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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 min 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 ± SEM, $p < 0.01$), and a myopic shift in resting refraction of 1.30 ± 0.39 D ($p < 0.05$) 90 min after instillation. Epinephrine caused a 2.76 ± 0.38 mm ($p < 0.01$) increase in pupil diameter with no change in resting refraction 60 min after instillation. Timolol maleate resulted in no significant change in either pupil diameter or resting refraction 60 min 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.

1. 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 et al., 2003; Gilmartin et al., 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, 1987; Tornqvist, 1967; Vasudevan et al., 2009). There is also significant inter-subject variations in this sympathetic facility (Gilmartin et al., 2002). The primate ciliary

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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 $beta_2$ subtype rather than $beta_1$, $alpha_1$ or $alpha_2$ subtypes (Hurwitz et al., 1972a; Stephens, 1985; van Alphen, 1976; Wax and Molinoff, 1987; Zetterstrom and Hahnenberger, 1988), although a small population of $alpha_1$ 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 $beta_1$ 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 et al., 1968; Gimpel et al., 1994; Hurwitz et al., 1972a; Paggiarino et al., 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 et al., 2002). 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 et al., 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, 1987; Gilmartin et al., 1984; Hurwitz et al., 1972a; 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 (Cyrlin et al., 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 et al., 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 et al., 2004; Lutjen-Drecoll and Kaufman, 1986). Chronic echothiophate use also results in a decreased accommodative response to pilocarpine (Croft et al., 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 et al., 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.

2. 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 et al., 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.

2.1. 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 min 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

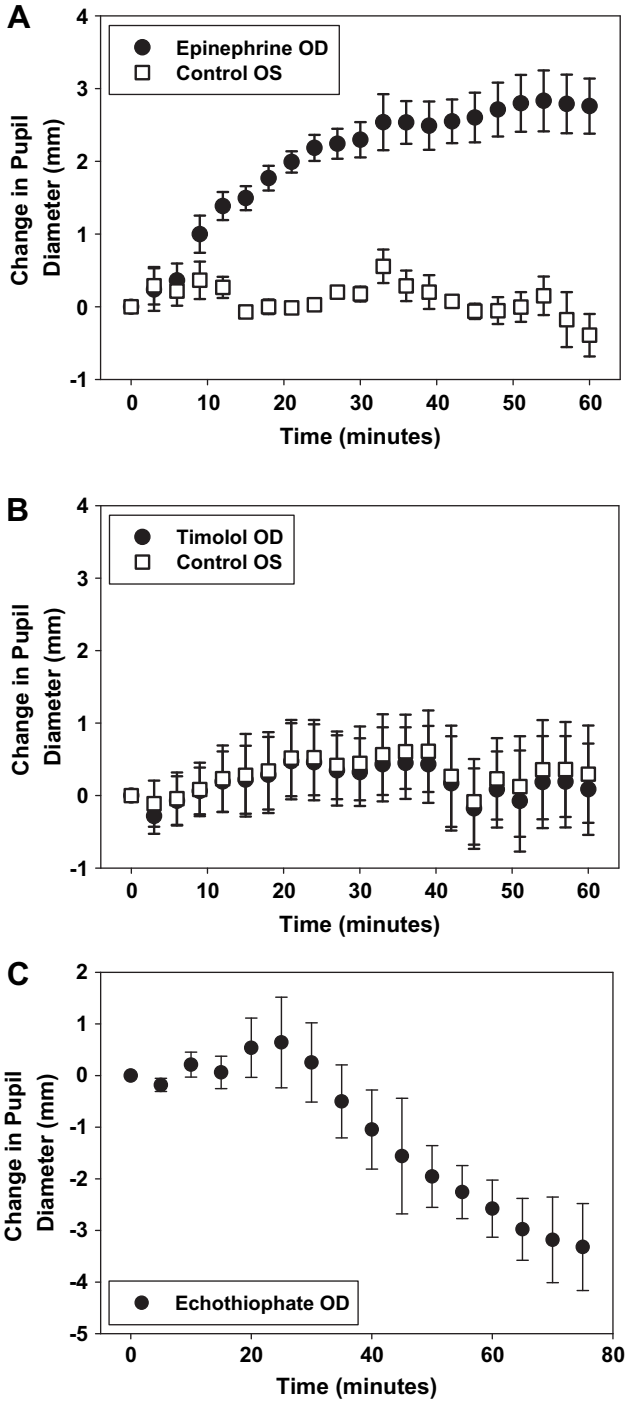

Fig. 1. Pupil diameter increased significantly by 2.76 ± 0.38 mm ($p < 0.01$, df = 3) in eyes treated with 2% epinephrine (A), was unchanged in eyes treated with 0.5% timolol maleate ($p = 0.91$) (B), and decreased significantly by 3.07 ± 0.65 mm ($p < 0.01$, df = 4) in eyes treated with 0.015% echothiophate (C).

