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Light-evoked Contraction of the Photosensitive Iris of the Frog[1]

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Abstract

Smooth muscle cells of the frog iris sphincter contain rhodopsin and contract in response to light. The mechanism of light-evoked contraction was studied with particular attention paid to the role of calcium. The contractile proteins of the sphincter smooth muscle cell can be activated by an increase in intracellular calcium. Light-evoked contraction, however, is not accompanied by a measurable change in membrane potential and occurs in the absence of extracellular Na^+ and/or Ca^{2+}, as well as in the presence of isotonic KCl. Maximum light-evoked tension is reduced by exposure to Ca^{2+}-free solutions containing EGTA and high K^+ and is restored by incubation in solutions containing Ca^{2+}. The restored response, which persists after return to Ca^{2+}-free solution, depends on the concentration of Ca^{2+} in the incubating solution and on the duration of the incubation. The results support the conclusion that light produces contraction of the iris sphincter by causing the release of Ca^{2+} from an intracellular storage site.

Vertebrate eyes are generally considered to contain two classes of photoreceptors: rods and cones. In both receptor types isomerization of photopigment initiates a chain of events that ultimately reduces the cation permeability of the outer segment surface membrane giving rise to hyperpolarizing potential change. Release of intracellular Ca^{2+} is thought to play a role in the generation of the response (Hagins, 1972; Gold and Korenbrot, 1980; Yoshikami *et al.*, 1980). In some vertebrates, e.g. frogs, the smooth muscle cells of the iris sphincter represent another type of photoreceptor (Weale, 1956; Seliger, 1962). They contain rhodopsin (Blaustein and Dewey, 1977) and contract in response to light independent of the retina and other neural elements (Barr and Alpern, 1963; Armstrong and Bell, 1968). The mechanism of the light-evoked contraction is not understood. Since the action spectrum of the contractile response closely resembles the absorption spectrum of rhodopsin, the sequence of events that culminates in smooth muscle contraction is thought to be initiated by photoisomerization of rhodopsin (Seliger, 1962; Barr and Alpern, 1963; Bito and Turansky, 1975). What these events are and how closely they resemble phototransduction in rods and cones has not been fully established. There is indirect evidence that the iris response involves a light-evoked increase in intracellular Ca^{2+} (Barr and Alpern, 1963; Bito and Turansky, 1975; Zucker and Nolte, 1978), but physiological experiments designed to test this point specifically have not been reported. In the present paper intracellular recordings and tension measurements have been used to investigate whether a membrane potential change is associated with light-evoked contraction and to examine the role of calcium in the generation of the response.

Materials and Methods

Dissection

Irises were obtained from 2- to 3-inch *Rana pipiens* that had been dark-adapted overnight. In dim red light pithed animals were decapitated and the eyes were removed and hemisected. In a dissection chamber containing frog Ringer's solution, the lens, suspensory ligaments, remnants of the retina, and the cornea were removed from the anterior half of the eye leaving the iris attached to a scleral ring. Irises were usually left attached to the sclera because, when removed from the ring, they usually contracted and could not be mounted on the tension transducer until they relaxed.

Tension measurements

Iris contraction was recorded with a photoelectric tension transducer (Hellam and Podolsky, 1969). Two stainless steel hooks were passed through the pupillary opening, one attached to the transducer and the other to a micromanipulator that provided mechanical ground. The hooks were coated with Epoxylite (Epoxylite 6001, Epoxylite Corp., South El Monte, CA) to insulate them electrically and to reduce the tendency of tissue to stick to them. The output of the transducer was linear from 8 mg down to at least 1 mg. The sensitivity of the transducer was 85 mv/mg (83 mV/dyne; tension is given in units of mass throughout this report). Unless stated otherwise, irises were stimulated with a focused spot of white light (irradiance 3.54×10^3 erg cm^{-2} sec^{-1}) that covered the entire preparation.

Solution changes

The solution bathing an iris could be changed in the dark with the apparatus illustrated in Figure 1. A Plexiglas disk containing eight evenly spaced, 1.5-ml wells rotated on a platform that could be raised and lowered under the iris. Solution changes produced mechanical artifacts in the tension record (Fig. 1, *inset*). As the well was lowered, the full weight of the iris on the transducer hook caused the tension trace to go off scale. Hence, the chart recorder was shut off (sharp upward pen deflection in the record) while an iris was between wells (<30 sec). When tension recording was resumed, the recorder pen moved rapidly downward. The return of tension to its original level typically followed a gradual time course with tension moving first above and then below the base line. This appeared to be a response to stretch. At times, the tension returned to a new base line, presumably when the preparation shifted slightly on the transducer as it was reimmersed. Although these artifacts made it difficult to detect small changes in tension, they were more predictable than those produced when solutions were changed by other methods.

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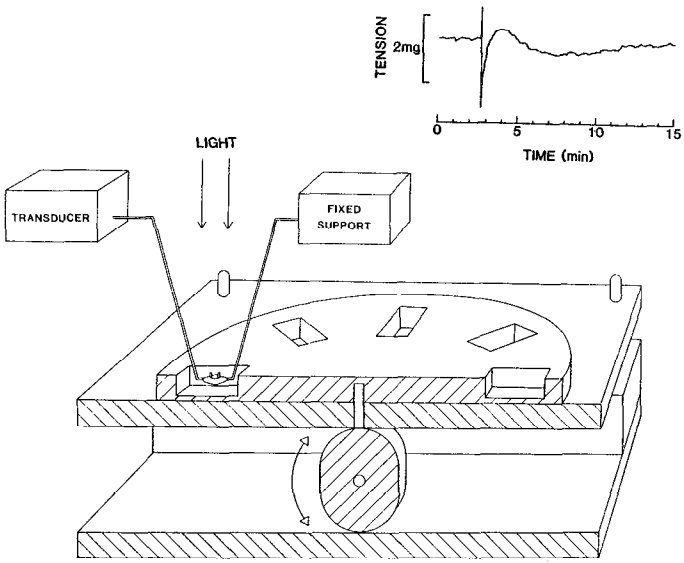


