Effect of Diltiazem on Lid Tension During Light-Flash-Induced Eye Blinks in the Rabbit

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Purpose. The purpose of this study was to examine the effect of the Ca blocker diltiazem in combination with the Ca chelator EGTA (ethyleneglycol-bis-ta-amino-ethyl ether]N,N'-tetracetic acid) on eyelid tension generated during the light-induced eyelid reflex in the adult rabbit. The goal is to develop nontoxic, nonsurgical therapies for blepharospasm.

Methods. Blinks were triggered in the alert rabbit, and tension was measured with a strain gauge attached to the eyelid. Strain gauge output was amplified, digitized, and stored on computer for later analysis. Experiments consisted of a set of trials in which identical light stimuli were delivered at 0.1 Hz for 5 min.

Results. In control trials, blink tensions decreased dramatically for the first seven to nine blinks and then leveled off, indicating that with respect to muscle tension output, blinks contained both rapidly habituating and nonhabituating components. After diltiazem and EGTA were injected in the lid, blinks were reduced 30% to 90% over those in control trials. Reductions could not be explained by injection trauma or irritation due to fluid volume, because injections of saline alone did not produce such tension depression.


Benign essential blepharospasm is a variably progressive, bilateral, involuntary focal cranial dyskinesia of unknown etiology. It is characterized by spasmodic, forceful, and involuntary contractions of the orbicularis oculi (OO) muscles, frequently resulting in prolonged eyelid closure and severe visual disability.

Blepharospasm often is associated with and may progress to Meige's syndrome, a form of craniofacial dystonia involving eyelid, facial, and oromandibular movements. Surgical and nonsurgical methods have been developed to alleviate benign essential blepharospasm symptoms, but none of them have been entirely satisfactory. Among these, facial nerve transection has a high recurrence rate and untoward side effects, including partial facial paralysis. Myectomy results in numbness of the forehead, chronic lymphedema in the periorbital region, and exposure keratitis. Pharmacotherapy (antipsychotics, stimulants, sedatives, antimuscarinics, antihistamines, anticonvulsants and anti-Parkinsonian drugs) has been useful to ameliorate specific types of blepharospasm by weakening the OO; however, efficacy seems to be limited and temporary in most cases, and significant side effects are a problem here as well.1
One fairly successful nonsurgical approach uses botulinum A toxin (Oculinum, Botox), which induces the best temporary relief for orbicularis spasms. Here too, however, there are undesirable side effects, including lagophthalmos, exposure keratitis, and diffusion of toxin to undesirable locations, causing ptosis and diplopia. Although there may be no entirely satisfactory symptomatic, nonsurgical treatment for benign essential blepharospasm, the development of effective alternative nonsurgical therapies with less toxic drugs would be desirable, particularly those in which the risk of spread and involvement of adjacent tissue is minimized.

The benzothiazepine Ca\(^{2+}\) channel blocker diltiazem in combination with the Ca\(^{2+}\) chelator EGTA (ethyleneglycol-bis-ta-amino-ethyl ether) N,N'-tetracetic acid) has been shown to reduce tonic contractions in extraocular muscle (EOM) in vitro. Diltiazem-EGTA also was found to reduce the contractility of EOM in vivo (and J. Jacoby, unpublished observations). The effect in vivo might not be due exclusively to the effect on the tonic fibers, because in other experiments carried out in EOM in vitro it was found that electrically stimulated twitch responses also were reduced in the presence of diltiazem with EGTA (and J. Jacoby, unpublished observations). The findings in EOM are consistent with findings in other studies demonstrating that in vitro diltiazem has a depressant effect on mammalian skeletal muscles. The results of these studies suggest the possibility that diltiazem-EGTA also might reduce the contractility of a mammalian twitch muscle in vivo, including the fast-twitch OO muscle. If so, diltiazem, a mild drug that has been in extensive clinical use for years, might provide a potential alternative, nontoxic, nonsurgical treatment for benign essential blepharospasm. To test this hypothesis, a study was undertaken to investigate the effect of diltiazem-EGTA on eyelid tension generated during the light-induced eye blink reflex in the adult rabbit. Some of the results reported here have been published previously in abstract form.

