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Autonomic Drugs and the Accommodative System in Rhesus Monkeys

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Abstract

Accommodation and pupil constriction result from parasympathetic stimulation from the Edinger-Westphal (EW) nucleus of the midbrain resulting in release of acetylcholine at the neuromuscular junctions of the ciliary muscle and iris. Cholinergic and adrenergic drugs can be applied topically to evaluate the effects on the pupil and accommodative system without input from the EW nucleus. This study is directed at characterizing how topical low dose echothiophate, an anti-cholinesterase inhibitor (i.e., an indirect cholinergic agonist), epinephrine, an adrenergic agonist, and timolol maleate, a beta adrenergic antagonist, affect pupil diameter, resting refraction and accommodative amplitude and dynamics in rhesus monkeys. The effects of 0.015% echothiophate, 2% epinephrine, 0.5% timolol maleate and saline on pupil diameter and resting refraction were measured in one eye each of four normal rhesus monkeys for 60-90 minutes following topical instillation. Pupil diameter was measured with infrared videography and refraction was measured with a Hartinger coincidence refractometer. Effects on static and dynamic EW stimulated accommodation were studied in three iridectomized monkeys (ages 5, 6 and 12 years) with permanent indwelling stimulating electrodes in the EW nucleus. Dynamic accommodative responses were measured with infrared photorefraction for increasing current amplitudes before and during the course of action of the pharmacological agents. Echothiophate caused a significant decrease in pupil diameter of 3.07 ± 0.65 mm (mean $\pm$ SEM, $p < 0.01$), and a myopic shift in resting refraction of 1.30 ± 0.39 D ($p < 0.05$) 90 minutes after instillation. Epinephrine caused a 2.76 ± 0.38 mm ($p < 0.01$) increase in pupil diameter with no change in resting refraction 60 minutes after instillation. Timolol maleate resulted in no significant change in either pupil diameter or resting refraction 60 minutes after instillation. There was no significant change in maximum EW stimulated accommodative amplitude after any agent tested. The amplitude vs. peak velocity relationship for accommodation was significantly different after echothiophate and timolol maleate, and for disaccommodation after echothiophate, epinephrine and timolol maleate. In conclusion, when tested objectively in anesthetized monkeys, epinephrine and timolol maleate did not alter resting refraction or accommodative amplitude, but did have small, significant affects on accommodative dynamics. This suggests that there is an adrenergic component to the accommodative system. Low dose echothiophate had significant effects on pupil diameter and resting refraction, with only small effects on the dynamics of the accommodative response.

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Keywords

accommodation; crystalline lens; Edinger-Westphal nucleus; photorefraction; parasympathetic system; autonomic nervous system

Introduction

Accommodation is controlled by the autonomic nervous system, driven by visual input and higher brain centers, and is stimulated by the parasympathetic system. When the Edinger-Westphal (EW) nucleus in the midbrain is stimulated, acetylcholine is released at the neuromuscular junctions of the ciliary muscle, causing the ciliary muscle to contract. EW stimulation in anesthetized monkeys results in the current spread to the oculomotor nucleus and the parasympathetically driven triad of convergence, pupil constriction and accommodation. The iris and ciliary muscle are dually innervated by parasympathetic and sympathetic neurons (Cogan, 1937; Stephens, 1985), although the role of sympathetic innervation in accommodation is not completely understood (Chen, Schmid and Brown, 2003; Gilmartin, Bullimore, Rosenfield, Winn and Owens, 1992). Most autonomic systems are comprised of both sympathetic and parasympathetic components, one being stimulatory and the other being inhibitory. However, the accommodative system is largely controlled by the parasympathetic system, with a small sympathetic component that has been shown to be directly related to the concurrent background parasympathetic activity (Gilmartin and Bullimore, 1987b; Tornqvist, 1967; Vasudevan, Ciuffreda and Gilmartin, 2009). There is also significant inter-subject variations in this sympathetic facility (Gilmartin, Mallen and Wolffsohn, 2002). The primate ciliary muscle primarily contains parasympathetic cholinergic receptors that are bound by endogenous acetylcholine. In the cynomolgous and rhesus monkey ciliary muscle, only about 1% of the nerve terminals are sympathetic (Ruskell, 1973). In humans, the ciliary muscle has been shown to contain sympathetic receptors of the β_2 subtype rather than β_1 , α_1 or α_2 subtypes (Hurwitz, Davidowitz, Chin and Breinin, 1972b; Stephens, 1985; van Alphen, 1976; Wax and Molinoff, 1987; Zetterstrom and Hahnenberger, 1988), although a small population of α_1 adrenergic receptors has been identified (Zetterstrom and Hahnenberger, 1988). Therefore, it may be possible to isolate sympathetic activity using a non-selective beta antagonist such as timolol maleate, as opposed to a β_1 specific drug, such as betaxolol HCl.