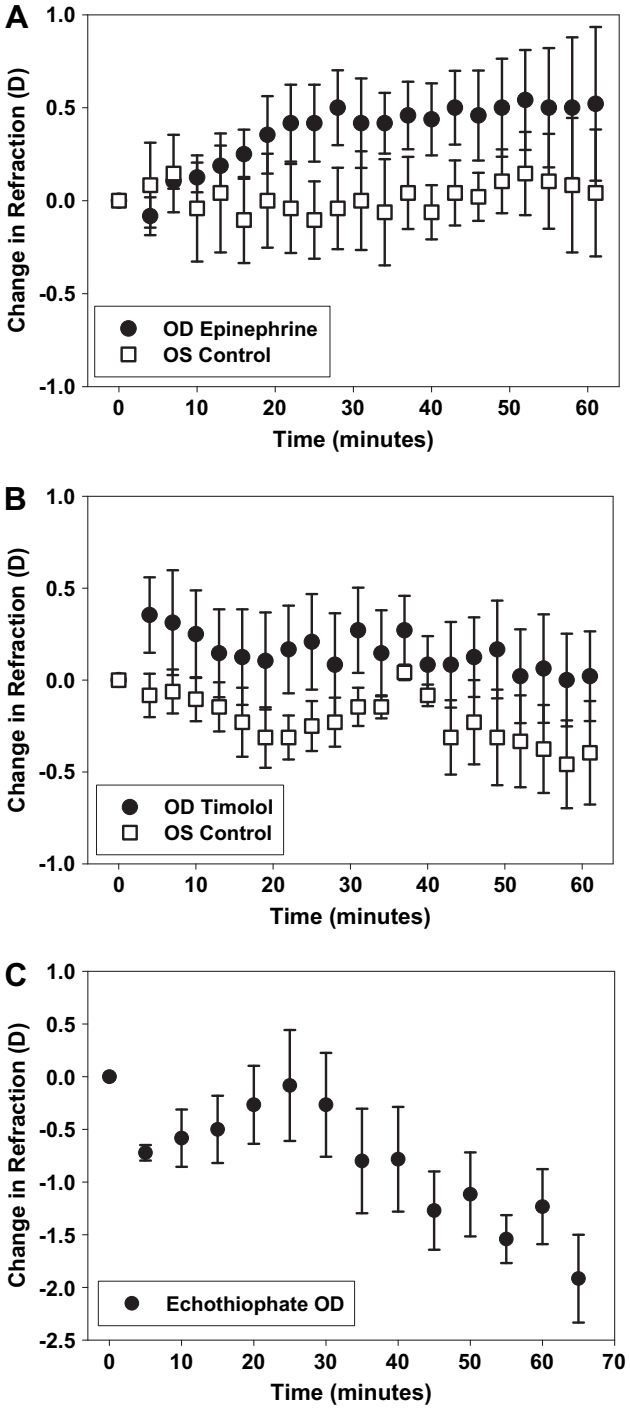

Fig. 2. Resting refraction was unchanged in eyes treated with 2% epinephrine ($p = 0.30$) (A) and 0.5% timolol maleate ($p = 0.94$) (B). There was a significant myopic shift in resting refraction of 1.30 ± 0.39 D in eyes treated with 0.015% echothiophate ($p < 0.05$, df = 4).

and prevent dehydration. Vital signs were monitored, including heart rate and SpO_2, 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 3 min after topical instillation of the drug in one eye for 60 min using video pupillography (Ostrin et al., 2004; Ostrin and Glasser, 2004). The eye was illuminated by 20 infrared diodes placed on the lower half of the camera lens. Gray-scale 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 3 min after topical instillation of the drug for 60 min. 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 min, another 0.1 ml was again applied to the cornea, and after 30 s, 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 5 min 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 1 min 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.

