The Effects of Phenylephrine on Pupil Diameter and Accommodation in Rhesus Monkeys

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PURPOSE. Phenylephrine is used to dilate the iris through α-adrenergic stimulation of the iris dilator muscle. Sympathetic stimulation of the ciliary muscle is believed to be inhibitory, decreasing accommodative amplitude. Investigations in humans have suggested some loss of functional accommodation after phenylephrine. It is unclear whether this loss is due to direct action of phenylephrine on the ciliary muscle or to secondary optical factors associated with mydriasis. The purpose of this study was to determine whether phenylephrine affects Edinger-Westphal (EW)-stimulated accommodation in rhesus monkeys.

METHODS. The time course for maximum mydriasis was determined by videographic pupillography after phenylephrine instillation in 10 normal rhesus monkeys. Static and dynamic EW-stimulated accommodative responses were studied in five iridectomized rhesus monkeys before and after phenylephrine instillation. Accommodative amplitude was measured with a Hartinger coincidence refractometer. Dynamic accommodative responses were measured with infrared photorefraction, and functions were fitted to the data to determine peak velocity versus accommodative response relationships.

RESULTS. The maximum dilated pupil diameter of 8.39 ± 0.23 mm occurred 15 minutes after administration of phenylephrine. In iridectomized monkeys, postphenylephrine accommodative amplitudes were similar to prephenylephrine amplitudes. Dynamic analysis of the accommodative responses showed linear peak velocity versus accommodative amplitude relationships that were not statistically different before and after phenylephrine.

CONCLUSIONS. α-Adrenergic stimulation causes a strong pupil dilation in noniridectomized monkey eyes but does not affect EW-stimulated accommodative amplitude or dynamics in anesthetized, iridectomized rhesus monkeys. (Invest Ophthalmol Vis Sci. 2004;45:215–221) DOI:10.1167/iovs.03-0704

Phenylephrine is a sympathomimetic agent that is used clinically to dilate the iris without cycloplegia. Phenylephrine (2.5%) is used diagnostically for fundus examination, and 10% phenylephrine is used therapeutically to dilate the iris, be- cause optometrists normally fail to measure small pupil diameters. The effects of phenylephrine on accommodative amplitude and dynamics have not been unequivocally demonstrated.

Accommodation is the dioptric change in power of the crystalline lens that allows the eye to focus on near objects after contraction of the ciliary muscle. It is controlled by the parasympathetic division of the autonomic nervous system. An accommodative effort results in parasympathetically driven convergence, pupil constriction, and accommodation. The ciliary muscle is dually innervated by parasympathetic and sympathetic neurons.1,2 The role of sympathetic innervation in accommodation is not well understood. It has been suggested that sympathetic stimulation of the ciliary muscle is inhibitory, thereby decreasing accommodative amplitude.3 Patients with unilateral Horner’s syndrome (paralysis of the oculosympathetic pathway causing facial anhydrosis, ptosis, and pupillary constriction) have greater accommodative amplitudes in the affected eye relative to the unaffected eye.4

Phenylephrine is a relatively specific α1-adrenergic receptor agonist. The α1a-receptor subtype has a higher affinity for phenylephrine than the α1b-receptor subtype. Only approximately 1% of the nerve terminals in the monkey ciliary muscle are sympathetic.5 In humans, the ciliary muscle sympathetic receptors have been shown to be of the β2 subtype rather than β1, α1, or α2 subtypes.6,5–8 A small population of α1-adrenergic receptors has been identified in humans.5 Because there are so few α-adrenergic receptors on the ciliary muscle, it is unlikely that phenylephrine has a significant effect on accommodation.