Figure 1. Tension recording and solution changes. The iris was suspended anterior side upward between two stainless steel hooks, one attached to a tension transducer and the other to a fixed support. The apparatus used to change solutions is shown in partial cross-section. The upper platform could be raised and lowered by means of a cam. When the platform was in the raised position the preparation was immersed in the solution contained in one of eight wells milled into a Plexiglas disk. To change solution the platform was lowered, the disk was rotated to the desired well, and the platform was raised. The *inset* shows typical artifacts recorded by the tension transducer during a solution change beginning at $t = 2.7$ min (both wells contained normal Ringer's solution; see the text for details).

Intracellular electrical recording

Microelectrodes with long (2 to 5 cm) flexible shanks and resistances of 200 to 400 megohms were pulled on a Livingston horizontal puller and filled with either 4 M potassium acetate or 3 M KCl. Control recordings made from contracting muscle cells in the frog stomach and heart indicated that these microelectrodes were suitable for penetrating small contractile cells without causing damage.

Intracellular recordings from sphincter smooth muscle cells were obtained most easily when the iris was penetrated from the posterior surface. For these experiments, the iris was pinned anterior-side down on a layer of Sylgard in a small Plexiglas well. Light stimuli were delivered to the iris from below. For simultaneous measurements of membrane potential and tension, the iris was mounted on the hooks of the tension transducer anterior-side down and illuminated from below.

Extracellular electrical recording

For extracellular electrical measurements, irises with the cornea still attached were pinned, cornea-side down, over a loop of silver wire (ground electrode) resting on a layer of Sylgard at the bottom of a Plexiglas chamber. Tungsten wire electrodes, blunt-tipped glass electrodes filled with Ringer's solution, and silver wires positioned near the pupillary margin were used in different experiments as recording electrodes.

Solutions

The normal composition of Ringer's solution was: NaCl, 117 mM; KCl, 2.5 mM; $CaCl_2$, 2 mM; MOPS (3-(*N*-morpholino)propanesulfonic acid), 4 mM, pH 7.4. Na^+-free Ringer's solution was made by a 1:1 replacement of NaCl with choline chloride. Ca^{2+}-free solution contained 0 Ca^{2+} and 2 mM EGTA (ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid). Increases in the concentration of K^+ or Ca^{2+} or the addition of tetraethylamonium bromide (TEABr) were compensated for by the iso-osmotic replacement of NaCl. Hypertonic Ringer's solution was made by the addition of 250 mM sucrose (2 times) or 125 mM sucrose (1.5 times). Nifedipine, D600, formamide, A23187, and caffeine when used were added to standard Ringer's solution at the indicated concentration. In order to avoid results due to effects on autonomic nerve endings in the iris, atropine (1.4 μM), propranolol (3.3 μM), and phenoxybenzamine (3.6 μM) were added to all solutions. At these concentrations autonomic blocks did not alter resting tension and did not affect the amplitude, latency, or time course of light-evoked contractions.

The drugs used were: atropine sulfate (Burroughs Wellcome, Co., Research Triangle Park, NC); formamide, caffeine, TEABr, and MOPS (Sigma Chemical Co., St. Louis, MO); propranolol hydrochloride (Ayerst Laboratories, Inc., New York, NY); phenoxybenzamine hydrochloride (Smith Kline and French Laboratories, Philadelphia, PA); A23187 (Calbiochem-Behring Corp., La Jolla, CA); nifedipine (Pfizer Laboratories, New York, NY); and D600 (Knoll Pharmaceutical Co., Whippany, NJ).

Ca^{2+} Depletion and Loading Experiments

Solutions. All solutions contained 80 mM K^+, 20 mM EGTA, and 6 mM free Mg^{2+}. Potassium hydroxide was combined (4:1) with EGTA, $MgCl_2$ and $Ca(OH)_2$ (when used) were added, and pH was adjusted to 7.0 with MOPS. The amount of Ca^{2+} and Mg^{2+} required to obtain the desired free concentrations of these ions was calculated with stability constants from Fabiato (1981) and Godt (1974). For some experiments, solutions were made with Na^+ instead of K^+. When used, nifedipine or D600 was added without substitution to the desired solution.

Depletion and loading protocols. In these experiments light-adapted frogs were used, and irises, prepared in room light, were initially depleted of Ca^{2+} as follows. While in low Ca^{2+} solution (pCa 8), they were exposed to light for 15 min, dark-adapted for 5 min, re-exposed to light for 5 min, and then dark-adapted for 20 min. At this time loading experiments were started. The protocol for loading will be described later.

Results

Basic observations

Resting tension in darkness. The resting tension recorded from a freshly mounted iris showed small rapid tension fluctuations superimposed on larger, more gradual changes (Fig. 2). Figure 2, *A* and *B*, displays the tension fluctuations at different gains and time bases. The tension record in Figure 2*C* is from the same iris but recorded 90 min later. It shows that, with time, the spontaneous changes declined in frequency and amplitude. Since this was a consistent observation, irises were routinely mounted on the tension transducer 1½ to 2 hr before an experiment was started.