Previous studies in monkeys and humans have shown equivocal results with respect to sympathetic effects on accommodation. It has been suggested that sympathetic stimulation of the ciliary muscle is inhibitory in both monkeys and humans, thereby decreasing accommodative amplitude (Chin, Ishikawa, Lappin, Davidowitz and Breinin, 1968; Gimpel, Doughty and Lyle, 1994; Hurwitz, Davidowitz, Chin and Breinin, 1972a; Paggiarino, Brancato and Newton, 1993; Zetterström, 1984). A previous study showed that alpha adrenergic stimulation through topical instillation of phenylephrine causes a strong pupil dilation in monkey and human eyes, but does not affect EW stimulated accommodative amplitude or dynamics in anesthetized, iridectomized rhesus monkeys or objectively measured voluntary accommodative amplitude in humans (Do, Kasthurirangan, Ostrin and Glasser, 2002; Ostrin and Glasser, 2004). Several investigators have demonstrated a more subtle effect of sympathetic stimulation in humans. Gilmartin concluded that the sympathetic system provides a small, slow component to accommodation (Gilmartin, 1986). It has been found that timolol maleate, a beta adrenergic antagonist, is capable of increasing post-task regression to baseline refraction following sustained reading (Winn, Culhane, Gilmartin and Strang, 2002). The sympathetic contribution to accommodation may only be evident following prolonged near work, allowing a build-up of sympathetic inhibitory activity over a background of parasympathetic activity, which helps to return the

accommodative system to its baseline, resting refraction (Gilmartin and Bullimore, 1987a; Gilmartin, Hogan and Thompson, 1984b; Hurwitz, Davidowitz, Chin and Breinin, 1972b; Tornqvist, 1967).

Historically, adrenergic and cholinergic drugs have played an important clinical role in the ophthalmic and vision research fields, including the management of glaucoma and binocular disorders and in the study of accommodation. Timolol maleate is a common first line therapy in glaucoma treatment to reduce intraocular pressure by reducing aqueous production (Demailly and Arrata, 1980). Concomitant use with epinephrine has been shown to decrease intraocular pressure further (Cyril, Thomas and Epstein, 1982). Although no longer used, echothiophate was one of the first pressure lowering drugs for glaucoma (Krishna and Leopold, 1959), and has more recently been used in the treatment of binocular disorders (Kim, Kim and Hwang, 2005). Beta-blockers have become a mainstay in the management of glaucoma, because they are not known to constrict the pupil or cause an accommodative spasm as expected with cholinergic drugs like pilocarpine or echothiophate.

Despite therapeutic uses for autonomic drugs, there are potentially undesirable effects of long term use. In nonhuman primate eyes, long-acting cholinesterase inhibitors such as echothiophate iodide have been shown to cause an increase in IOP, collapse and densification of the trabecular meshwork, alterations in the shape and orientation of Schlemm's canal and the ciliary muscle, and discontinuity between ciliary muscle bundles and trabecular beams (Gabelt, Hennes, Seeman, Tian and Kaufman, 2004; Lutjen-Drecoll and Kaufman, 1986). Chronic echothiophate use also results in a decreased accommodative response to pilocarpine (Croft, Kaufman, Erickson-Lamy and Polansky, 1991). It is possible that these structural and functional changes are due to cellular effects of elevated levels of acetylcholine, inhibition of enzymes or changes in aqueous composition (Lutjen-Drecoll and Kaufman, 1979; Walinder and Bill, 1969).

Previous investigators have attempted to dissociate the cholinergic processes of outflow facility, accommodation and miosis through the use of various cholinergic agonists and antagonists (Gabelt and Kaufman, 1992). They concluded that dissociation of these processes may not be possible because all three responses are mediated by the cholinergic M3 receptor subtype. However, they were able to dissociate miosis and accommodation to intracameral pilocarpine by delivering the drug in a dose dependant manner, with a maximal pupil constriction occurring with a 10-20 fold lower dose of pilocarpine than needed for accommodation. This doesn't necessarily indicate that the sphincter muscle is more sensitive than the ciliary muscle, because other factors, such as drug delivery method, may play a role. Here, a low concentration of echothiophate delivered topically is used in an attempt to selectively produce miosis with little to no effects on accommodation. An agent capable of producing miosis with no other ocular effects might have clinical implications for increasing depth of field or reducing aberrations in conditions such as presbyopia or corneal scarring following refractive surgery.

It is of interest to understand the effects of the autonomic stimulation and blockage on accommodation and pupil diameter. Characterization of how these drugs affect the pupil and accommodative system will lead to a better understanding of the regulation in the anterior segment and the effects of autonomic drugs used for therapeutic purposes. This study was aimed at determining the effects of low dose echothiophate, an indirect cholinergic agonist, epinephrine, a non-selective adrenergic agonist, and timolol maleate, a beta-adrenergic antagonist on pupil diameter, resting refraction and accommodative amplitude and dynamics in rhesus monkeys using a protocol similar to prior studies of the effects of pharmacological agents on the accommodative system (Ostrin, Frishman and Glasser, 2004; Ostrin and Glasser, 2004). Through the study of accommodative dynamics and possible changes after

instillation of specific autonomic agents, important characteristics of the response properties can be assessed, such as velocities and time constants, which cannot be determined with static accommodative measures.

Materials and Methods

Experiments were performed on 12 anesthetized rhesus monkeys (*Macaca mulatta*). The effects of the drugs on pupil diameter and resting refraction were determined in nine rhesus monkeys with intact irides. The effects of the drugs on accommodation was assessed in three rhesus monkeys that had previously undergone complete iridectomy and had a permanent indwelling electrode in the EW nucleus of the midbrain (Crawford, Terasawa and Kaufman, 1989; Kaufman and Lütjen-Drecoll, 1975). All experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were conducted under an institutionally approved animal protocol. Experiments performed on the same animals took place at least one week apart.

Pupil Diameter and Resting Refraction

Monkeys were anesthetized with 10 mg/kg intramuscular ketamine and 0.5 mg/kg intramuscular acepromazine. Anesthesia was supplemented with 6.25 mg/kg ketamine approximately every 30 minutes throughout the duration of the experiment as needed. For epinephrine and timolol maleate experiments, the drug was topically instilled in the right eye, and the left eye served as a control in four monkeys (#112, #114, #54, #58). Echothiophate experiments were performed in the right eye only of five monkeys (#34, #87, #74, #96, #118). The eyes were held open with a lid speculum, and contact lenses were placed on the eyes to maintain corneal clarity and prevent dehydration. Vital signs were monitored, including heart rate and SpO₂, during all experiments.