### MATERIALS AND METHODS

Adult New Zealand white rabbits, both male and female and weighing 3.0 to 5.0 kg, were selected to serve as experimental animals. They were handled and housed in the animal facility of New York University Medical Center in compliance with the ARVO and NIH Guidelines for the Care and Use of Laboratory Animals.

**Animal Preparation**

For the measurement of tension generated during a rabbit eye blink, a strain gauge was connected to the rabbit’s eyelid via a 5-0 silk suture placed at midposition of the upper eyelid margin. The suture was deep to the OO muscle, but did not pierce the palpebral conjunctiva. For placement of the suture, the hair on the eyelid was trimmed, and then the surface of the eyelid was cleaned with sterile swabs soaked in betadine followed by 70% ethanol. Suturing was carried out using sterile techniques under ketamine-xylazine sedation (final concentrations: ketamine HCl, 43 mg/ml; xylazine HCl, 8.6 mg/ml; acepromazine, 1.4 mg/ml) at a dosage of 0.5 ml/kg delivered intramuscularly. The topical anesthetic proparacaine HCl was applied to the lid shortly before the suture was made. After suturing, an antibiotic gel (tobramycin) was applied to the suture site, and the eyelid was allowed to heal for 48 hr before carrying out blink tension experiments. Blinks were triggered by a bright, brief light flash. The rabbit was dark adapted for at least 0.5 hr before a blink trial to maximize any response to the light flash, and throughout the blink trials the room remained dark. Trials also were carried out without distracting noises or movements in the room to minimize any head movement artifacts in the recorded tension responses.

For the injection of solutions into the eyelid, the room remained dark and the rabbit was placed in a restrainer without any sedation. A 5% solution of proparacaine HCl was applied at the suture site and circumferentially around the eye on the upper and lower eyelids, using a sterile swab. A sterile 27 G needle attached to a tuberculin syringe was used to inject into the lid a total volume of 0.10 to 0.15 ml of: 0 Ca-added saline (in mmol/l: NaCl, 136; KCl, 5; MgSO\(_4\), 3; imidazole-SO\(_4\), 5; glucose, 11) with 15 to 30 mmol/l diltiazem and 5 mmol/l EGTA added, or diltiazem or EGTA alone, or normal saline alone. Four injections were made 5 mm away from the eyelid margin, one each at the superior and inferior aspects of the medial and lateral canthus. Immediately after injections, more proparacaine was applied at the injection sites, and the rabbit was then given a 5-min rest period before the onset of the experimental blink trial.

For all blink trials, the animal was alert. No general sedation was given, because even ketamine, the usual drug of choice for rabbits, and believed to have no direct effect on skeletal muscle, was noted to decrease significantly the amplitude of light-flash-induced blinks (J. Jacoby, unpublished observations). For control blink trials, that is, those trials that were not immediately preceded by eyelid injections of any kind, proparacaine was applied to the eyelid at 8 and 5 min before the start of the control trial to reproduce as closely as possible the exact conditions of the experimental trials.

For the blink trial, the rabbit was placed in a heavily padded restrainer that minimized head movements without causing physical discomfort. Although movement was minimized by the restrainer, it was not designed to immobilize the rabbit, which could move...
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both its head and its body if it chose to do so. When such movements occurred for more than a few seconds, it was assumed that the animal was experiencing distress. The blink trial was terminated and the animal was removed from the restrainer.

The strain gauge was attached to the suture in the rabbit's upper eyelid through a 1-inch long metal pin with an open loop at its free end. For any set of trials, the strain gauge-eyelid attachment was adjusted to eliminate slackness and generate a maximal blink tension response. The corresponding baseline tension was monitored and adjusted to the same level for subsequent trials within a series.