Light response. Figure 3 shows the typical response of a dark-adapted iris to a 5-min step (Fig. 3*A*) and a 1.4-sec flash (Fig. 3*B*) of bright light. Tension developed during the step peaked in less than 30 sec and then declined to a plateau that was maintained until the light was turned off. In some experiments the plateau was not

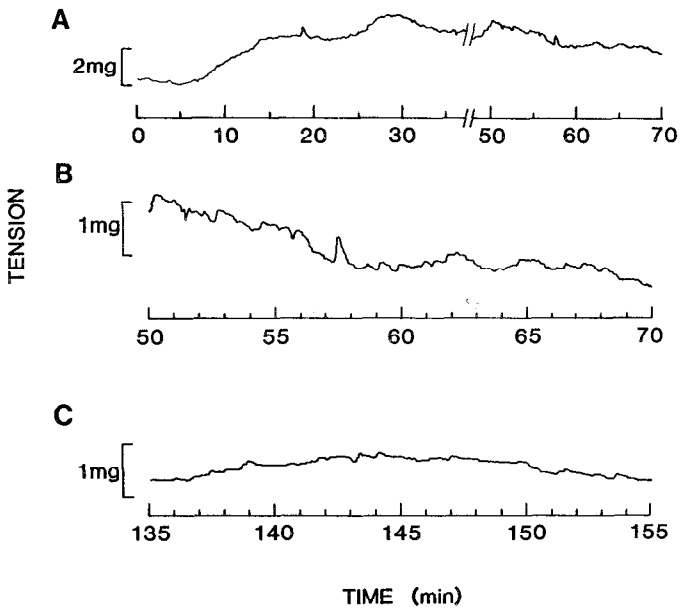


Figure 2. Spontaneous changes in resting tension in darkness. *A*, Slow changes in tension recorded shortly after an iris was mounted on the tension transducer. *B*, Expanded portion of the trace in *A* from 50 min to 70 min showing small rapid fluctuations superimposed on the slow tension changes. *C*, Resting tension recorded 1½ hr later. All records are from the same iris bathed in normal Ringer's solution (without autonomic blockers).

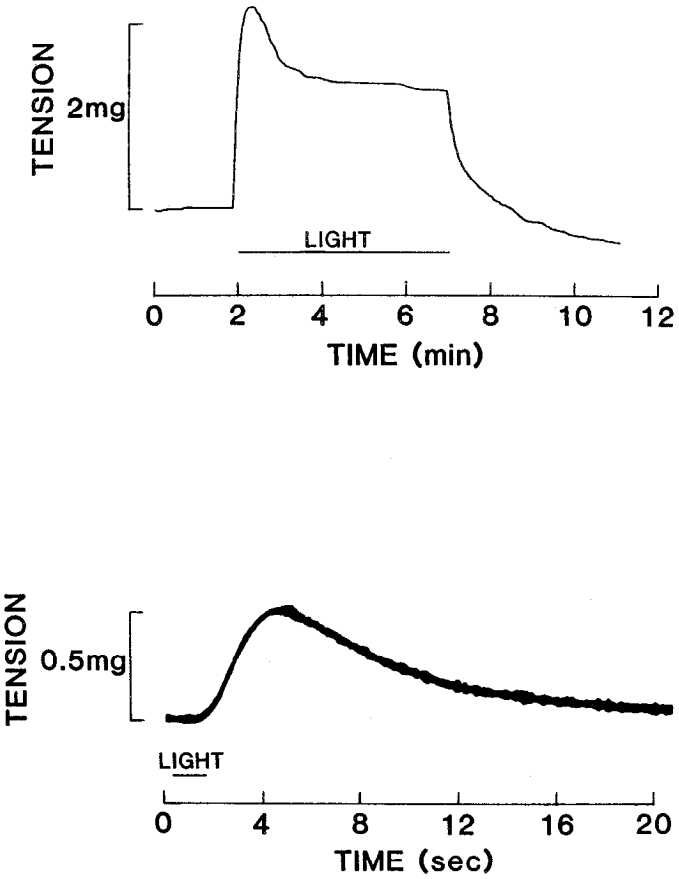


Figure 3. Iris light responses. *A*, Tension developed by an iris during a 5-min light step. The response shown is typical of those observed in most irises. In a few cases tension either increased or decreased gradually during the plateau phase of the response. *B*, Tension developed by a second iris in response to a 1.4-sec light flash.

maintained, and tension either slowly increased or decreased with time. Maximum light-evoked tension varied in different irises as did the peak-to-plateau ratio. Maximum amplitude contractions evoked by 1.4-sec flashes had a mean latency of 1.03 ± 0.18 sec (±SD) and a time to peak of 4.9 ± 0.3 sec. Dimmer flashes evoked smaller responses with longer latencies. The observed light responses were similar to those reported by Barr and Alpern (1963).

Control of contraction by intracellular Ca^{2+}

Exposure of visceral smooth muscle to solutions containing high K or Ca^{2+} ionophores produces contractions that are triggered by an increase in intracellular Ca^{2+} due, at least in part, to the movement of extracellular Ca^{2+} into the cells (Pressman, 1972; Bolton, 1979; Kuriyama, 1981). Calcium's ability to initiate contraction of sphincter smooth muscle was tested by comparing the effect of Ca^{2+} ionophores and high K^+ solutions on resting dark tension in the presence and absence of extracellular Ca^{2+}. It was necessary to investigate this tissue because iris sphincter, unlike other types of smooth muscle, is embryologically derived from neural ectoderm rather than mesoderm.

A23187. The calcium ionophore A23187 was dissolved in dimethylsulfoxide (DMSO) and added to Ringer's solution to give a final concentration of 10^{-5} M in approximately 0.1% (v/v) DMSO. Solvent alone (0.1% and 0.2% DMSO, v/v) had no effect on resting tension. Bathing the iris in standard Ringer's solution containing A23187 caused, after a latency of approximately 2 min, a slowly developing sustained contraction (Fig 4*A*). In the presence of ionophore, maximum tension depended on extracellular [Ca^{2+}]. In the experiment of Figure 4*B*, an iris was bathed sequentially in A23187 solutions containing 2, 1, 0.5, 0.1, and 0 mM Ca^{2+}. Tension was greatest in 2 mM Ca^{2+} and decreased as extracellular Ca^{2+} was reduced.