To determine the effects of 2% epinephrine and 0.5% timolol maleate on pupil diameter, three static images of the eyes were captured before and every three minutes after topical instillation of the drug in one eye for 60 minutes using video pupillography (Ostrin, Frishman and Glasser, 2004; Ostrin and Glasser, 2004). The eye was illuminated by 20 infrared diodes placed on the lower half of the camera lens. Grayscale images of the retroilluminated pupil were captured using an infrared sensitive charge-couple device (CCD) camera. Calibrated pupil diameters were measured offline using image analysis software (Optimas, Media Cybernetics, Silver Springs, MD). Resting refraction was measured in both eyes with a Hartinger coincidence refractometer (HCR) (Zeiss, aus JENA, Germany) (Fincham, 1937) three times before and every three minutes after topical instillation of the drug for 60 minutes. Following baseline measurements, the contact lens was removed from the right eye, and 0.1 ml of 2% epinephrine or 0.5% timolol maleate was topically instilled into the eye using a canula on a 1 ml syringe. After 1 minute, another 0.1 ml was again applied to the cornea, and after 30 seconds, the contact lens was replaced to resume measurements.

For echothiophate experiments, ten baseline images of the right eye were captured before drug instillation, and three images were captured every five minutes until no further change in pupil diameter was noted by evaluation of the live images on the computer. Following baseline measurement, the contact lens and speculum were removed, and 0.1 ml of 0.015% echothiophate was topically instilled into the eye with a canula on a 1 ml syringe, and the eye was closed for one minute to allow ocular penetration. The contact lens and speculum were replaced, and measurements resumed. Immediately following each set of images captured, resting refraction was measured three times with the HCR. For each time period, repeated pupil and resting refraction measurements were averaged.

Dynamic Accommodation Experiments

The three monkeys used for dynamic accommodation testing had previously undergone bilateral, complete iridectomies (Kaufman and Lütjen-Drecoll, 1975) and had stimulating electrodes surgically implanted in the EW nucleus of the midbrain (Crawford, Terasawa and Kaufman, 1989; Glasser and Kaufman, 1999). The monkeys are used in multiple experimental protocols (Glasser and Kaufman, 1999; Vilupuru and Glasser, 2002; Vilupuru and Glasser, 2003) and the iridectomies (Kaufman and Lütjen-Drecoll, 1975), justification for them (Bito, Kaufman, DeRousseau and Koretz, 1987) and absence of an effect on centrally stimulated accommodation (Crawford, Kaufman and Bito, 1990) have been described previously. The monkeys were 5 (#111), 6 (#38) and 12 (#4) years old.

Monkeys were initially anesthetized with 10 mg/kg intramuscular ketamine and 0.5 mg/kg intramuscular acepromazine. Surgical depth anesthesia was maintained for the duration of the experiment with intravenous propofol (initial bolus of 1.5 mg/kg followed by constant perfusion at 0.5 mg/kg/min). Accommodation experiments were performed on one eye of each monkey. The contact lens was placed on the cornea of the test eye, and sutures were tied beneath the lateral and medial rectus muscles to reduce eye movements during accommodation (Glasser and Kaufman, 1999; Vilupuru and Glasser, 2002).

Static accommodative responses to increasing stimulus currents were measured with the HCR before and 60 minutes after topical instillation of 0.2 ml 2% epinephrine, 0.2 ml 0.5% timolol maleate and saline, and 70 minutes after topical instillation of 0.1 ml 0.015% echothiophate to determine a pre- and post-drug accommodative stimulus response function. The pre-treatment stimulus response function was later used to calibrate the dynamic photorefractive measurements, and to compare the accommodative responses for each stimulus amplitude for the pre- and post-treatment conditions. First, baseline, resting refraction was measured three times with the HCR. A pre-treatment accommodative stimulus response function was then measured by stimulating accommodation with approximately ten increasing current amplitudes delivered to the EW nucleus, ranging from 0 μ A up to the amplitude sufficient to produce the maximum accommodative response available to each monkey, using two-second long stimulus trains. For each stimulus amplitude, accommodation was stimulated three times in succession and measured with the HCR and averaged. Baseline refractions and the stimulus response function were measured again 60 minutes following epinephrine, timolol maleate or saline instillation, and 70 minutes following echothiophate instillation.

Dynamic accommodative responses were measured with infrared photorefractive to determine the relationship between the peak velocity and the amplitude of the accommodative responses (a main sequence relationship) (Vilupuru and Glasser, 2002). Calibrated photorefractive (Schaeffel, Farkas and Howland, 1987; Schaeffel, Wilhelm and Zrenner, 1993) was performed at a 0.3 m working distance and analyzed over 40% of the iridectomized pupil diameter (Vilupuru and Glasser, 2002). Before drug instillation, a sequence of dynamic accommodative responses to increasing stimulus currents was recorded with infrared photorefractive, using the same stimulus current amplitudes as those used for the static HCR measured stimulus response function. Accommodative responses to eight increasing stimulus amplitudes were recorded spanning the full accommodative range for each monkey. For each stimulus amplitude, responses to five, four-second long stimulus trains were recorded. The first two of the five responses were not considered further, but the last three responses were averaged and analyzed. For each amplitude measured in the HCR stimulus response function, the slope of the luminance profile extracted from the photorefractive images in the central 40% of the pupil, recorded while the eye was accommodated, was determined. The relationship between the photorefractive measured slope and HCR measured refraction provided the calibration function which was used to

convert each video frame of the dynamically measured photorefractive images to refraction, recorded at 30 Hz.