Experimental Setup

A white light source, originating from a 300 W projector lamp, was used to trigger the blink reflex. The onset and duration of the light stimulus was controlled by a Uniblitz shutter (A. W. Vincent Assoc. Inc, Rochester, NY), which was placed 9 inches from the eye. The light from the lamp was focused on the shutter opening by a condensor lens and a fiberoptic light guide. The light passing through the shutter was focused so that a 2.5-cm diameter circle of light would fall on the pupil. The shutter was triggered to open by a transistor-to-transistor logic (TTL) stimulus controlled by the investigator.

Both the TTL stimulus signal and the voltage output from the strain gauge were recorded on a pen recorder and simultaneously digitized and stored on computer. The voltage output from the strain gauge was first amplified by a Gould transducer amplifier (Gould Electronics, East Rutherford, NJ), and then low-pass band-filtered at 2.5 kHz using a two-pole bessel filter. Both the TTL stimulus and the amplified, filtered output from the strain gauge were recorded and stored in digital form as a running, "real time" record using the Axotape program (Axon Instruments, Burlingame, CA), a system consisting of an analog-to-digital converter (Labmaster) and data acquisition and analysis software.

Experimental Protocol

The experimental protocol was designed to elicit eye blinks at as high a frequency as possible, to maximize any putative effect on OO contractility by the use-dependent Ca-blocker diltiazem. For our set-up and light source, a light flash of 500-msec duration was necessary to elicit reliably a strong blink response, and the greatest frequency at which this stimulus could be delivered without failures in most cases was 0.1 Hz. A blink trial thus consisted of a train of repetitive light flash stimuli at 0.1 Hz delivered over 5 to 6 min. Every experiment consisted of a set of blink trials that included two to four control trials, an experimental trial following 5 min after injections, then usually one to three post-injection control trials.

The entire, uninterrupted tension record from each trial, including the quiescent intervals between blinks, was saved in data files on computer. For a given blink, various components of the tension response were measured from the stored files off-line, including maximum tension amplitude (peak tension), time from the beginning of the stimulus to onset of tension rise (latency), time from the onset of tension rise to maximum tension (time to peak), and time from maximum, to one-half maximum tension during the declining phase of the tension (half relaxation time).

Statistical Analysis

The differences between trials for a given variable were compared in two ways: (1) all responses in a trial were averaged and compared with the averaged value for the blinks in other trials, using standard parametric statistics; and (2) each blink of a trial was compared with the same blink in another trial (based on temporal order) using the nonparametric Wilcoxon's signed rank test for matched pairs.16,17

RESULTS

Tension Generated During Light-Flash-Induced Eye Blink

The effect of diltiazem on rabbit OO contractility in vivo was evaluated indirectly, by recording isometric lid tension during an eye blink triggered with a bright, brief flash of light. There has been no systematic investigation of the tensions generated during rabbit blinks, so the characteristics of a normal blink were examined. It also was necessary to characterize blink tension responses to a train of light-flash stimuli corresponding to the experimental protocol used in this study. As expected, individual blink tension responses were complex; furthermore, the relative amplitude of a blink response was found to depend on recent blink history, or the number of blinks immediately preceding it within a short time period.

Shape of an Individual Tension Response. The rising phase of a blink tension response consisted of at least three to five components that were resolvable due to differences in latency of onset. The relaxation phase usually contained at least two components. Figure 1A illustrates a typical tension record, the third response in a control blink trial, in which there are four clearly discernible components in its rising phase, and two components in the relaxation phase. A characteristic of all responses, shown here, is that the first, or earliest component of the tension is of very small amplitude relative to the total tension response. Despite a constant tension maintained on the lid by the strain gauge, in some blink trials this first small component is obscured by a small, transient tension drop (not shown), which initiates approximately 40 to 50 msec earlier.
FIGURE 1. Tension records from Axotape files of light-stimulated blinks from a control blink trial. (A) Third blink. (B) 9th blink. (C) 16th blink. (D) 26th blink. Stimulus shown at bottom is 500 msec in duration. (Vertical scale bar = 10 g tension.)

than that of the tension rise, or about 45 to 55 msec after the onset of the light stimulus. It is assumed that this brief tension drop corresponds to the relaxation of the levator palpebrae superioris muscle.