Responses to elevated extracellular K^+. Figure 5*A* shows a typical response to high K^+ (50 mM) Ringer's solution. It consists of three phases: an initial rapid contraction, a relaxation, and a second, slowly developing sustained contraction. Although the general characteristics of the high K^+ contracture were similar in all preparations, details of the tension changes varied greatly from iris to iris. Nevertheless, K^+ contractures always required extracellular Ca^{2+}. Figure 5, *B* and *C*, shows the response of an iris to high K^+ in the absence and presence of Ca^{2+}. The results of these experiments and those with A23187 indicate that, in spite of its unique embryological origin, the contractile proteins of sphincter smooth muscle can be activated by a rise in intracellular Ca^{2+}.

Electrical measurements

The contractile responses of the iris sphincter to A23187 and high K^+ depend on the presence of extracellular Ca^{2+} and thus presumably involve the movement of Ca^{2+} across the sarcolemma of the smooth muscle cells. This suggests that light-evoked contraction of the cells might also depend on Ca^{2+} influx and might be accompanied by a membrane potential change.

Penetration and resting potentials. It was difficult to record from cells when the iris was penetrated from its anterior surface because electrodes usually broke, possibly upon contact with guanine crystals in the iridophore cells (see Fig. 1 in Nolte and Pointner, 1975). When the iris was approached from its posterior surface, electrodes first encountered a layer of cells with low resting potentials (−5 to −20 mV) followed by cells with resting potentials ranging from −40 to −80 mV. Electrodes advanced further into the iris often broke and very rarely recorded resting potentials. The superficial cells with low membrane potentials were probably pigment epithelial cells since cells with −5- to −20-mV resting potentials were found when an electrode was inserted into the iris very near the pupillary margin, a region composed of only posterior pigment epithelium (Nolte and Pointner, 1975). The cells with −40- to −80-mV resting potentials were considered to be smooth muscle cells because they had resting potentials similar to those of other types of smooth muscle and were found in the part of the iris where the sphincter muscle is located. Tissue opacity due to a high density of screening pigment prevented the use of intracellularly injected dyes to confirm the position of the recording electrode. The average resting potential of 93 cells considered to be sphincter smooth muscle cells was −60 ± 13 mV. Similar values were obtained by Bülbring and Hooton (1954) using albino rabbits, in which it is possible to visually identify the sphincter muscle within the iris.

Intracellular recording during light-evoked contractions. Light-evoked contraction of the iris was not accompanied by an obvious or consistent change in membrane potential. Figure 6 shows simultaneous recordings of tension from the whole iris and membrane potential from four different smooth muscle cells. No potential change is seen before the onset of tension in any of the records. In two of them (Fig. 6, *A* and *D*), the electrode came out of the cell during contraction; in the other two (Fig. 6, *B* and *C*) the electrode stayed in the cell, but no reproducible potential changes are apparent. Similar electrical measurements from 93 cells, some at a higher gain, failed to reveal any consistent changes of membrane potential during light-evoked contractions.

Several attempts were made to block contraction of the iris sphincter so that membrane potential could be recorded without contraction-induced artifacts. Caffeine (10 mM) or 1 M formamide (Cordoba et al., 1979) greatly reduced the mechanical response of the iris to light without changing the resting membrane potential but did not reveal any light-induced potential changes (18 and 11 cells, respectively). Light-evoked contractions were also reduced by bathing the iris in hypertonic Ringer's solution but, under these conditions, intracellular recordings were not possible, presumably because the cells were shrunken.