Following the baseline recordings, the test drug was instilled topically as described above. For epinephrine, timolol maleate and saline, the dynamic accommodative response was recorded every five minutes for 60 minutes for a fixed stimulus current amplitude previously determined to elicit maximum accommodation. For echothiophate, the dynamic accommodative response was recorded every 10 minutes for 70 minutes for three different stimulus current amplitudes – one previously determined to elicit a maximum response, and two previously determined to elicit sub-maximal responses. The stimulation and measurement procedures are described above.

At 60 minutes (epinephrine, timolol maleate and saline) or 70 minutes (echothiophate) post-treatment, a dynamic accommodative stimulus-response function was recorded for amplitudes spanning the full range available to each monkey, using the same stimulus current amplitudes as the pre-treatment sequence.

Dynamic Accommodation Analysis

The effects of the saline and each of the three drugs on dynamic accommodation were evaluated in terms of peak velocity of accommodation and disaccommodation (Vilupuru and Glasser, 2002). Amplitude of accommodation, peak velocity of accommodation and peak velocity of disaccommodation were determined for each stimulus amplitude of the pre-treatment sequence, for the repeated stimuli recorded during the 60-70 minutes following instillation, and for the post-treatment stimulus amplitude sequence using methods described previously and is explained only briefly here (Vilupuru and Glasser, 2002). The accommodative response amplitude was determined for each stimulus by taking the difference between the baseline refraction and the accommodated refraction.

The accommodative response was plotted as a function of time, and exponential curves shown in equations (1) and (2) below, were fitted to the accommodation phases and the disaccommodation phases (Vilupuru and Glasser, 2002):

$$\text{Accommodation} = A(1 - e^{-x/\tau}) + bx + cx^2 \quad (1)$$

$$\text{Disaccommodation} = A(e^{-x/\tau}) + bx + cx^2 \quad (2)$$

where A is the response amplitude, x is the time, and τ is the time constant. The derivative of these functions with respect to time gives the velocity profile of the responses, and the maximum velocity achieved is the peak velocity.

Results

Pupil and Resting Refraction

All values for pupil diameter, resting refraction and maximum accommodative amplitude are given as mean $\pm$ SEM, and tested for statistical significance with paired t-tests. Prior to 2% epinephrine instillation, pupil diameter was 5.57 ± 0.49 mm and resting refraction was $+2.85 \pm 1.54$ D. Sixty minutes after instillation, pupil diameter had increased to 8.33 ± 0.15 mm, a significant increase of 2.76 ± 0.38 mm ($p < 0.01$, $df = 3$) (figure 1), and resting refraction was not significantly different at $+3.37 \pm 1.17$ D ($p = 0.30$, $df = 3$) (figure 2). Prior to 0.5% timolol maleate instillation, pupil diameter was 4.55 ± 0.81 mm and resting refraction was $+2.71 \pm 1.42$ D. Sixty minutes after instillation, there was no significant change in pupil diameter, 4.63 ± 0.27 mm ($p = 0.91$, $df = 3$) or resting refraction, $+2.73 \pm 1.60$ D ($p = 0.94$, $df = 3$).

Prior to 0.015% echothiophate instillation, pupil diameter was 4.76 ± 0.63 mm and resting refraction was $+2.68 \pm 2.18$ D. Measurements were recorded until the change in pupil diameter plateaued, as observed from the live images, and ranged from 60 to 90 minutes (74 ± 5.3 minutes). At this time, pupil diameter was 1.69 ± 0.12 mm, a significant decrease of 3.07 ± 0.65 mm ($p < 0.01$, $df=4$), and resting refraction was $+1.38 \pm 2.33$, a significant myopic shift of 1.30 ± 0.39 D ($p < 0.05$, $df=4$).

Maximum Accommodative Amplitude

There was no significant difference in centrally stimulated maximum accommodative amplitude, measured with the HCR, before and after instillation of saline or any drug. Maximum accommodative amplitude prior to saline was 10.91 ± 1.46 D and after saline was 10.39 ± 1.33 ($p=0.17$, $df=2$), a decrease of 0.52 ± 0.25 D; prior to 2% epinephrine was 11.03 ± 1.44 D and after epinephrine was 11.00 ± 1.85 ($p=0.97$, $df=2$), a decrease of 0.03 ± 0.67 D; prior to 0.5% timolol maleate was 11.86 ± 1.52 D and after timolol maleate was 11.42 ± 1.46 D ($p=0.06$, $df=2$), a decrease of 0.44 ± 0.10 D; and prior to 0.015% echothiophate was 10.69 ± 0.96 and after echothiophate was 11.71 ± 0.56 ($p=0.30$, $df=3$), an increase of 1.02 ± 0.80 D. For increasing current amplitudes, Bland-Altman analysis (Bland and Altman, 1986) shows a mean difference of $+0.76 \pm 0.96$ D before and after saline, $+0.50 \pm 0.76$ D before and after epinephrine, $+0.52 \pm 0.56$ D before and after timolol maleate and -1.03 ± 1.20 D before and after echothiophate (figure 3). However, all differences were within the 95% confidence intervals for each drug tested.