Several aspects of accommodation have been studied in relation to the sympathetic contribution in both humans and monkeys, including effects on tonic accommodation, maximum accommodative amplitude, and dynamic response. It has been proposed that stimulation of the sympathetic system affects focus objects beyond the tonic position of accommodation,9 and that the sympathetic system inhibits the ciliary muscle to achieve “distance accommodation.”10 Later termed the sympathetic range of accommodation.11 It has also been suggested that the β-adrenergic system plays a role in conjunction with the parasympathetic system in maintaining the level of tonic accommodation.12,13 Pharmacological studies with the β-adrenergic antagonists timolol maleate and isoprenaline and the parasympathetic antagonist tropicamide have led to the suggestion that, although the sympathetic system is involved in tonic accommodation, variations in the state of tonic accommodation among individuals are not determined by sympathetic tone, but by parasympathetic tone.14,15 Phenylephrine has been reported both not to change the resting state of accommodation16 and to cause a myopic shift in resting accommodation.17 Gilmartin18 concluded that the sympathetic system does not affect tonic accommodation, but provides a small, slow component of accommodation. The β-adrenergic antagonist timolol maleate is capable of increasing post-task regression to baseline refraction after a sustained reading task,19 which, in agreement with previous studies,16 suggests that the sympathetic contribution to accommodation may be evident after prolonged near work. This allows a build-up of sympathetic inhibitory activity over a background of parasympathetic activity, helping return the accommodative system to its baseline refraction.
Several studies have suggested that phenylephrine affects accommodative amplitude. Studies in humans have reported average decreases up to 3 diopters in maximum, subjectively measured accommodative amplitude. Many of these studies used the push-up technique to determine the near point. This requires the subject to report when an approaching target is too close to read clearly. Although the push-up test is used clinically to measure accommodation and is a functional subjective measure of near vision, it does not unequivocally determine the dioptric change in power of the eye. There are many confounding factors that influence the results of the push-up test, such as pupil size and ocular aberrations. When measuring accommodative amplitude after phenylephrine, the iris dilates, and the depth of field is greatly reduced. This may explain the perceived reduction in subjectively measured accommodation.

Even objective measurement of accommodation may not unequivocally prove that phenylephrine has an effect on the accommodative plant when a human subject is required to provide an accommodative response to a visual stimulus. The subject must see the distant and near targets clearly to produce the appropriate accommodative response. The response amplitude may be affected by decreased depth of field and increased ocular aberrations due to mydriasis rather than from a decrease in accommodative ability. Therefore, an objectively measured decrease in maximum amplitude or change in accommodative dynamics may result from the effect that mydriasis has on the subject’s perception of blur. Investigators who have examined the effects of sympathetic agents on various aspects of stimulus driven dynamic accommodation responses in humans have reported both no change in the step-response times and slowed response times after phenylephrine administration.

The effect of sympathetic agonists on accommodation has also been studied in monkeys, with contradictory results. Vervet monkeys had a reduced centrally stimulated accommodative amplitude after 5% levarterenol, an alpha-adrenergic agonist. This was attributed to a beta-agonist effect of the drug, rather than an alpha-agonist effect, as the effect was blocked by a beta-blocker but not an alpha-blocker. Others have found that accommodation is “relatively unaffected” by 10% phenylephrine, or decreases by up to 7 diopters with brain-stimulated accommodation after 1 drop of 2.5% phenylephrine. This decrease was attributed to vasoconstriction of the ciliary body vasculature, reducing the volume of the ciliary body and depressing accommodative amplitude.

Central stimulation of accommodation in anesthetized monkeys offers an opportunity to establish the direct pharmacological action of a drug on the physiology of the ciliary muscle. Open-loop accommodation can be stimulated in anesthetized monkeys with an electrode implanted in the Edinger-Westphal (EW) nucleus of the midbrain. The accommodative response achieved is not affected by pupil size or visual feedback, can be rigorously controlled by stimulus amplitude, and can be accurately quantified with objective measurements, such as infrared photorefraction. Rhesus monkeys represent a unique model for human accommodation because they have high accommodative amplitudes and an accommodative mechanism similar to that of humans. The intraocular adrenergic receptor characteristics closely resemble those of the human eye. Because rhesus monkeys and humans are most frequently used in accommodation studies, it is of interest to understand the effects of phenylephrine on the accommodative plant of rhesus monkeys and humans, rather than in cynomolgus or vervet monkeys. In the present study, the effects of 10% phenylephrine on centrally stimulated accommodation were studied in rhesus monkeys to determine the effects on amplitude, dynamics, and resting state. This study may differentiate effects of phenylephrine on the ciliary muscle versus effects due to secondary optical factors resulting from mydriasis.