If iris smooth muscle cells were damaged by microelectrode

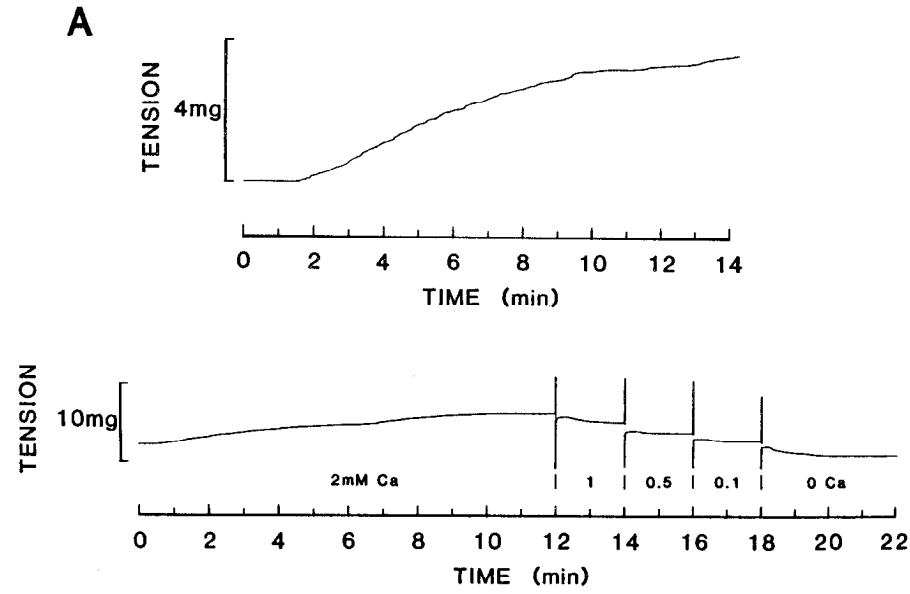
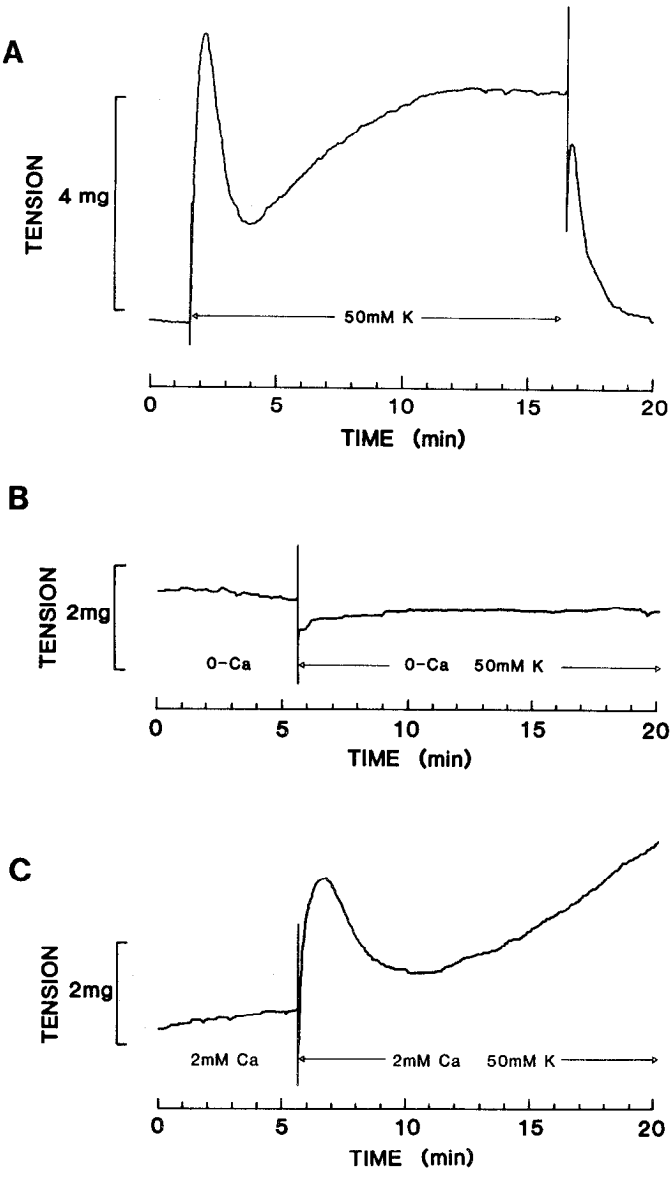


Figure 4. Effect of Ca^{2+} ionophore. The contractile response of an iris to 10^{-5} M A23187 in normal Ringer's solution is shown in *A*. *B* shows the dependence of the tension developed in A23187 on external $[Ca^{2+}]$. All solutions contained 10^{-5} M A23187. External Ca^{2+} concentrations were as follows: 0 to 12 min, 2 mM Ca^{2+}; 12 to 14 min, 1 mM Ca^{2+}; 14 to 16 min, 0.5 mM Ca^{2+}; 16 to 18 min, 0.1 mM Ca^{2+}; 18 to 22 min, 0 Ca^{2+}, 2 mM EGTA. Experiments were conducted in darkness.



impalement or if light-induced inward and outward currents exactly balanced one another, any potential changes produced by light might not have been detected. To determine whether this was the case, cells were impaled in the presence of elevated extracellular Ca^{2+} and in the presence of tetraethylamonium ion. Any membrane depolarization accompanying Ca^{2+} influx should be augmented in increased external Ca^{2+}. High Ca^{2+} solutions have also been found to stabilize the resting potential of the cells in other preparations and to facilitate electrical recording (Stefani and Steinbach, 1969; Singer and Walsh, 1980). Extracellular tetraethylamonium ion blocks K^+ channels in many cells and therefore might reveal a membrane depolarization that is ordinarily obscured by an outward K^+ current. Experiments conducted in the presence of 16 and 32 mM Ca^{2+} (10 and 14 cells, respectively) or 50 mM TEABr and 8 mM Ca^{2+} (17 cells) did not unmask any light-induced changes in membrane potential, nor did those conducted in the presence of 50 mM TEABr and 10 mM caffeine (28 cells).

Extracellular recording. The possibility that a sampling problem was responsible for our failure to observe membrane potential changes associated with light-evoked contraction was investigated with standard extracellular recording techniques. Using this approach we were unable to record any extracellular potential changes before or during light-evoked contraction of the iris. With the methods used, electroretinograms were routinely recorded when small remnants of retina were left attached to the iris. This was true when the recording electrode was 1 or 2 mm away from a piece of retina that was as small as or smaller than the sphincter muscle.

Light responses in different bathing solutions

Since inward movement of Ca^{2+} would be expected to be accompanied by membrane depolarization, the above results suggest that light-evoked contraction is not triggered by Ca^{2+} influx. This is

Figure 5. Calcium dependence of high K contracture. The typical response of an iris to high K^+ in Ringer's solution containing 2 mM Ca^{2+} consists of an initial contraction followed by relaxation and a second contraction (*A*). Both contractile phases of the contracture are absent in Ca^{2+}-free solution (*B*) and are restored by the addition of Ca^{2+} (*C*). *A* was recorded from a different iris than *B* and *C*. The differences in the appearance of the high K^+ contracture in *A* and *C* are typical of variations observed from iris to iris. Experiments were conducted in darkness. High K^+ solutions contained 50 mM K^+, and Ca^{2+}-free solutions contained 0 Ca^{2+} with 2 mM EGTA.