Decrement in Blink Tension Amplitude Over 3 to 8 min at 0.1 Hz Stimulus Frequency. A blink “trial” consisted of the set of blink tension responses to light flashes of constant amplitude and duration delivered every 10 sec (0.1 Hz) over 5 to 6 min. All trials were initiated only after the rabbit had been rested for at least 30 min in a quiet, darkened room. Nevertheless, with every blink trial there was a sharp and immediate decrement in the amplitude of the response over the course of the first seven to nine blinks (70–90 sec), followed by a very slight decrease or no decrease at all over the succeeding 10 to 30 blinks. Figure 1A–D illustrates this point by showing the 3rd, 9th, 16th, and 26th blinks, respectively, from a blink trial (Fig. 2, open circles), in which there is no reduction in amplitude between the 16th and 26th blink.

Figure 2 plots all the blinks within a trial as a function of blink number in a set of two consecutive control trials, to emphasize the predictable, stereotypical nature of the decrement in blink tension over the course of a trial. The inset to Figure 2 shows part of an actual tension record consisting of the first 10 blinks of one of the plotted trials (open circles). In summary, under the conditions of the experimental protocol used, there were two components to the pattern of tension responses in a blink trial: one that was subject to immediate and rapid habituation, and a second part, fairly insensitive to habituation, in which there was only a gradual decline or in some trials no significant decline at all over the course of 25 to 35 blinks triggered at 0.1 Hz (Fig. 2B).

Effect of Diltiazem With EGTA on Blink Tension

The effect on the light-induced blink of the benzothiazepine Ca-blocker diltiazem in combination with the Ca chelator EGTA, was investigated by injecting the drugs directly into the rabbit’s eyelid and measuring tension during a blink trial. Each such experiment (n = 7), was composed of a set of blink trials separated by 30-min rest periods as follows: two to four initial control blink trials were carried out to establish an average tension response; the eyelid was then injected with a saline solution with (n = 7) or without (saline control, n = 7) drugs, immediately followed by an “experimental” blink trial. Finally, in most cases, one to four blink trials were carried out subsequent to the experimental trial to chart the recovery from the effects of the injection. For each experiment in which the two drugs were injected into an eyelid, a parallel experiment was carried out on the same lid in which a control saline only was injected. These saline injections, undertaken within several days of their “companion” diltiazem experiments, were made to assess the possible effect of injection trauma and the fluid volume alone on blink performance. The results of these experiments are summarized in Table 1.

In six of six experiments in which a total of 0.1 ml of fluid was injected into the lid, injections of saline...
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### TABLE 1. Change in Blink Tension After Injections in the Eyelid

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Change in Peak Tension</th>
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<tbody>
<tr>
<td></td>
<td>Drug Injection</td>
</tr>
<tr>
<td>15 mmol/l diltiazem + 5 mmol/l EGTA</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>-55.4%* (n = 56)</td>
</tr>
<tr>
<td>2.</td>
<td>-45%* (n = 48)</td>
</tr>
<tr>
<td>3.</td>
<td>-42%* (n = 52)</td>
</tr>
<tr>
<td>4. Without 5 min wait</td>
<td></td>
</tr>
<tr>
<td>First 7 blinks</td>
<td>-4% (n = 14)</td>
</tr>
<tr>
<td>8th–28th blinks</td>
<td>-31%*† (n = 39)</td>
</tr>
<tr>
<td>30 mmol/l Diltiazem + 5 mmol/l EGTA</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>-64%*† (n = 57)</td>
</tr>
<tr>
<td>6.</td>
<td>-26%* (n = 56)</td>
</tr>
<tr>
<td>7. 0.4 ml fluid volume</td>
<td>-90%*† (n = 57)</td>
</tr>
<tr>
<td>8. 15 mmol/l diltiazem alone</td>
<td>-20%* (n = 41)</td>
</tr>
<tr>
<td>9. 5 mmol/l EGTA alone</td>
<td>-13%‡ (n = 53)</td>
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* Significance P < .001, Wilcoxon’s signed rank for matched pairs.
† Every “experimental” blink lower than its corresponding blinks in two preceding control trials.
§ Based on comparison of experimental trial with 1st and 3rd, rather than 2nd and 3rd of three control blink trials because of persistent head movements in the 2nd control trial.