There was no change in the accommodative amplitude to a maximum accommodative stimulus, measured every five minutes for 60 minutes after epinephrine, timolol maleate or saline instillation ($p=0.43$, $p=0.51$, $p=0.49$, respectively), or the accommodative amplitude to an approximately 1/3, 2/3, and maximum accommodative stimulus measured every 10 minutes for 70 minutes after echothiophate instillation ($p=0.20$, $p=0.11$, $p=0.98$, respectively) (figure 4) as measured with infrared photorefraction. While there are slight differences in the baseline accommodative response between experiments, seen in figure 4A, there is no change in the accommodative response with time for each agent tested.

Accommodative Dynamics

Dynamic analysis of the accommodative and disaccommodative responses measured with infrared photorefraction showed linear peak velocity vs. amplitude relationships before and after drug treatment (figure 5). Statistical software was used to determine whether the slopes or intercepts of the main sequences were changed after treatment (SigmaStat 3.5). Saline had no effect on the dynamics of accommodation (slope: $p=0.593$; intercept: 0.466) or disaccommodation (slope: 0.359; intercept: 0.422). However, the slope of the relationship decreased significantly after epinephrine for disaccommodation ($p < 0.0001$), after timolol maleate for accommodation ($p=0.05$) and disaccommodation ($p < 0.0001$), and after echothiophate for disaccommodation ($p < 0.01$). There was a significant decrease in y-intercept after echothiophate for accommodation ($p < 0.05$), indicating that the entire curve shifted down.

Discussion

Rhesus monkeys provide a unique animal model of human accommodation because the anterior segment anatomy, receptor type distribution and accommodative mechanism closely resemble that of humans (Glasser and Kaufman, 1999; Koretz, Bertasso, Neider, True-Gabelt and Kaufman, 1987; Koretz, Neider, Kaufman, Bertasso, DeRousseau and Bito, 1987; van Alphen, 1976). Furthermore, rhesus monkeys provide a method by which accommodation can be stimulated repeatedly and rigorously controlled. Open loop accommodation confers

advantages as far as understanding the physiological effects of the drugs independent of the behavioral/psychophysical effects (Ostrin and Glasser, 2004; Vilupuru and Glasser, 2002). Detailed analysis of the dynamic accommodative response allows evaluation of the direct pharmacological action of a drug on the physiology of the iris and ciliary muscle in the absence of visual feedback which can also influence accommodation. In the current study, it has been demonstrated that the iris is affected by adrenergic (mydriasis) and cholinergic (miosis) stimulation and accommodative dynamics are slightly affected by low dose indirect cholinergic stimulation, beta adrenergic antagonism and non-selective adrenergic stimulation, with no effects on static accommodation (table 1).

Here, epinephrine was shown to have no effect on resting refraction in anesthetized monkeys, in agreement with previous studies (Crawford, Gabelt, Kaufman and Bito, 1990). A previous study performed in humans showed that timolol maleate causes a hyperopic shift in resting refraction, a decrease in accommodative amplitude, and a significant decrease in accommodative dynamics (Weber, Tuinenburg and Van Der Heijde, 1989). Still other reports suggest timolol maleate causes a myopic shift in tonic accommodation (Gilmartin, Hogan and Thompson, 1984a). Here, no change was found in either resting refraction or centrally stimulated accommodative amplitude in anesthetized monkeys with open loop accommodation. This study has, however, confirmed that there is a slight decrease in the peak velocity of accommodation and disaccommodation. Subtle changes in accommodative dynamics were demonstrated with all drugs evaluated here, but not with saline control. It may be possible that the altered dynamics resulted from changes in receptor configuration due to the various binding sites of the drugs, or from alterations in aqueous production or blood flow within the ciliary body, which could alter the volume and dynamics of the ciliary body. Although IOP was not evaluated here, timolol maleate is known to decrease IOP and cause changes in ciliary body and choroidal vasculature (Owens, Winn, Gilmartin and Pugh, 1991). These effects may have caused the subtle changes in accommodative dynamics following timolol maleate instillation. In the absence of any voluntary control over the accommodative system, resting refraction and maximum amplitude, as well as pupil diameter, are not affected by beta adrenergic antagonism.

Echothiophate is an anti-cholinesterase inhibitor, which is an indirect cholinergic agonist. In high doses, echothiophate causes ciliary muscle spasm and pupil constriction. Here in anesthetized monkeys, one drop of a low concentration (0.015%) echothiophate results in a strong miotic response and a small myopic shift in resting refraction. A previous study in humans showed that echothiophate in concentrations of 0.06-0.25% resulted in miosis, and therefore improved reading ability in presbyopic subjects (Cohen, 1966). Improved reading ability was also reported by one subject using topical echothiophate (Caplan, 2004). A decrease in pupil diameter may aid near reading in presbyopic patients due to an increase in depth of field, although it is unclear what the long term effects of chronic echothiophate use might be, and further investigation is warranted to determine if it is an appropriate treatment in presbyopic patients.

Previous studies of adrenergic drugs have demonstrated that beta sympathetic stimulation by isoproterenol significantly decreased centrally stimulated accommodation by over 50% in vervet monkeys (Hurwitz, Davidowitz, Chin and Breinin, 1972a). It was also found that levarterenol, a non-selective sympathetic agonist with higher affinity for alpha rather than beta receptors, decreased accommodation, and the effect was blocked by propranolol, a beta-blocker (Hurwitz, Davidowitz, Pachter and Breinin, 1972). These studies suggest that beta adrenergic stimulation can greatly decrease accommodation. It is unclear why the current study has found only subtle effects of beta adrenergic stimulation, evaluated with epinephrine, on accommodative dynamics and no effect on accommodative amplitude, as compared to previous studies with different sympathetic drugs. It may be that with the drug

instillation protocol presented here, which is similar to dosages used in humans for therapeutic purposes as opposed to stronger concentrations, the drugs maintain specificity and do not saturate all receptors in the anterior segment. There may also be species differences in anterior segment receptors between vervet and rhesus monkeys.