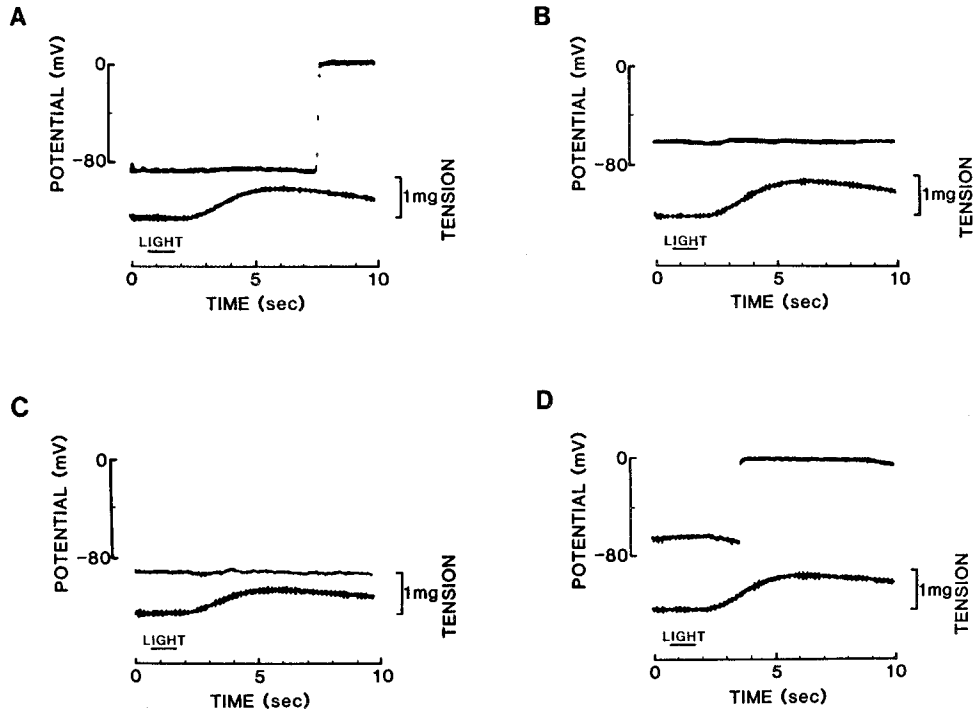


Figure 6. Membrane potential records made during light-evoked contractions. *A* to *D* are simultaneous recordings of membrane potential and contractile tension in four different experiments. The *upper trace* in each record is membrane potential, the *lower trace* is contractile tension. The *bar* indicates the delivery of a 1.4-sec flash of white light. Identical experiments were conducted in different external solutions (see the text) and also failed to reveal any membrane potential changes associated with light-evoked contraction.

consistent with observations that light-evoked contractions are not reduced by the Ca^{2+} channel blockers nifedipine (10^{-5} M) and D600 (10^{-6} M) and, unlike the contractions evoked by high K^+ or A23187, occur in Ca^{2+}-free solutions (Fig. 7*A*). Light responses in Ca^{2+}-free solutions also occur with or without Mg^{2+}, in the presence of high concentrations of EGTA (20 and 60 mM), and when the cells are depolarized by high K^+ (Fig. 7*B*). When experiments in low Ca^{2+} were continued for long periods of time there was a slow decline in the amplitude of the light response (Fig. 7*C*). Responses evoked every 2 min by 1.4-sec flashes declined to half-maximum over times ranging from 8 to 44 min. The rate of decline appeared to depend upon the frequency of stimulation, but variations from iris to iris made quantitation impractical.

Loading and unloading of intracellular Ca^{2+} stores

Although extracellular Ca^{2+} is not required for light-evoked contraction, the slow decline of the response in Ca^{2+}-free solution suggests that an intracellular Ca^{2+} pool may be involved. If this is the case, making the plasma membrane more permeable to Ca^{2+} and exposing irises bathed in Ca^{2+}-free solution to long-duration light steps should deplete the pool more rapidly. Figure 8 shows that the light response of an iris bathed in Ca^{2+}-free, Na^+-free solution containing 8 mM K^+ and 20 mM EGTA was reduced considerably by a single 5-min light step but not by a 1-min step. The different effect of the 5-min versus the 1-min conditioning light was not due to a change in adaptational state since similar results were obtained when longer (≥30 min) dark intervals were used between light exposures.

These results are consistent with the existence of a light-controlled Ca^{2+} pool and would be unlikely if the decline of the light response during long exposures to Ca^{2+}-free solution were due to cell injury. The following experiments provide further physiological evidence for the involvement of a releasable Ca^{2+} store by demonstrating that the light responses of Ca^{2+}-depleted irises can be restored by re-exposing them to Ca^{2+}. Figure 9 shows that the peak light response of a Ca^{2+}-depleted iris (i.e., one treated as in Fig. 8*A*) increases after incubation in the dark for 15 min in high K^+ solution containing 7.9 mM Ca^{2+}. When irises were bathed in Ca^{2+} solutions that contained 80 mM Na^+ instead of K^+ there was little, if any, increase