Experimental trials were compared with the two preceding control trials. Unless otherwise indicated, there was a 5 min wait after injections before the experimental trial was initiated, and the total volume of fluid injected was 0.1–0.12 ml.

containing either 15 or 30 mmol/l diltiazem and 5 mmol/l EGTA resulted in a highly significant reduction in isometric blink tension (Table 1). Paired experiments carried out within several days of the drug experiment, in which saline only was injected into the same eyelid, produced no effect or small reductions in tension. Table 1 shows the results of these experiments. Figures 3 to 6 show the data from one such pair of drug and control saline experiments, corresponding to experiment number 2 in Table 1.

In Figure 3, a diltiazem-EGTA injection experiment, sample tension responses are shown superimposed for two control trials (Figs. 3A, B), the experimental (or injection) trial (Fig. 3C), and the third post-injection trial (Fig. 3D). Figure 4 contains a plot of tension as a function of blink number within a trial for the same experiment as shown in Figure 3. The inset to Figure 4 shows amplitude histograms representing the average blink tension for each trial. For all the experiments, there were two consistent characteristics to the change in tension response after diltiazem-EGTA injections, and these are illustrated in the records shown in Figure 3: (1) a large reduction in tension amplitude, but (2) no change in the gross shape of the response compared with control blinks.

Figures 5 and 6 show the actual tension records and plotted data, respectively, of the companion saline injection experiment to that shown in Figures 3 and 4. The records in Figure 5 show no reduction in tension after injection of saline alone, and this was the case in four of six such experiments. A small but significant reduction in blink tension (over preceding controls), however, was observed in the remaining two experiments in which 0.1 ml of saline alone was injected (see Table 1). One example is shown in Figure 7. Unlike the large reduction in tension after injection with diltiazem-EGTA (Fig. 7, left panel), in this and one other case, the reduction was small. These findings suggest that there may be some effect of the fluid volume alone on blink tension; however, the results, as illustrated in Figure 7, are equivocal.

Less equivocal were the results of another pair of experiments, shown in Figure 8, which involved injection of four times the usual volume of saline (0.4 ml) into the lid. This experiment was carried out for two purposes: to determine (1) if the putative effect of drug on the orbicularis oculi muscle would be augmented by “flooding” its extracellular environment with the solution, thus creating a higher effective concentration of drug at the muscle sarcolemma and a longer washout time; and (2) whether, in the case of saline alone, the fluid volume itself might have a tendency to depress blink tension.

Injection of 0.4 ml saline containing 30 mmol/l diltiazem/5 mmol/l EGTA resulted in a 90% reduction in blink tension (Table 1). Furthermore, 25% of the light stimuli failed to elicit any tension response at all in the experimental trial (These nonresponses were not included in the calculation of the 90% reduction). The profound depression of blink tension did not recover fully for several days. After injection of saline alone, there was a significant 32% reduction in blink tension.
FIGURE 3. Blink tension responses from experiment 2 (Table 1), diltiazem-EGTA injection. The 2nd, 5th, 9th, 12th, 17th, and 24th blinks are superimposed for: (A) control 1; (B) control 2; (C) experimental; and (D) third post-experimental trials. Scaling as in Figure 1.

tension (Fig. 8, right panel), suggesting a real effect of fluid volume on depressing the blink response. In contrast to the aftermath of drug injection, however, there was a dramatic recovery to 160% of original control levels in the trial immediately after the experimental trial (30 min after experimental trial). This large "overshoot" recovery of blink tension after saline injection was consistent with that seen in several other saline experiments (in which only 0.1 ml fluid volume was injected). No other injections of 0.4 ml saline were attempted because of the obvious physical discomfort to the rabbit.

Two additional experiments were carried out, one each in which either 15 mmol/l diltiazem or 5 mmol/l EGTA alone was injected into the lid (Table 1, numbers 8 and 9, respectively). In each case there was a small but significant reduction over control tension. However, the effect was less pronounced than with the drugs in combination. Further experiments injecting each drug separately are necessary to evaluate these preliminary findings.