2.2. 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 et al., 1989; Glasser and Kaufman, 1999). The monkeys are used in multiple experimental protocols (Glasser and Kaufman, 1999; Vilupuru and Glasser, 2002, 2003) and the iridectomies (Kaufman and Lütjen-Drecoll, 1975), justification for them (Bito et al., 1987) and absence of an effect on centrally stimulated accommodation (Crawford et al., 1990b) 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 min after topical instillation of 0.2 ml 2% epinephrine, 0.2 ml 0.5% timolol maleate and saline, and 70 min 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 photorefraction 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 μA 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 min following epinephrine, timolol maleate or saline instillation, and 70 min following echothiophate instillation.

Dynamic accommodative responses were measured with infrared photorefraction to determine the relationship between the peak

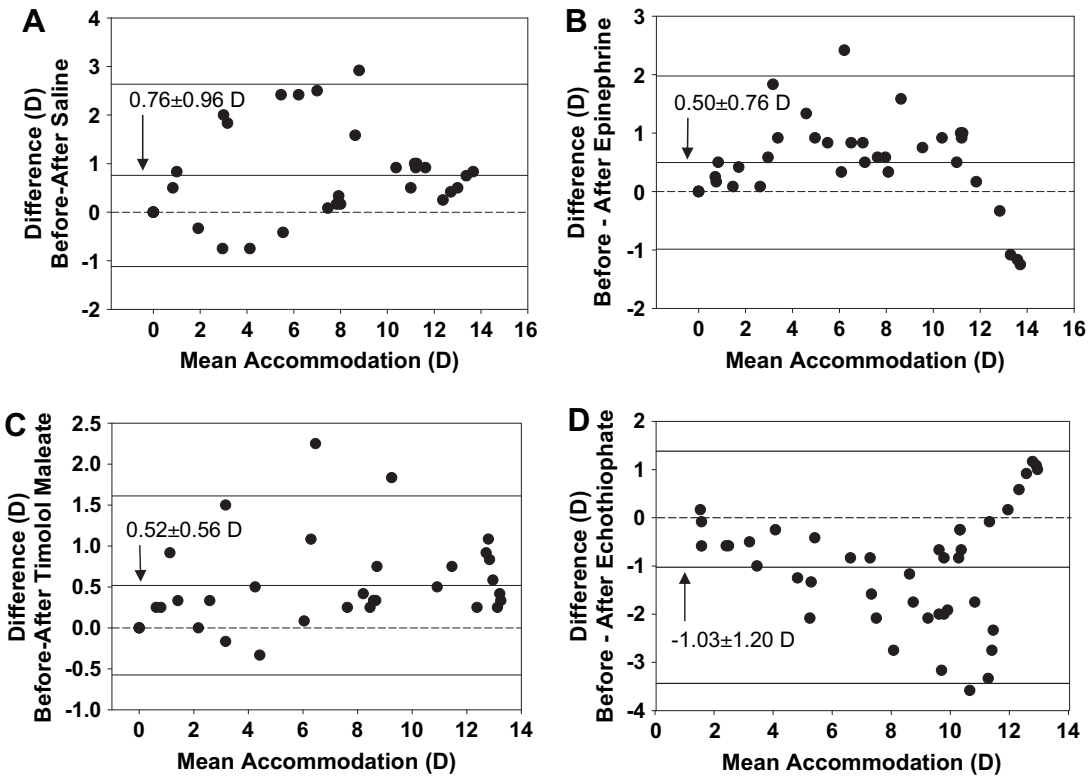

Fig. 3. Bland-Altman analysis of the mean difference of the static accommodative responses to increasing stimulus currents before and after drug instillation was unchanged following instillation of saline (A), 2% epinephrine (B), 0.5% timolol maleate (C) and 0.015% echothiophate (D). The difference in 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.

velocity and the amplitude of the accommodative responses (a main sequence relationship) (Vilupuru and Glasser, 2002). Calibrated photorefraction (Schaeffel et al., 1987, 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 photorefraction, 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 photorefraction images in the central 40% of the pupil, recorded while the eye was accommodated, was determined. The relationship between the photorefraction measured slope and HCR measured refraction provided the calibration function which was used to convert each video frame of the dynamically measured photorefraction 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 5 min for 60 min for a fixed stimulus current amplitude previously determined to elicit maximum accommodation. For echothiophate, the dynamic accommodative response was recorded every 10 min for 70 min 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 min (epinephrine, timolol maleate and saline) or 70 min (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.

2.3. 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 min 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):

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

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

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.

