To investigate the autonomic components of the pupillary light reflex in humans, we used infrared pupillometry combined with a partial local cholinergic (tropicamide) or alpha-adrenergic (thymoxamine) blockade. The pupillary response curve was analyzed using parameters identical or similar to those employed previously to study the autonomic components of the pupillary light reflex. Troplamide increased baseline pupil area and affected five of the eight measured parameters. Thymoxamine lowered baseline pupil area but did not affect any of the parameters. We found the expected cholinergic contribution to the constrictive phase of the pupillary light reflex but no evidence for peripheral alpha-adrenergic activity during redilation. We propose that redilation primarily involves parasympathetic relaxation, modulated by cholinergic inhibition of the dilator muscle and central sympathetic inhibition of the Edinger-Westphal nucleus.

While it has been long known that constriction of the pupil to a light flash depends on parasympathetic outflow from the Edinger-Westphal (E-W) nucleus, the autonomic control of the individual phases of the human pupillary light reflex is not well established. Lowenstein and Loewenfeld employed pharmacological agents and surgical ablation to study the pupillary light reflex in cats and monkeys and described two phases of constriction and primary and secondary redilatory phases. They hypothesized that the constrictive phases were due to a parasympathetic activation modified at its conclusion by a superimposed central sympathetic inhibition of the E-W nucleus. The primary redilation was thought to be due to parasympathetic relaxation, while the secondary redilation was thought to be due to an increase in peripheral sympathetic activity. Observations in a small number of patients with neurological lesions suggest that this model also may apply in humans.

Information relevant to possible mechanisms underlying the pupillary light reflex in humans also is available from pharmacological analysis of human intraocular muscle. Several studies have demonstrated a cholinergic stimulation of the constrictor muscle and an alpha-adrenergic stimulation of the dilator muscle, as required by the Lowenstein and Loewenfeld model. In vitro study has also demonstrated beta-adrenergic inhibition of both muscles, alpha-adrenergic inhibition of the sphincter and cholinergic inhibition of the dilator. The physiological contribution to the light reflex of these latter effects, observed in vitro, is unknown. Other workers have found that the beta-adrenergic blocker timolol does not affect either baseline pupil area or the pupillary light reflex.

In the present study we measured the pupillary light reflex by infrared pupillometry after applying agents to produce a parasympathetic or sympathetic blockade. Our data confirm the expected cholinergic contribution to constriction but fail to confirm the expected alpha-adrenergic contribution to the redilatory phase. Instead we find that the recently described cholinergic inhibition of the dilator muscle may contribute to the rate of redilation. We present a hypothesis to explain redilation in the human pupillary light reflex that includes this cholinergic inhibition.

**Materials and Methods**

The pupillary light reflex was studied in 27 normal subjects (16 males and 11 females) between the ages of 20 and 40 years. The experimental protocol for this study was approved by the UCSF Committee on Human Research and informed consent was obtained in writing.

Pupil area was measured every 50 msec by infrared video pupillometry (Micromeasurements, Inc., Berkeley, CA). The light stimulus was a square-wave pulse of collimated white light, generated by a glow-
Table 1. Pupillary light reflex parameters in normals, drug and comparative normal subgroups*