No clear role for the sympathetic system was demonstrated in accommodative dynamics, as both accommodative and disaccommodative responses slowed with beta-adrenergic antagonism and disaccommodative responses slowed with non-selective adrenergic stimulation. However, as shown here and in previous studies (Ostrin and Glasser, 2007; Ostrin and Glasser, 2004), resting refraction and accommodative responses to step stimuli are not affected by the sympathetic system, although accommodative dynamics can be altered through pharmacological manipulations.

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Reference List

- Bito LZ, Kaufman PL, DeRousseau CJ, Koretz J. Presbyopia: an animal model and experimental approaches for the study of the mechanism of accommodation and ocular aging. *Eye*. 1987; 1(Pt 2): 222–30. [PubMed: 3308526]
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986; 1:307–10. [PubMed: 2868172]
- Caplan J. PI monovision for presbyopia. *Binocular Vision Strabismus Quarterly*. 2004; 19:133. [PubMed: 15320858]
- Chen JC, Schmid KL, Brown B. The autonomic control of accommodation and implications for human myopia development: a review. *Ophthalmic Physiol Opt*. 2003; 23:401–22. [PubMed: 12950887]
- Chin NB, Ishikawa S, Lappin H, Davidowitz J, Breinin GM. Accommodation in monkeys induced by midbrain stimulation. *Invest Ophthalmol*. 1968; 7:386–96. [PubMed: 4969601]
- Cogan D. Accommodation and the autonomic nervous system. *Arch Ophthalmol*. 1937; 18:739–66.
- Cohen SW. Management of errors of refraction with echothiophate iodide. *Am J Ophthalmol*. 1966; 62:303–12. [PubMed: 5944748]
- Crawford K, Gabelt BT, Kaufman PL, Bito LZ. Effects of various anesthetic and autonomic drugs on refraction in monkeys. *Curr Eye Res*. 1990; 9:525–32. [PubMed: 2387164]
- Crawford K, Terasawa E, Kaufman PL. Reproducible stimulation of ciliary muscle contraction in the cynomolgus monkey via a permanent indwelling midbrain electrode. *Brain Res*. 1989; 503:265–72. [PubMed: 2605519]
- Crawford KS, Kaufman PL, Bito LZ. The role of the iris in accommodation of rhesus monkeys. *Invest Ophthalmol Vis Sci*. 1990; 31:2185–90. [PubMed: 2211015]
- Croft MA, Kaufman PL, Erickson-Lamy K, Polansky JR. Accommodation and ciliary muscle muscarinic receptors after echothiophate. *Invest Ophthalmol Vis Sci*. 1991; 32:3288–97. [PubMed: 1748559]
- Cyrlin MN, Thomas JV, Epstein DL. Additive effect of epinephrine to timolol therapy in open angle glaucoma. *Arch Ophthalmol*. 1982; 100:414–8. [PubMed: 7065957]
- Demailly P, Arrata M. Long-term treatment with timolol ophthalmic solution in open-angle glaucoma. *Ophthalmologica*. 1980; 181:221–7. [PubMed: 7231889]
- Do T, Kasthurirangan S, Ostrin LA, Glasser A. The Effects of Phenylephrine on Accommodation in Humans. *Optometry and Vision Science*. 79s abstract 174. 2002.
- Fincham EF. The coincidence optometer. *Proc Phys Soc (London)*. 1937; 49:456–68.

