	0.741 ± 0.250	1.70 ± 0.18[b]	2.04 ± 0.47[a]	2.38 ± 0.88
(CG/PCN)/N	6	0.83 ± 0.12	0.47 ± 0.08[c]	0.59 ± 0.16[a]	0.76 ± 0.38	0.59 ± 0.08[c]	0.71 ± 0.16	0.86 ± 0.30

Total outflow facility (C; μl/min/mm Hg) was measured by two-level constant pressure perfusion in operated (CG/PCN) and contralateral control (N) eyes 6 or more months after unilateral CG/PCN. After determining baseline facility (C_0), successive doses of eserine (A) or pilocarpine (B) were given and facility measured for 45 min following each dose; subscripts indicate dose in μg. When calculating the post-drug/baseline facility ratios, the measured post-drug facilities shown were adjusted downward by 15% to compensate for the facility increase induced by perfusion itself.

The seven eserine experiments were run first; 1–2 months later, when the anterior chamber was free of biomicroscopically visible cells or flare, six of the seven monkeys were perfused with pilocarpine. Data are mean ± SEM facility for n paired eyes. Ratio significantly different from 1.0 by the two-tailed paired t-test: [a]$P < 0.10$, [b]$P < 0.02$, [c]$P < 0.005$.

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.[2] It is possible that there are multiple receptor subtypes in the primate ciliary muscle for which PILO exhibits some selectivity.[9] PILO is known to be somewhat selective for the M_1 subtype in other systems.[10] Assuming that longitudinal and circular fibers of the ciliary muscle can contract independently of one another,[11] 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.[2] 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.[12] Furthermore, ES has recently been shown to interact directly with the nicotinic acetylcholine receptor.[13] Collectively, these findings suggest the possibility that ES may have stimulated neurotransmitter release from noncholinergic nerves in the ciliary muscle (eg, VIP[14]) or from adrenergic,[15] parasympathetic[15] or sensory[15] 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[3]).

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 reinnervated eyes.[1,2] 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

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Rod ERG Diurnal Rhythm in Some Patients With Dominant Retinitis Pigmentosa

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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

Previous studies of normal human subjects who were monocularly entrained to a 14 hr light: 10 hr dark cycle showed that a diurnal rhythm existed in the rod electroretinogram (ERG) of the entrained eye.[1,2] B-wave sensitivity was significantly lower 1.5 hr after light onset compared to other times of day. A diurnal variation in the rod ERG of normal, light entrained pigmented rats has been observed, with a-wave and b-wave sensitivities lowest at the time of day when phagosomes in the pigment epithelium were most numerous and rod outer segments were shortest.[3] A normal rod ERG diurnal rhythm was recently reported in Royal College of Surgeons (RCS) pigmented rats with a defect in the capacity of the pigment epithelium to phagocytize outer segment discs; this finding suggested that the reduction in ERG sensitivity following light onset in humans was not directly due to a diurnal rhythm in phagosome frequency, but, instead, might occur as a consequence of an alteration in rod photoreceptor function.[4] The presence of a diurnal rhythm in the rod ERG of normal humans, together with the findings in rats, raised the possibility of monitoring functional changes associated with rod outer segment renewal in patients with retinal diseases involving the rod photoreceptor. Initial studies were conducted on patients with dominant disease as these patients have abnormal, but often easily detectable, rod ERGs in the early stages.

Materials and Methods. Three siblings (ages 25, 26 and 32) from a family with dominant retinitis pigmentosa with complete penetrance, one patient (age 22) from a second family with dominant retinitis pigmentosa with complete penetrance, and one patient (age 54) from a family with dominant retinitis pigmentosa with reduced penetrance were studied. These patients had corrected visual acuities between 20/20 and 20/40, full or nearly full visual fields to a V-4e white test light with the Goldmann perimeter, and final dark-adapted rod thresholds that were elevated 0.5 to 2 log units above normal to an 11° white test light in all regions tested with a Goldmann-Weekers adaptometer. Full-field rod ERGs to blue light were reduced 50% to 90% below our lowest normal amplitude (normal range: 100–275 μV); full-field cone ERGs to 30 Hz white flicker were normal in amplitude in the three siblings and reduced 50% to 75% below our lowest normal amplitude in the other two patients (normal range: 50–125 μV).[5] All patients had delayed rod b-wave implicit times (normal range: 71–108 msec); and the oldest patient also had delayed cone b-wave implicit time (normal range: 25–32 msec).[5]

In the three siblings rod loss exceeded cone loss based on both ERG testing with full-field stimuli and psychophysical testing with 2° blue and red stimuli; however, they did not admit to night blindness at the age of testing. The other patient with complete penetrance had comparably reduced rod and cone ERG responses and comparable elevations of her rod and cone psychophysical thresholds; she acknowledged nightblindness by age 11. The patient with reduced penetrance also had rod and cone responses that were comparably reduced, and she reported that night-