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>C (%)†</th>
<th>mrc†</th>
<th>mrd†</th>
<th>d2 (%)</th>
<th>t†</th>
<th>tmrc</th>
<th>tmrd†</th>
<th>rec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLS (27)</td>
<td>43.9</td>
<td>43.4</td>
<td>13.2</td>
<td>7.9</td>
<td>0.296</td>
<td>0.515</td>
<td>1.29</td>
<td>83.4</td>
</tr>
<tr>
<td>SE</td>
<td>1.2</td>
<td>2.4</td>
<td>0.7</td>
<td>0.6</td>
<td>0.007</td>
<td>0.007</td>
<td>0.02</td>
<td>1.5</td>
</tr>
<tr>
<td>Tropic (4)</td>
<td>9.7‡</td>
<td>19.8‡</td>
<td>8.1‡</td>
<td>7.2</td>
<td>0.339‡</td>
<td>0.543</td>
<td>1.195</td>
<td>95.06‡</td>
</tr>
<tr>
<td>SE</td>
<td>2.3</td>
<td>3.7</td>
<td>2.2</td>
<td>1.0</td>
<td>0.010</td>
<td>0.017</td>
<td>0.075</td>
<td>2.8</td>
</tr>
<tr>
<td>lgNLs (5)</td>
<td>38.5</td>
<td>52.4</td>
<td>15.7</td>
<td>6.1</td>
<td>0.255</td>
<td>0.511</td>
<td>1.283</td>
<td>81.8</td>
</tr>
<tr>
<td>SE</td>
<td>2.7</td>
<td>5.1</td>
<td>1.5</td>
<td>0.7</td>
<td>0.016</td>
<td>0.007</td>
<td>0.054</td>
<td>3.7</td>
</tr>
<tr>
<td>Thymox (5)</td>
<td>50.9</td>
<td>34.7</td>
<td>11.2</td>
<td>6.3</td>
<td>0.298</td>
<td>0.493</td>
<td>1.228</td>
<td>86.7</td>
</tr>
<tr>
<td>SE</td>
<td>1.5</td>
<td>5.7</td>
<td>0.8</td>
<td>1.3</td>
<td>0.009</td>
<td>0.014</td>
<td>0.036</td>
<td>2.9</td>
</tr>
<tr>
<td>smNLs (11)</td>
<td>46.3</td>
<td>32.7</td>
<td>10.2</td>
<td>8.8</td>
<td>0.306</td>
<td>0.500</td>
<td>1.269</td>
<td>82.8</td>
</tr>
<tr>
<td>SE</td>
<td>1.7</td>
<td>2.3</td>
<td>0.8</td>
<td>1.2</td>
<td>0.010</td>
<td>0.010</td>
<td>0.035</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Tropicamide compared to large normals (lgNLs), thymoxamine compared to small normals (smNLs). For parameters independent of pupil area, comparison with the entire control group did not alter findings of statistical significance.

modulator tube (Sylvania R-1131C; Mountainview, CA), focused in the plane of the pupil and contained within the pupil (i.e., a Maxwellian-view stimulus).15,16 The duration of the stimulus (200 msec) was chosen to be shorter than the latency to the light reflex4 (Table 1) to further ensure elimination of variation in stimulus intensity. Microcomputer-controlled tracking was used to compensate for minor head and eye movements. Pupil area data were stored in a PDP-8 computer.

Pupil area was found to be stable after 5 min of adaptation to a constant dim level of illumination (0.34 ft-candles, measured by a Tektronix J-16 photometer probe located on the wall one meter in front of the subject). Starting shortly before a trial, the subject fixated on a red light-emitting diode, coaxial with the stimulus and placed at optical infinity to eliminate accommodation effects. The subject pressed a button to begin each recording period, which continued until 5 sec after the stimulus was delivered. Length of the prestimulus interval was randomly set

Fig. 1. A series of pupillary light reflex responses from a typical subject. Responses to six 200 msec 1200 cd/m² stimuli (S) presented at 1 min intervals at time t = 0. Pupil area (PA) measured at 20 Hz.

Fig. 2. A single response to light (top) and its time differential (below): BPA, mean baseline pupil area over 1 sec prior to flash; tᵣ, time to constriction (latency); C (%), size of constriction (as % of BPA); d₂ (%), % secondary redilation (% redilation occurring between 2.2-2.6 sec after the stimulus (S); rec (%), % redilation at 5 sec after the stimulus; mrc, tmrc, maximum rate of constriction and its time after S and; mrd, tmrd, maximum rate of primary redilation and its time after S.
at either 3 or 4 sec in order to reduce effects of flash anticipation on the pupillary response. Up to 12 responses were obtained at 1 min intervals. Baseline pupil area was determined from the prestimulus recording.