- Gabelt BT, hennes EA, seeman JL, Tian B, Kaufman PL. H-7 Effect on Outflow Facility after Trabecular Obstruction Following Long-Term Echothiophate Treatment in Monkeys. *Investigative Ophthalmology and Vision Science*. 2004; 45:2732–6.
- Gabelt BT, Kaufman PL. Inhibition of outflow facility and accommodative and miotic responses to pilocarpine in rhesus monkeys by muscarinic receptor subtype antagonists. *J Pharmacol Exp Ther*. 1992; 263:1133–9. [PubMed: 1469625]
- Gilmartin B. A review of the role of sympathetic innervation of the ciliary muscle in ocular accommodation. *Ophthalmic Physiol Opt*. 1986; 6:23–37. [PubMed: 2872644]
- Gilmartin B, Bullimore MA. Sustained near-vision augments inhibitory sympathetic innervation of the ciliary muscle. *Clinical Vision Science*. 1987b; 1:197–208.
- Gilmartin B, Bullimore MA. Sustained near vision augments inhibitory sympathetic innervation of the ciliary muscle. *Clinical Vision Science*. 1987a; 1:197–208.
- Gilmartin B, Bullimore MA, Rosenfield M, Winn B, Owens H. Pharmacological effects on accommodative adaptation. *Optom Vis Sci*. 1992; 69:276–82. [PubMed: 1565427]
- Gilmartin B, Hogan RE, Thompson SM. The effect of Timolol Maleate on tonic accommodation, tonic vergence, and pupil diameter. *Invest Ophthalmol Vis Sci*. 1984a; 25:763–70. [PubMed: 6724847]
- Gilmartin B, Hogan R, Thompson S. The effect of timolol maleate on tonic accommodation, tonic vergence and pupil diameter. *Invest Ophthalmol Vis Sci*. 1984b; 25:763–70. [PubMed: 6724847]
- Gilmartin B, Mallen EA, Wolffsohn JS. Sympathetic control of accommodation: evidence for inter-subject variation. *Ophthalmic Physiol Opt*. 2002; 22:366–71. [PubMed: 12358304]
- Gimpel G, Doughty MJ, Lyle WM. Large sample study of the effects of phenylephrine 2.5% eyedrops on the amplitude of accommodation in man. *Ophthalmic Physiol Opt*. 1994; 14:123–8. [PubMed: 8022593]
- Glasser A, Kaufman PL. The mechanism of accommodation in primates. *Ophthalmology*. 1999; 106:863–72. [PubMed: 10328382]
- Hurwitz BS, Davidowitz J, Chin NB, Breinin GM. The effects of the sympathetic nervous system on accommodation. I. Beta sympathetic nervous system. *Arch Ophthalmol*. 1972a; 87:668–74. [PubMed: 4402365]
- Hurwitz BS, Davidowitz J, Pachter BR, Breinin GM. The effects of the sympathetic nervous system on accommodation. II. Alpha sympathetic nervous system. *Arch Ophthalmol*. 1972; 87:675–8. [PubMed: 4402366]
- Hurwitz B, Davidowitz J, Chin N, Breinin G. The effects of the sympathetic nervous system on accommodation: 1. Beta sympathetic nervous system. *Arch Ophthalmol*. 1972b; 87:668–74. [PubMed: 4402365]
- Kaufman PL, Lütjen-Drecoll E. Total iridectomy in the primate in vivo: surgical technique and postoperative anatomy. *Invest Ophthalmol Vis Sci*. 1975; 14(10):766–71.
- Kim TW, Kim JH, Hwang JM. Long-term outcome of patients with large overcorrection following surgery for exotropia. *Ophthalmologica*. 2005; 219:237–42. [PubMed: 16088244]
- Koretz JF, Bertasso AM, Neider MW, True-Gabelt B, Kaufman PL. Slit-lamp studies of the rhesus monkey eye. II Changes in crystalline lens shape, thickness and position during accommodation and aging. *Exp Eye Res*. 1987; 45:317–26. [PubMed: 3653294]
- Koretz JF, Neider MW, Kaufman PL, Bertasso AM, DeRousseau CJ, Bito LZ. Slit-lamp studies of the rhesus monkey eye. I. Survey of the anterior segment. *Exp Eye Res*. 1987; 44:307–18. [PubMed: 3582515]
- Krishna N, Leopold IH. Echothiophate (phospholine) iodide (217-MI) in the treatment of glaucoma: further observations. *AMA Archives of Ophthalmology*. 1959; 62:300–13. [PubMed: 13669812]
- Lutjen-Drecoll E, Kaufman PL. Echothiophate-induced structural alterations in the anterior chamber angle of the cynomolgus monkey. *Investigative Ophthalmology and Vision Science*. 1979; 18:918–29.
- Lutjen-Drecoll E, Kaufman PL. Biomechanics of echothiophate-induced anatomic changes in monkey aqueous outflow system. *Graefe's Archives of Clinical and Experimental Ophthalmology*. 1986; 224:564–75.

- Ostrin LA, Glasser A. Effects of Pharmacologically Manipulated Amplitude and Starting Point on Edinger-Westphal Stimulated Accommodative Dynamics in Rhesus Monkeys. *Invest Ophthalmol Vis Sci.* 2007; 48:313–20. [PubMed: 17197548]
- Ostrin LA, Frishman L, Glasser A. Effects of pirenzepine on pupil size and accommodation in rhesus monkeys. *Invest Ophthalmol Vis Sci.* 2004; 45:3620–8. [PubMed: 15452069]
- Ostrin LA, Glasser A. The effects of phenylephrine on pupil diameter and accommodation in rhesus monkeys. *Invest Ophthalmol Vis Sci.* 2004; 45:215–21. [PubMed: 14691176]
- Ostrin LA, Glasser A. Comparisons between pharmacologically and Edinger-Westphal-stimulated accommodation in rhesus monkeys. *Invest Ophthalmol Vis Sci.* 2005; 46:609–17. [PubMed: 15671289]
- Owens H, Winn B, Gilmartin B, Pugh JR. Effect of a topical beta-adrenergic receptor antagonist on the dynamics of steady-state accommodation. *Ophthalmic Physiol Opt.* 1991; 11:99–104. [PubMed: 2062546]
- Paggiarino DA, Brancato LJ, Newton RE. The effects on pupil size and accommodation of sympathetic and parasympatholytic agents. *Ann Ophthalmol.* 1993; 25:244–53. [PubMed: 8363290]
- Ruskell GL. Sympathetic innervation of the ciliary muscle in monkeys. *Exp Eye Res.* 1973; 16:183–90. [PubMed: 4198985]
- Schaeffel F, Farkas L, Howland HC. Infrared photoretinoscope. *Appl Opt.* 1987; 26:1505–9. [PubMed: 20454351]
- Schaeffel F, Wilhelm H, Zrenner E. Inter-individual variability in the dynamics of natural accommodation in humans: relation to age and refractive errors. *J Physiol.* 1993; 461:301–20. [PubMed: 8350267]
- Stephens KG. Effect of the sympathetic nervous system on accommodation. *Am J Optom Physiol Opt.* 1985; 62:402–6. [PubMed: 2990216]
- Tornqvist G. The relative importance of the parasympathetic and sympathetic nervous systems for accommodation in monkeys. *Invest Ophthalmol Vis Sci.* 1967; 6:612–7.
- van Alphen GW. The adrenergic receptors of the intraocular muscles of the human eye. *Invest Ophthalmol.* 1976; 15:502–5. [PubMed: 6403]
- Vasudevan B, Ciuffreda K, Gilmartin B. Sympathetic inhibition of accommodation after sustained nearwork in subjects with myopia and emmetropia. *Investigative Ophthalmology and Vision Science.* 2009; 50:114–20.
- Vilupuru AS, Glasser A. Dynamic accommodation in rhesus monkeys. *Vision Res.* 2002; 42:125–41. [PubMed: 11804637]
- Vilupuru AS, Glasser A. Dynamic accommodative changes in rhesus monkey eyes assessed with A-scan ultrasound biometry. *Optom Vis Sci.* 2003; 80:383–94. [PubMed: 12771664]
- Walinder PE, Bill A. Influence of intraocular pressure and some drugs on aqueous flow and entry of cycloleucine into the aqueous humor of vervet monkeys (*Cercopithecus ethiops*). *Invest Ophthalmol.* 1969; 8:446. [PubMed: 4978725]
- Wax MB, Molinoff PB. Distribution and properties of beta adrenergic receptors in the human iris-ciliary body. *Invest Ophthalmol Vis Sci.* 1987; 28:420–30. [PubMed: 3030954]
- Weber J, Tuinenburg AE, Van Der Heijde GL. Effect of timolol on the amplitude and dynamics of accommodation. *Doc Ophthalmol.* 1989; 72:341–7.
- Winn B, Culhane HM, Gilmartin B, Strang NC. Effect of beta-adrenoceptor antagonists on autonomic control of ciliary smooth muscle. *Ophthalmic Physiol Opt.* 2002; 22:359–65. [PubMed: 12358303]
- Zetterström C. The effect of phenylephrine on the accommodative process in man. *Acta Ophthalmol.* 1984; 62:872–8. [PubMed: 6524312]
- Zetterstrom C, Hahnenberger R. Pharmacological characterization of human ciliary muscle adrenoreceptors in vitro. *Exp Eye Res.* 1988; 46:421–30. [PubMed: 3350077]