**Methods**

Experiments were performed on 15 rhesus monkeys (*Macaca mulatta*). Ten monkeys with normal intact irides were used to ascertain the effects of phenylephrine on pupil diameter. Five monkeys that had been surgically iridectomized and had stimulating electrodes surgically implanted in the EW nucleus of the brain were used to test the effects of phenylephrine on accommodation. All experiments conformed to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and were conducted under an institutionally approved animal protocol.

**Phenylephrine Dosage Experiment**

**Animal Preparation.** Ten 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, as required. Monkeys were placed prone with the head upright and facing forward in a head holder. The eyelids were held open with a speculum. A custom made PMMA contact lens (Metro Optics, Austin, TX) was placed on the cornea to maintain clarity and hydration.

**Phenylephrine Dosage Determination.** In one noniridectomized monkey (118), four topical phenylephrine concentrations and dosage protocols were used to determine the extent and time course of pupil dilation in four different sessions, each at least 3 days apart. The protocols were (1) one 0.1-mL dose (approximately 2 drops) of 2.5% phenylephrine; (2) two 0.1-mL doses of 2.5% phenylephrine 2 minutes apart; (3) one 0.1-mL dose of 10% phenylephrine; and (4) two 0.1-mL doses of 10% phenylephrine 2 minutes apart. The effects of phenylephrine were determined by measuring the infrared, retroilluminated pupil diameters. Images of the pupil were captured with a video camera placed 0.3 meters from the eye (Cohu, San Diego, CA). The eye was illuminated with a bank of 20 infrared diodes placed behind a knife-edge aperture shielding the lower half of the camera lens. Ten prephenylephrine images were captured over 2 minutes and saved to a computer by frame grabber card and image analysis software (Optimas; Media Cybernetics, Silver Springs, MD). The contact lens was removed and phenylephrine was topically instilled. The cornea was irrigated with saline 30 seconds after administration of the last dose of phenylephrine, and the contact lens was replaced. Three images were captured every 5 minutes for 65 minutes, except with protocol 4, which was observed for 120 minutes. The calibrated images were later analyzed off-line.

**Pupil Diameter Measurements.** Once the appropriate phenylephrine protocol was determined to give maximum pupil dilation in a short time, this same protocol was tested in one eye each of 10 noniridectomized rhesus monkeys. The time course and extent of mydriasis were determined by videographic pupillography before and every 5 minutes after topical phenylephrine administration, as described earlier.

**Accommodation Experiments**

The five monkeys used for accommodation testing had undergone bilateral complete iridectomies and had stimulating electrodes surgically implanted in the EW nucleus of the midbrain. The monkeys were used repeatedly in multiple experimental protocols and the iridectomies, justification for it, and absence of an effect on centrally stimulated accommodation have been described previously. The EW-implemented monkeys were 3 (monkey 111), 4 (monkey 20), 5 (monkeys 38 and 70) and 11 (monkey 4) years old. Accommodative responses were stimulated and measured before and after topical instillation of phenylephrine. As a control, the protocol was repeated...
on one monkey (monkey 111) with topical instillation of saline instead of phenylephrine.

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 per minute). The contact lens was placed on the cornea, and sutures were tied beneath the lateral and medial rectus muscles to reduce eye movements during accommodation.32,56

**Static Accommodation Measurements.** Static EW-stimulated accommodative responses were measured before and 30 minutes after instillation of phenylephrine to determine the stimulus response function. The static Hartinger stimulus response function was later used to calibrate the dynamic photorefraction measurements and to compare the accommodative responses for each stimulus amplitude before and after phenylephrine instillation. First, baseline refraction was measured three times with a Hartinger coincidence refractometer (Carl Zeiss Meditec, Jena, Germany). An accommodative stimulus response function was then measured. Accommodation was stimulated using 2-second stimulus trains, with 10 current amplitudes ranging from 0 μA up to an amplitude sufficient to produce the maximum accommodative response available to each monkey. For each stimulus amplitude, accommodation was stimulated and measured three times in succession and averaged. Baseline refractions and the stimulus response function were measured again 30 minutes after phenylephrine instillation.