Nine subjects also were tested after conjunctival administration, in the consensual eye, of one to two drops of either 0.5% tropicamide (Alcon Laboratories, Forth Worth, TX) or 0.5% thymoxamine (Warner-Lambert, Morris Plains, NJ). These agents produce a parasympathetic or sympathetic (alpha-adrenergic) block, respectively.17,18 All subjects were examined by tangential light beam to detect the presence of a narrow anterior chamber, before administration of tropicamide. The only adverse reaction noted following either drug was the routinely observed transient burning sensation with thymoxamine.18,19 The pupillary light reflex was studied during a partial blockade (ie, 10 to 15 min after drug administration) rather than at the time of maximal effect, after 30 min. This allowed us to obtain responses during a blockade but at levels of baseline pupil area comparable to some normal subjects. This simplified the data analysis since several parameters

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**Fig. 3.** (A, B) Five analysis parameters as a function of baseline pupil area (BPA) in normal (X) and after partial parasympathetic (tropicamide, △) or sympathetic (thymoxamine, □) block. Dotted lines indicate classification of normals into small, medium and large BPA groups. Small BPA normals (Xsm) were compared to subjects treated with thymoxamine. Large BPA normals (Xlg) were compared to subjects treated with tropicamide (see Table 1).
used in the analysis are dependent on pupil area in a nonlinear fashion,20 confounding an interpretation of control and drug data from the same individual. In addition, since at the limits of pupil size mechanical factors can influence pupillary response,21 it was important to compare groups with comparable baseline pupil area.

For each pupillary response a smoothed curve22 of pupil area versus time and its time differential curve were plotted. These curves were analyzed using parameters either identical to or similar to those found by Lowenstein and Loewenfeld to reflect the autonomic components of the pupillary light reflex: baseline pupil area (BPA), latency to constriction (tc), size of constriction [C(%)], maximum rate of constriction and its latency (mrc, tmrc), maximum rate of primary redilation and its latency (mrd1, tmrd1), and percentage recovery [rec(%)].3 Under our experimental conditions, employing a short, Maxwellian-view stimulus, unlike the stimulus used in the study of Lowenstein and Loewenfeld,3 the secondary redilation was often partially overlapped by the primary redilation. This has been observed by others employing a short stimulus.23 Therefore, we used the percent redilation occurring during the time interval 2.2–2.6 sec after the stimulus (ie, 2.0 to 2.4 sec after the end of

Fig. 3 (continued) (C, D).
See legend under Figure 3 (A, B).
the stimulus) to measure secondary redilation [d2(%)]. This time interval was selected because: (1) in some curves there was a distinct secondary redilation noted at this time interval; (2) evidence for a secondary redilation at this time interval was consistently seen in the derivative curve; and (3) this time interval corresponds to the time during redilation of the distinct secondary phase observed by Lowenstein and Loewenfeld.3

Statistical analysis was done using the student t-test.

Results

Mean baseline pupil area for the normal subjects was 31.9 ± 2.0 mm² (mean ± SE). For tropicamide-treated subjects the mean baseline area was 53.9 ± 2.6 mm², and for thymoxamine-treated subjects the mean was 19.2 ± 2.6 mm². Thus, both drugs produced the expected autonomic block (P < 0.05).

Several sequential measurements of the pupillary light reflex in a single subject are shown in Figure 1. The latency, maximum rates of change, time to peak, and times of maximum rate of change showed little variation from trial to trial. There was minor nonsystematic fluctuation in baseline pupil area. Figure 2 demonstrates a single response with its time differential and illustrates the parameters used to analyze the response.