Effect of Diltiazem With EGTA on Latency of Blink Reflex

Diltiazem is known to affect other neuromuscular sites aside from the muscle sarcolemmal Ca channel, including the acetylcholine receptor and also possibly presynaptic transmitter release.\textsuperscript{11,18,19} Thus, the action of diltiazem–EGTA on muscle activation in vivo may be complex. Such effects, if prominent, would be expected to increase the delay between the stimulus and the onset of the twitch, or blink latency, and also slow the rate of rise in muscle tension, or time to reach peak tension.

The average latency of the control light-induced blink reflex was measured and found to vary from rab-
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FIGURE 5. Blink tension responses from experiment 2 (Table 1), saline injection. The 2nd, 5th, 9th, 14th, and 22nd blinks are superimposed for: (A) control 1; (B) control 2; (C) experimental, and (D) post-experimental trials. Scaling as in Figure 1.

bit to rabbit from approximately 85 to 105 msec, and the range of latencies typically fluctuated 15 to 20 msec around these averages. Unlike the striking effect of repetitive stimulation on the amplitude of maximal blink tension, blink latency neither increased nor decreased significantly during the course of a blink trial. An example is shown in Figure 9, data from the same experiment as that shown in Figure 4. There were only four sets of experiments in which changes in latency after diltiazem-EGTA injection were measured. In two other cases, latency changes were not measured because the tension rise was preceded in many blinks by a small dip in tension 45 to 60 msec after the stimulus, thus obscuring the onset of tension rise. In the seventh case, injection of 0.4 ml fluid, the responses after drug injection were too small to make latency measurements meaningful.

The results of drug injection were variable. There was a substantial, statistically significant increase in latency compared to the two preceding controls in only two of four experiments. Figure 9 shows data from the same experiment whose tension data are plotted in Figure 4. Despite the highly significant drop in blink tension in this experiment, there is a significant increase in latency only with respect to the immediately preceding control (inset), but not with respect to the first control trial. As can be seen in Figure 9, the first two blinks of the first control are extremely large, but even without these values, the average latency from the first trial is still slightly greater and statistically no different from that of the experimental trial. In experiments with injections of saline alone, there was no effect in three experiments, but in one, an increased latency was observed. In conclusion, there appears to be a trend toward longer latency after diltiazem-EGTA injection, but thus far the results are inconclusive.

FIGURE 6. Blink tension versus blink number, trials from same experiment as in Figure 5. Open circles, control 1 (C1). Filled circles, control 2 (C2). Open triangles, experimental (E). Filled triangles, post-experimental (P). Inset: average tension + standard error for each trial.
Effect of Diltiazem With EGTA on Time to Peak and Relaxation of Blink Reflex

The time to reach peak tension (TTP) and half relaxation time (RLT) of the tension response also were investigated. There was no increase in TTP in any experiment after drug injection, and in fact there actually was a reduction in TTP after drug injection, correlated more with tension amplitude than with treatment. Figure 10 shows some of the data from one experiment, in which TTP is plotted as a function of blink tension. The experimental data (open triangles), fall along a continuum that includes control (filled circles) and post-injection (filled squares) data.

FIGURE 7. Blink tensions from experiment 1 (Table 1). Left: diltiazem-EGTA. Right: saline injections. Symbols as in Figure 6, except at left: open boxes, control 3 (C3); and at right: open circles, control 3 (C3); filled circles, control 4 (C4). Insets: average tension.

FIGURE 8. Average tension for each trial from experiment 7 (Table 1), in which 0.4 ml fluid volume was injected into the eyelid. Left: diltiazem-EGTA. Right: saline injections. P3 was run 2.5 hr after the experimental trial.
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The decline in blink tension was examined as well, by measuring RLT. There was no consistent pattern of change in this variable either as a function of blink number, or after drug injection. In control trials, there was a wide variability in RLT, but the average RLT for a trial fell within the range of 700 to 740 msec (time from stimulus to half relaxation). RLT was not calculated as time from peak to half relaxation, for the following reason: The tension response typically consisted of a plateau region, such that the time of the peak tension value could fluctuate as much as 150 msec. Even if one used the last occurrence of the maximum tension value, this might fall at the beginning or end of the plateau, thus creating misleading estimates of the true pattern of the relaxation of tension. The only unambiguous measure, therefore, was stimulus onset, or tension onset to half relaxation.