**Dynamic Accommodation Measurements.** To study the accommodative dynamics, infrared photorefraction was used to determine the relationship between the peak velocity and the amplitude of the accommodative responses (a main sequence relationship). Calibrated photorefraction was performed at a 0.3-m working distance and analyzed over 40% of the iridectomized pupil diameter. Before the instillation of phenylephrine, a sequence of dynamic accommodation responses to increasing stimulus currents was recorded with infrared photorefraction, using the same stimulus current amplitudes as those used for the Hartinger stimulus response function. Accommodative responses to eight stimulus amplitudes were recorded spanning the full accommodative range. For each stimulus amplitude, responses to five, 4-second stimulus trains were recorded. The first two of the five responses were recorded but not analyzed.

After the baseline recordings, two doses of 0.1 mL 10% phenylephrine were instilled topically, separated by 2 minutes, as described earlier. Dynamic accommodation was measured every 2 minutes for 20 minutes, at a stimulus amplitude previously determined to elicit maximum accommodation. The stimulation and measurement procedures were the same as those already described.

At 25 minutes after phenylephrine, a dynamic accommodation stimulus response sequence was recorded for amplitudes spanning the full range available to each monkey, using the same stimulus amplitudes as the prephenylephrine sequence.

**Dynamic Accommodation Analysis.** The effects of phenylephrine on dynamic accommodation were established in terms of peak velocities of accommodation and disaccommodation. Amplitude of accommodation, peak velocity of accommodation, and peak velocity of disaccommodation were determined for each stimulus amplitude of the prephenylephrine sequence, for the repeated single-amplitude stimuli after instillation of phenylephrine, and for the postphenylephrine stimulus amplitude sequence, using methods described previously. Maximum accommodative response was determined for each stimulus amplitude 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 were fitted to the accommodation phases and the disaccommodation phases. The derivative of these functions with respect to time gives the velocity profile of the responses. The $V_{\text{max}}$ achieved is the peak velocity. The sequence of analysis was as follows.

**RESULTS**

**Phenylephrine Dosage Experiments**

Figure 1 shows pupil diameter as a function of time after instillation of phenylephrine for the four different administration protocols tested in one noniridectomized eye of monkey 118. Two doses of 10% phenylephrine elicited the most dilation in the shortest time. Pupil diameter was measured for 120 minutes, over which period the mydriasis persisted (data not shown). From this experiment, it was determined that two doses of 10% phenylephrine were appropriate to elicit maximum mydriasis, and this protocol was tested in one eye each of 10 noniridectomized rhesus monkeys. Average pupil diameter of $8.39 \pm 0.23 \text{ mm}$ was recorded within 15 minutes (Fig. 2), and irides remained dilated for at least 65 minutes. A mean increase in pupil diameter of $3.82 \pm 1.01 \text{ mm}$ from baseline was achieved.

**Accommodation Experiments**

In the five monkeys in which accommodation was measured, dynamic accommodative responses to a single maximum stimulus amplitude were measured with photorefraction every 2 minutes after instillation of phenylephrine. There was no significant difference in amplitude between the average of three stimuli before phenylephrine and the average of the last three
stimuli at 16, 18, and 20 minutes after phenylephrine ($t$-test: $t = -0.6685, df = 4, P = 0.54$). Although the average results from the five monkeys receiving phenylephrine showed no significant change in amplitude 20 minutes after phenylephrine, individual monkeys showed changes from baseline (Table 1), with a decrease in three monkeys, an increase in two monkeys, and an increase in the saline control monkey.

Dynamic analysis of the accommodative responses measured with infrared photorefraction showed linear peak velocity versus amplitude relationships that were not significantly different before and 25 minutes after phenylephrine for accommodation (Fig. 3A, $P = 0.52$) or disaccommodation (Fig. 3B, $P = 0.24$). Although peak velocity and amplitude are both independent variables, linear regression analysis, as opposed to orthogonal regression analysis, was performed because the three accommodative responses were averaged before fitting the functions to generate the amplitude and peak velocity data. Therefore, information about variance, which is required for orthogonal regressions, was not available. However, the variance was not likely to differ before and after phenylephrine, and therefore the equation from the linear regression is not likely to differ from the equation that an orthogonal regression would yield.