The values for the parameters for normal and drug-treated subjects are listed in Table 1 and plotted as a function of baseline pupil area in Figure 3 (A–E). For statistical analyses drug-treated groups of subjects were compared only to the subset of normal subjects who had a baseline area comparable to that of the drug-treated subjects, that is, tropicamide-treated subjects were compared with controls with large baseline area and thymoxamine-treated subjects were compared with controls with small baseline area (Fig. 3). Using this comparison, tropicamide significantly affected five of the eight parameters, decreasing the size of constriction [C(%)], the maximum rate of constriction (mrc) and the maximum rate of primary redilation (mrd), and increasing the latency to constriction (t_c) and the percentage recovery [rec(%)]. The measure of secondary redilation [d2(%)], the time to maximum rate of constriction (tmrc), and the time to maximum rate of primary redilation (tmrd) were unaffected by tropicamide.

Thymoxamine treatment, despite significantly lowering baseline pupil area, failed to produce significant differences in any of the parameters.

Discussion

In this study we used a partial, local cholinergic or alpha-adrenergic blockade to investigate the autonomic components of the pupillary light reflex in humans. Baseline pupil area, measured under mesopic light conditions, was similar to that observed by others.24 The cholinergic antagonist tropicamide significantly increased baseline pupil area. This effect was probably due to antagonism of the known cholinergic stimulation of the sphincter muscle, but an effect on the recently described cholinergic inhibition of the dilator muscle may have contributed. The alpha-adrenergic antagonist thymoxamine significantly decreased baseline pupil area. This effect was probably due to antagonism of the known alpha-adrenergic stimulation of the dilator, but antagonism...
of alpha-adrenergic inhibition of the sphincter, which has been reported,\textsuperscript{10-12} may have contributed.

Since the size of the pupillary constriction to light is known to be a function of pupil area,\textsuperscript{21} we analyzed, as well, the dependence on pupil area of all the individual parameters in our analysis. Absolute magnitude of constriction, as mentioned above, is highly dependent on pupil area ($r = -0.8$), while the percentage constriction [C($\%$)] was only moderately correlated with area ($r = -0.4$). The maximum rate of constriction, mrc, and the maximum rate of primary redilation, mrd$_1$, both absolute measures, were greater at larger pupil area ($r = 0.8$ and 0.7, respectively), presumably secondary to the increased constriction which occurred in a reflex of essentially the same duration. The two percentage measures, secondary redilation [d$_2$(\%) and percentage recovery [rec$(\%)$], were independent of pupil area. Of the temporal parameters, only latency ($t_c$) was a function of pupil area, inversely so, as has been previously observed.\textsuperscript{23,25} The values we observed for the parameters [except d$_2$(\%)], a parameter we devised] were similar to those previously observed.\textsuperscript{3,26,27}

Because of the dependence of some of the parameters on pupil area, we compared the data from drug-treated subjects with that from control subjects who had a baseline pupil area comparable to that of the drug-treated subjects.

Tropicamide affected five of the eight parameters: C $(\%)$, mrc, mrd$_1$, $t_c$, and rec $(\%)$. The decreases in C $(\%)$ and mrc and the increase in $t_c$ are readily explained by the well established antagonism by tropicamide of the cholinergic parasympathetic activation of the sphincter pupillae muscle. The maximum rate of primary redilation, mrd$_1$, which is thought to represent parasympathetic relaxation, was also decreased. This decrease in mrd$_1$ probably was due to the significantly attenuated constriction after tropicamide in two of the four subjects. Unexpectedly, the percentage recovery, rec $(\%)$, was increased in the presence of tropicamide. This observation would not be expected if redilation was due primarily to parasympathetic relaxation and peripheral sympathetic activity. This finding can be explained, however, by assuming that cholinergic inhibition of the dilator pupillae\textsuperscript{10-12} plays a role in the light reflex. If phasic cholinergic inhibition of the dilator normally is present during redilation then a cholinergic blocker such as tropicamide would be expected to decrease this inhibition, thereby enhancing redilation. Since rec$(\%)$ was measured at 5 sec after the stimulus, we conclude that this cholinergic inhibition of the dilator still would be present or have its effect still present at 5 sec post-stimulus.