DISCUSSION

The results of this study demonstrate that, when injected into the rabbit eyelid, diltiazem in combination with EGTA significantly reduces the tension generated during the light-stimulated blink reflex, demonstrating indirectly that these drugs act to reduce the contractility of the OO muscle. This reduction is both striking and consistently observed. Parallel experiments in which saline alone is injected into the eyelid do not produce this result, indicating that the reduction in OO contractility is not a response to the trauma of injections or the pressure or discomfort of the fluid volume in the lid.

Crucial to this study was the ability to establish a stimulus protocol for the blink trials that was identical for each and every trial, to be able to compare one trial
with another, and also to be able to make a paired comparison of data between trials. In our experimental trials, a light flash of constant luminance and duration sufficient to elicit an eye blink reliably was delivered to the eye every 10 sec. The blink reflex showed immediate habituation, in that even with the second response the peak tension was reduced over that of the initial response. Our experimental design probably maximized this effect for the following reasons. It is known that habituation to a light stimulus is reduced if the animal is distracted between stimuli, presumably because the predictability of the stimulus is thereby less apparent to the rabbit. In our trials, however, because of the jumpiness of rabbits and the fact that the animal was restrained but not actually immobilized, we took pains to minimize any noises, movements, or other distracting stimuli in the room that might cause the animal to move. Any “distraction” to minimize habituation would have had to be nonstartling to the rabbit and also stereotypical to minimize variation, which itself would tend to become habituating. Because of the predictable character of the stimulus protocol and recording conditions, the pattern and time course of the habituation were consistent and predictable from one trial to another. This allowed for the investigation not only of any general effect of the drugs on blink tension overall, but also of any possible difference in effect of the drugs on habituating and nonhabituating components of the response.

Our results show that diltiazem with EGTA reduces the blink tension throughout a blink trial. However, in general, the effect is proportionally more pronounced for the early, nonhabituated portion of the trial. There also is a substantial reduction in the blink-to-blink variability of the tension response in the experimental trial in comparison to control and postexperimental trials. These effects could be explained by an average reduction in the safety factor for generating an action potential and triggering contraction in a muscle fiber during neuromuscular transmission in the presence of the drugs. There was one case in which no reduction in tension was seen until the eighth blink in the experimental trial, but this was an experiment in which a blink trial was initiated immediately after drug injection, rather than after the usual 5-min waiting period. In this one case, the delay in effect of the drugs on tension is more likely due to diffusion time than to any use-dependent factor, such as spontaneous eye blinks during the 5-min waiting period in the other experiments. Rabbits do not exhibit much spontaneous blinking and, aside from the eyelid-closing during injection, the experimental animals were rarely observed to blink during the 5-min rest period after injection.

Electromyographic (EMG) studies have established that there are two clearly distinguishable components to the blink reflex, regardless of the stimulus: an early response (R1), whose amplitude but not duration is related to the intensity of the stimulus, and that is resistant to habituation, and the second, the so-called late response (R2), of variable duration, which is subject to strong habituation. The latter pathway is related to perception of a physical threat, and the magnitude of this component of the response is reduced as the stimulus becomes predictable and thus no longer perceived as a potential threat. In our experiments, the tension that was generated during a blink exhibited at least four to five components. These could be subdivided further into those components that showed great decrement during the course of a blink trial and those that were resistant to decrement during a blink trial, the latter including the initial small component and a second component unmasked during the course of the trial. The early small component persists throughout the blink trial with no increase in latency, and in the late blinks (after 2 min) becomes almost completely separated from the later part of the response, due primarily to the great reduction in amplitude of the late component and the persistence of the small, but robust early component.