**Table 1. Accommodative Amplitude before and 20 Minutes after 10% Phenylephrine Measured with Photorefraction**

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Amplitude Before (diopters)</th>
<th>Amplitude After (diopters)</th>
<th>Difference (diopters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8.30 ± 0</td>
<td>7.56 ± 0.04</td>
<td>-0.74</td>
</tr>
<tr>
<td>20</td>
<td>11.40 ± 0.04</td>
<td>11.10 ± 0.06</td>
<td>-0.29</td>
</tr>
<tr>
<td>38</td>
<td>10.99 ± 0.16</td>
<td>12.30 ± 0.24</td>
<td>+1.32</td>
</tr>
<tr>
<td>70</td>
<td>11.85 ± 0.35</td>
<td>15.12 ± 0.25</td>
<td>+3.27</td>
</tr>
<tr>
<td>111</td>
<td>13.02 ± 0.04</td>
<td>12.10 ± 0.02</td>
<td>-0.91</td>
</tr>
<tr>
<td>Mean</td>
<td>11.11 ± 1.75</td>
<td>11.64 ± 2.72</td>
<td>+0.53 ± 1.77</td>
</tr>
<tr>
<td>111 control</td>
<td>11.57 ± 0.07</td>
<td>11.87 ± 0.07</td>
<td>+0.30</td>
</tr>
</tbody>
</table>

For the five monkeys receiving 10% phenylephrine and the saline control, accommodative responses to a single maximum stimulus amplitude measured with infrared photorefraction showed no significant difference in amplitude between the three prephenylephrine stimuli and the last three stimuli at 16, 18, and 20 minutes after phenylephrine ($t$-test: $t = -0.6685, df = 4, P = 0.54$), although individual monkeys showed differences, including the saline control monkey. Data are expressed in diopters (mean ± SD).

**Figure 2.** Pupil diameters were measured in one eye each of 10 noniridectomized monkeys after two 0.1-mL doses of 10% phenylephrine. Mean maximum dilation was achieved after 15 minutes.

**Figure 3.** The linear relationship (from linear regressions) between peak velocity and accommodative amplitude (main sequence relationship) was not significantly different before and 25 minutes after phenylephrine instillation for (A) accommodation ($P = 0.52$) or (B) disaccommodation ($P = 0.24$).

**Figure 4.** Baseline refraction measurements were similar before and 30 minutes after instillation of 10% phenylephrine ($t$-test: $t = -1.4931, df = 4, P = 0.2097$).
All monkeys had hyperopic unaccommodated refractions (1-7 D) through the contact lens, and, on average, the resting refractions were not significantly different before and 30 minutes after instillation of 10% phenylephrine (Fig. 4, t-test: $t = -1.49$, $df = 4$, $P = 0.21$).

Average static accommodation responses from the four highest stimulus current amplitudes, measured with the Hartinger coincidence refractometer, were not significantly different before and 30 minutes after the instillation of phenylephrine (Fig. 5, t-test: $t = -0.1050$, $df = 4$, $P = 0.9214$). Average accommodative amplitude before phenylephrine was $11.73 \pm 1.78$ D and after phenylephrine was $11.75 \pm 1.50$ D. Figure 6 shows the maximum accommodative amplitude achieved before phenylephrine versus the amplitude achieved for the same stimulus amplitude after phenylephrine. Orthogonal regression analysis results in a slope of $1.017$ and an intercept of $-0.476$ ($r = 0.9898$) that are not statistically different from the 1:1 line (95% confidence interval).

**DISCUSSION**

The goal of this study was to determine whether phenylephrine, an $\alpha_1$-adrenergic agonist, has a direct effect on the ciliary muscle. It is known that phenylephrine dilates the iris through sympathetic stimulation of the dilator muscle, but its effects on the ciliary muscle are not well understood. Prior studies have reported a large range of differing effects of phenylephrine on accommodation, including decreases of up to 3 D in humans, $^{16,21,25,28,46,47}$ and up to 7 D in monkeys. $^{3,20}$ Results from the present study indicate that, although there are individual differences before and after phenylephrine, these differences are not systematic and therefore are probably not due to phenylephrine. Within the resolution of the methods, there are no systematic, significant effects of phenylephrine on accommodative amplitude, dynamics, or resting position. Similarly, response amplitude to each stimulus current amplitude, measured with a Hartinger coincidence refractometer, did not change after phenylephrine.
Tornqvist directly stimulated the cervical sympathetic nerve in cynomolgus monkeys. These results differ from those found in other studies of electrically stimulated accommodation in monkeys.

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References