Thymoxamine did not significantly affect any of the parameters, although it did decrease baseline pupil area. If secondary redilation were due to peripheral sympathetic activity, we would predict a decrease in the secondary redilation measure, d$_2$(\%), in the presence of thymoxamine. Since we did not see any change in d$_2$(\%), we also looked at redilation in thymoxamine-treated subjects during the time period of 2.6-5.0 sec post-stimulus to see if there was a later effect of thymoxamine, but none was seen. The percentage recovery, rec$(\%)$, was also unaffected in thymoxamine-treated subjects, further suggesting a minimal or absent contribution by peripheral alpha-adrenergic activity (either dilator stimulation or sphincter inhibition) to the redilatory phase. The thymoxamine-treated pupils were compared to normal pupils of similar size since this was the only appropriate control available. It is possible that normal small pupils, which have increased central parasympathetic tone, have a generally inhibited sympathetic system and thereby an inhibited dilator activity. A small pupil also might have diminished dilator activity because of the length-tension curve properties for the stretched dilator muscle. While it is not possible to rule out these effects, if they were operative in the normal small pupil controls, we should have observed an increased value for d$_2$(\%) (our measure to detect secondary active sympathetic redilation) in normal pupils with a larger BPA, but this was not seen (ie, d$2\%$ was independent of BPA).

While it is true that adrenergic antagonists such as thymoxamine are not pure in terms of receptor action (alpha vs. beta or between alpha subclasses) or might have other actions, the biochemical evidence suggests strongly that thymoxamine as employed by us would have been expected to affect sympathetic activity of the dilator. It is possible that the difference between our finding and that of Lowenstein and Loewenfeld is due to the different stimulus used. In their study\textsuperscript{3} they employed a prolonged (1 sec) non-Maxwellian-view stimulus. Another possibility is that in the ablation techniques they employed, on which their interpretation was based, other components besides alpha-adrenergic efferents were affected (eg, cholinergic inhibitory outflows\textsuperscript{28}). Our data do bring into question the role of peripheral sympathetic activity in the mechanism for the finding of a secondary redilation of the pupil following a light stimulus. It should be emphasized that there is ample evidence that active central and peripheral sympathetic activity is necessary for the phenomenon of reflex pupillary dilatation in response to sensory or psychosensory stimuli.\textsuperscript{29}

It is possible that redilation represents predominantly a continual return to baseline tone (ie, parasympathetic relaxation) modulated by changes in cholinergic inhibition of the dilator as described above. This model, however, does not easily explain
the often observed abrupt attenuation in the rate of redilation observed in mid-redilation (Fig. 1). This attenuation, however, could be the result of a sudden decrease in the level of the increased central sympathetic inhibition of the E–W nucleus, which is thought to commence with and be responsible for the secondary phase of constriction. A decrease in this ongoing central sympathetic inhibition during the period of increased parasympathetic tone compared to baseline would result in a decreased rate of redilation. Indeed, Lowenstein and Loewenfeld proposed the mechanism of rapidly alternating levels of central sympathetic inhibition to explain the cyclical changes in pupil area that they observed during pupillary light reflexes in excited monkeys.

In summary, we have demonstrated the feasibility of employing pupillometry combined with pupillary pharmacological blockade to dissect the individual parasympathetic and sympathetic contributions to the pupillary light reflex in humans. We found the expected cholinergic contribution to the constrictive phase but no evidence for peripheral sympathetic activity during secondary redilation. We propose that redilation may be a complex event involving parasympathetic relaxation, cholinergic inhibition of the dilator muscle and central sympathetic inhibition of the Edinger–Westphal nucleus.

Key words: pupil, human, autonomic, light reflex, cholinergic, adrenergic

References