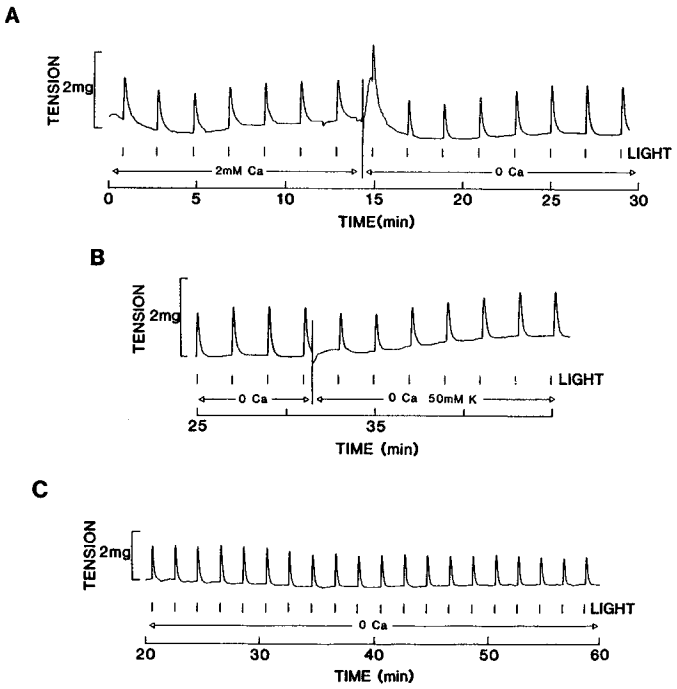


Figure 7. Light responses in Ca^{2+}-free solutions. White light flashes of 1.4 sec duration (*bars*) were delivered to the iris every 2 min throughout the experiments. *A*, At $t = 14$ min the external solution was changed from normal Ringer's solution to Ca^{2+}-free solution. *B*, Continuation of the record in *A*. AT $t = 32$ min the external solution was changed to high K^+ (50 mM), Ca^{2+}-free solution. *C*, Record from a second iris showing gradual decline of the light response during prolonged exposure to Ca^{2+}-free solution. All Ca^{2+}-free solutions contained 2 mM EGTA.

in the light response and only a small contraction developed during the incubation. Nifedipine (10^{-5} M) and D600 (0.2 mM) inhibited the recovery of the light response and reduced the contraction during incubation in solutions containing Ca^{2+} and K^+. The latter result

suggests that external Ca^{2+} gains access to the light-sensitive intracellular pool through surface membrane Ca channels opened in the presence of high K^+. Loading of the pool in the presence of Na^+ might be much slower because these channels are closed and because Na:Ca exchange would tend to buffer intracellular Ca^{2+}.

Loading versus Ca^{2+} concentration. Ca^{2+}-depleted irises were incubated in different Ca^{2+} solutions (as in Fig. 9), and the difference in the peak light-evoked tension before and after Ca^{2+} incubation was used as a measure of loading. Measurements were made in sets of three. In the first and third measurements, an iris was exposed to the same Ca^{2+} concentration. The average loading of the light-controlled Ca^{2+} pool that took place was determined and compared with the loading during the second measurement. In the experiment illustrated in Figure 10, the loading in pCa 2.7 was 40% of the average loading in pCa 2.1. The results of 27 loading experiments on 13 irises showed that loading was a graded function of the Ca^{2+} concentration with little or no loading occurring in solutions with pCa > 5.

Loading versus time. The amount of loading of the Ca^{2+} pool that took place during 5-, 10-, and 15-min incubations of Ca^{2+}-depleted irises in pCa 2.1 was compared. In five sets of experiments on three irises, the average loading for 5-min Ca^{2+} incubations was 55% of the loading after 15-min incubations. No differences were seen between 10- and 15-min incubations. Incubation times shorter than 5 min were not studied because of possible effects of light adaptation. In normal Ringer's solution, light sensitivity after a 1-min light exposure takes approximately 5 min to recover.

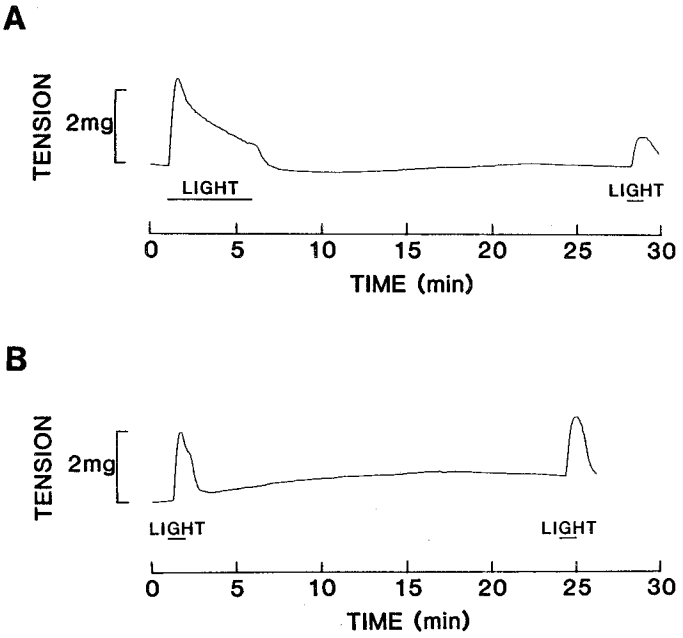


Figure 8. Calcium depletion. Exposure of Ca^{2+}-depleted irises to light caused the amplitude of subsequent light responses to be reduced. The decrease in light-evoked tension depended on the duration of the first light exposure. Responses of the iris to 1-min steps of light (*right*) were recorded 22 min after the iris was exposed to light (*left*) for 5 min (*A*) and 1 min (*B*). The reduced light response in *A* was not the result of light adaptation brought on by the 5-min light step since further recovery of the response did not occur when irises were allowed to dark adapt for longer periods of time. Between *A* and *B* the iris was incubated in a solution containing 7.9 mM Ca^{2+}. Low Ca^{2+} solution (pCa 8) contained no added Ca^{2+}, 20 mM EGTA, 80 mM K^+ (see "Materials and Methods").

Discussion

The results indicate that contraction of light-sensitive smooth muscle can be triggered by an increase in intracellular Ca^{2+}. They also demonstrate that light-evoked contraction is not associated with a membrane potential change and can occur in the absence of external Ca^{2+}. These findings suggest that the light response may involve the release of Ca^{2+} from intracellular storage sites. Further support for this hypothesis is provided by the Ca^{2+} experiments illustrated in Figures 8, 9, and 10 which show that the strength of light-evoked contraction varied in a manner consistent with the expected changes in the Ca^{2+} content of an intracellular pool. The site of this pool is unknown. It may be the sarcoplasmic reticulum which is especially well developed in iris sphincter smooth muscle (Bell and DiStefano, 1966; Gabella, 1976) and in smooth muscles capable of contracting in the absence of external Ca^{2+} (Devine et al., 1972). It is also possible that melanin granules in the sphincter muscle cells play a role in the storage of intracellular Ca^{2+} (Brown et al., 1975; Henkatt, 1975; Panessa and Zadunaisky, 1981).