Figure 1.

Pupil diameter increased significantly by 2.76 ± 0.38 mm ($p < 0.01$, $df = 3$) in eyes treated with 2% epinephrine (A), was unchanged in eyes treated with 0.5% timolol maleate ($p = 0.91$) (B), and decreased significantly by 3.07 ± 0.65 mm ($p < 0.01$, $df = 4$) in eyes treated with 0.015% echothiophate (C).

Figure 2.

Resting refraction was unchanged in eyes treated with 2% epinephrine ($p=0.30$) (A) and 0.5% timolol maleate ($p=0.94$) (B). There was a significant myopic shift in resting refraction of 1.30 ± 0.39 D in eyes treated with 0.015% echothiophate ($p<0.05$, $df=4$).

Figure 3.

Bland-Altman analysis of the mean difference of the static accommodative responses to increasing stimulus currents before and after drug instillation was unchanged following instillation of saline (A), 2% epinephrine (B), 0.5% timolol maleate (C) and 0.015% echothiophate (D). The difference in the accommodative response to each stimulus current between the baseline measurements and after drug or saline instillation is shown on the y axis. For each drug, there was no overall change in accommodative response after instillation.

Figure 4.

There was no change in the accommodative amplitude to a stimulus previously determined to produce maximum accommodation, measured every five minutes after epinephrine, timolol maleate or saline instillation ($p=0.43$, $p=0.51$, $p=0.49$, respectively), or the accommodative amplitude to an approximately 1/3, 2/3, and maximum stimulus amplitude measured every 10 minutes after echothiophate instillation ($p=0.20$, $p=0.11$, $p=0.98$, respectively) as measured with infrared photorefracton.

Figure 5.

Linear peak velocity vs. amplitude relationships before and after saline and drug treatments show a decrease in slope for disaccommodation after 2% epinephrine instillation, for accommodation and disaccommodation after 0.05% timolol maleate instillation and disaccommodation after 0.015% echothiophate instillation. There was a decrease in y-intercept for accommodation after 0.015% echothiophate instillation. * = significant difference in slope, + = significant difference in intercept

Table 1

Effects of various autonomic drugs on pupil diameter, resting refraction and accommodation in anesthetized, adolescent rhesus monkeys using the protocol presented in the current study and described previously (Ostrin and Glasser, 2007; Ostrin, Frishman and Glasser, 2004; Ostrin and Glasser, 2005; Ostrin and Glasser, 2004).

	Mechanism	Pupil Diameter	Resting Refraction	Accommodation
Adrenergic				
Epinephrine (2% topical) (current study)	Non-selective adrenergic agonist	Mydriasis	No effect	Subtle change in dynamics
Timolol maleate (0.5% topical) (current study)	Beta adrenergic antagonist	No effect	No effect	Subtle change in dynamics
Phenylephrine (10% topical) (Ostrin and Glasser, 2004)	Alpha 1 adrenergic agonist	Mydriasis	No effect	Subtle change in dynamics
Cholinergic				
Echothiophate (0.015% topical)	Anticholinesterase inhibitor	Miosis	Slight myopic shift	Subtle change in dynamics
Atropine (40% iontophoresis) (Ostrin and Glasser, 2007)	Non-selective cholinergic antagonist	Mydriasis	Slight hyperopic shift	Cycloplegia
Pirenzepine (2% subconj injection)(Ostrin, Frishman and Glasser, 2004)	Non-selective cholinergic antagonist (M1>M2-5)	Mydriasis	Hyperopic shift	Cycloplegia
Pilocarpine (6% topical)(Ostrin and Glasser, 2007)	Cholinergic agonist	Miosis	Large myopic shift	Ciliary muscle spasm
Carbachol (40% iontophoresis) (Ostrin and Glasser, 2005)	Cholinergic agonist	Miosis	Large myopic shift	Ciliary muscle spasm