Whether these early and late components of the tension response correspond to R1 and R2, respectively, of the EMG, remains to be determined. On the other hand, the range of latencies for the onset in tension rise in the rabbit blink reflex measured in this study agrees closely with measurements by Manning and Evinger of the latency of onset of eyelid movement. Furthermore, the invariant latency of the early small tension component is analogous to the lack of variation in latency of R1 after various protocols and treatments that affect the latency of R2 and the amplitude of both R1 and R2.

Manning and Evinger have proposed that the early part of the blink is controlled entirely by the preprogrammed early component R1, whereas R2 appears only when stimulus duration extends beyond the time of onset of the blink, altering the ongoing sensory input and thus triggering the late component and lengthening the duration of the blink. In our experiments, the duration of the light stimulus was 500 msec, which overlapped considerably the onset of the tension response that occurred 80 to 100 msec after stimulus onset. Under the above hypothesis, the presence of both components in these blink tension responses is predicted by the pulse protocol, if it is assumed that R2 and R1 give rise to the habituating and nonhabituating tension components, respectively.

The primary mechanism of action of diltiazem in the in vivo experiments reported here is not known, nor can it be resolved from these studies. In in vitro experiments with an EOM isolated in a chamber and attached to a strain gauge, twitch amplitude was signifi-
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...contractility with blocking activity removed. At higher concentrations and various conditions, diltiazem also has been reported to enhance twitch amplitude.\cite{24,25} In vivo, this might possibly be due in part to an inhibitory action of diltiazem on acetylcholinesterase at the neuromuscular junction.\cite{11,18} However, in vitro, the effect is more likely to be due to some other, as yet unknown property of diltiazem action probably related to its Ca\textsuperscript{2+}-blocking activity at the muscle membrane. Of possible significance, extracellular Ca\textsuperscript{2+} has been reported to be an open channel blocker of Na\textsuperscript{+} channels.\cite{26} One could hypothesize that under certain conditions and concentrations of drug, diltiazem might competitively remove this Ca\textsuperscript{2+}-mediated depression of Na\textsuperscript{+} channel activity, thus increasing the safety factor for triggering a muscle twitch with depolarization. By such an argument, the apparent twitch potentiation observed in these instances could be explained as contractility with blocking activity removed. At higher drug concentrations and longer incubation times, the blocking effect of diltiazem would presumably come to predominate. In this regard, in vitro experiments in which the muscle twitch amplitude is depressed after prolonged exposure to diltiazem always show an initial increase in twitch amplitude before the decline\cite{11} (and J. Jacoby, unpublished observations). In any case, for the in vivo condition, one would expect the concentration of diltiazem and the presence of a Ca\textsuperscript{2+} chelator to be critical to whether contractility is enhanced or depressed as a result of diltiazem treatment.

Whether diltiazem when applied in vivo acts to reduce muscle contractility predominantly presynaptically or postsynaptically, chronic, long-term treatment clearly reduces Ca\textsuperscript{2+} influx into muscle.\cite{27} This property of the Ca\textsuperscript{2+} blocker is undoubtedly responsible for recent findings that diltiazem can be beneficial in reducing damage caused by excess Ca\textsuperscript{2+} influx into muscle in various pathologic states.\cite{28,29}

In summary, the results from this study lend support to the possibility that chronic exposure of the OO muscle to diltiazem, particularly in the presence of a Ca\textsuperscript{2+} chelator such as EGTA, could provide long-term reduction in OO contractility. The use-dependent nature of this drug, at least with respect to its action at blocking the muscle Ca\textsuperscript{2+} channel, would make it an ideal candidate in the symptomatic treatment of benign essential blepharospasm, a condition characterized by chronic, pathologic activation of a muscle. Future experiments, in particular a detailed dose-response study and the development of a workable mechanism for the sustained release of diltiazem–Ca\textsuperscript{2+} chelator within the eyelid, will determine whether such an approach has a realistic clinical application.

**Key Words**
calcium blocker, diltiazem, eyeblink, eyelid, muscle tension.

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