3. Results

3.1. Pupil and resting refraction

All values for pupil diameter, resting refraction and maximum accommodative amplitude are given as mean ± 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 ± 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) (Fig. 1), and resting refraction was not significantly different at +3.37 ± 1.17 D ($p = 0.30$, df = 3) (Fig. 2). Prior to 0.5% timolol maleate instillation, pupil diameter was 4.55 ± 0.81 mm and resting refraction was +2.71 ± 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 ± 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 ± 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 min (74 ± 5.3 min). 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 ± 2.33, a significant myopic shift of 1.30 ± 0.39 D ($p < 0.05$, df = 4).

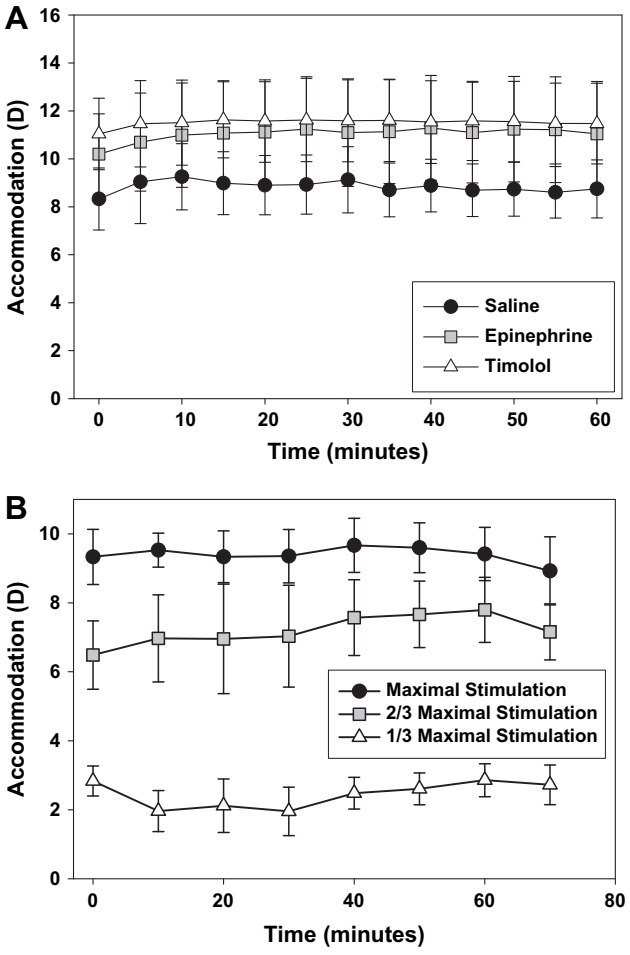

Fig. 4. There was no change in the accommodative amplitude to a stimulus previously determined to produce maximum accommodation, measured every 5 min after epinephrine, timolol maleate or saline instillation ($p = 0.43$, $p = 0.51$, $p = 0.49$, respectively), or the accommodative amplitude to an approximately 1/3, 2/3, and maximum stimulus amplitude measured every 10 min after echothiophate instillation ($p = 0.20$, $p = 0.11$, $p = 0.98$, respectively) as measured with infrared photorefraction.

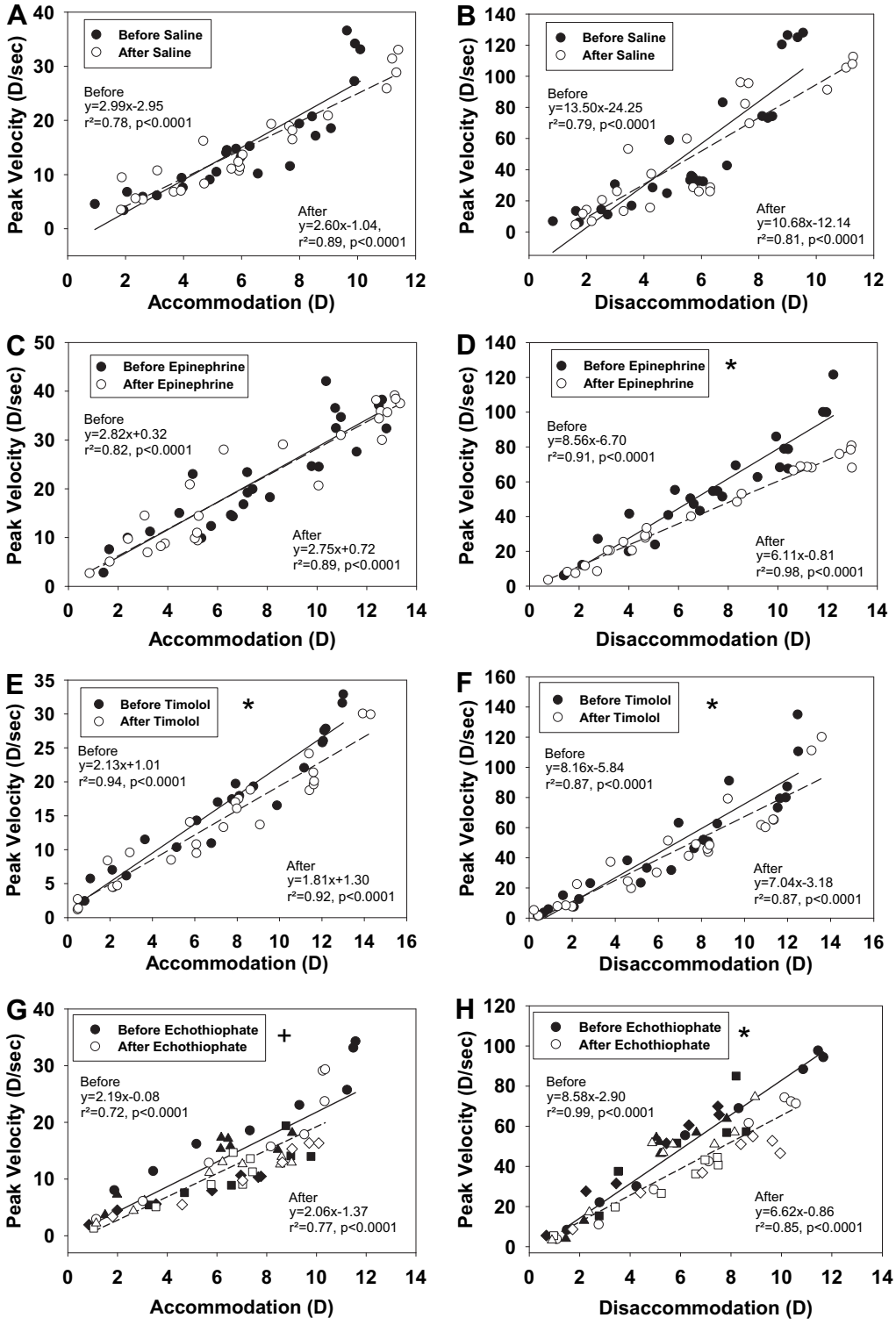

Fig. 5. Linear peak velocity vs. amplitude relationships before and after saline and drug treatments show a decrease in slope for disaccommodation after 2% epinephrine instillation, for accommodation and disaccommodation after 0.05% timolol maleate instillation and disaccommodation after 0.015% echothiophate instillation. There was a decrease in *y*-intercept for accommodation after 0.015% echothiophate instillation. * = significant difference in slope, $^{+}$ = significant difference in intercept.

3.2. 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 ± 0.96 D before and after saline, +0.50 ± 0.76 D before and after epinephrine, +0.52 ± 0.56 D before and after timolol maleate and −1.03 ± 1.20 D before and after echothiophate (Fig. 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 5 min for 60 min 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 min for 70 min after echothiophate instillation ($p = 0.20$, $p = 0.11$, $p = 0.98$, respectively) (Fig. 4) as measured with infrared photorefraction. While there are slight differences in the baseline accommodative response between experiments, seen in Fig. 4A, there is no change in the accommodative response with time for each agent tested.

3.3. 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 (Fig. 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: $p = 0.359$; 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 ($p < 0.05$), seen in that the entire curve shifted down.

4. 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 et al., 1987a,b; van Alphen, 1976). Furthermore, Edinger-Westphal stimulation 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 et al., 1990a). 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 et al., 1989). Still other reports suggest timolol maleate causes a myopic shift in tonic accommodation (Gilmartin et al., 1984). 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 et al., 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.

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, 2005, 2004; Ostrin et al., 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)	Alpha1 adrenergic agonist	Mydriasis	No effect	Subtle change in dynamics
Cholinergic				
Echothiophate (0.015% topical)	Anti-cholinesterase 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 et al., 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

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 isoproteronol significantly decreased centrally stimulated accommodation by over 50% in vervet monkeys (Hurwitz et al., 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 et al., 1972b). 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, 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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