In amphibian smooth muscle, activation of contraction has been estimated to take approximately 250 msec (Fay et al., 1979). The much longer latency of the iris light response (~1 sec) indicates that the link between rhodopsin isomerization and Ca^{2+} mobilization is slow and may include several steps. In vertebrate rods, light catalyzes the hydrolysis of cGMP as well as Ca^{2+} release (Hubbell and Bownds, 1979; Gold and Korenbrot, 1980; Yoshikami et al., 1980) which has led to speculation about the relationship between these two events (Fatt, 1982; Liebman et al., 1984). Recent work on rods and cones describing the presence of a cGMP-sensitive conductance in the outer segment membrane (Fesenko *et al.*, 1985; Nakatani and Yau, 1985) provides strong support for the hypothesis that the internal transmitter controlling the light-sensitive conductance in

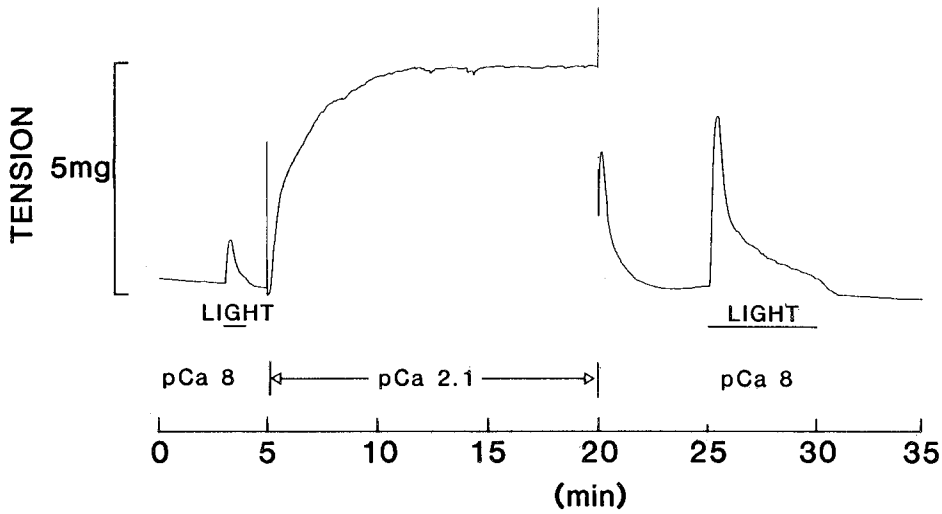


Figure 9. Restoration of the light response of a Ca^{2+}-depleted iris. The iris, depleted of Ca^{2+} as in Figure 8*A*, was exposed to light before (*left*) and after (*right*) a 15-min incubation in Ca^{2+}-containing solution (7.9 mM Ca^{2+}). The contracture during Ca^{2+} incubation presumably resulted from the movement of Ca^{2+} into the smooth muscle cells through channels opened in high K^+ solution. In other irises, recovery of the light response after Ca^{2+} incubation was evident as long as 20 min after they were returned to low Ca^{2+} solution (not shown).

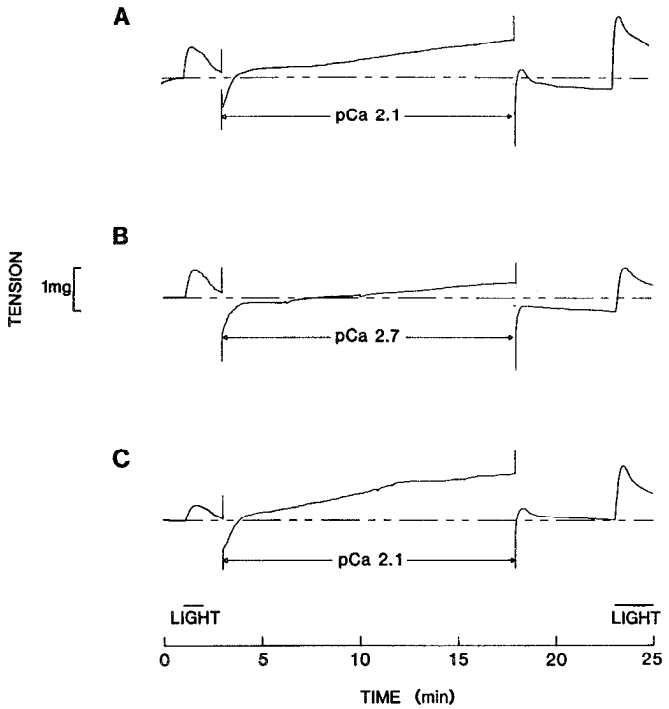


Figure 10. Loading versus Ca^{2+} concentration. The experiments shown compare loading of the light-controlled Ca^{2+} pool in pCa 2.7 (*B*) with loading in pCa 2.1 (*A*) and (*C*). Loading in pCa 2.7 was 40% of the average loading in pCa 2.1. Between experiments *A* and *B*, and *B* and *C*, the iris was allowed to dark adapt 20 min.

vertebrate photoreceptors is cGMP. Barr and Hunsicker (1983), however, report that changes in cGMP do not influence the light-evoked contraction of iris sphincter. This, along with our results indicating that light-evoked contraction is triggered by intracellular calcium release, argues against cGMP being causally related to the mobilization or action of calcium in sphincter smooth muscle. It is also possible that the transduction process in both light-sensitive smooth muscles and photoreceptors generates an intracellular signal that triggers, by different mechanisms, the activation of contractile proteins in smooth muscle and the stimulation of cGMP phosphodiesterase in photoreceptors. The latter part of this scheme, however, is difficult to reconcile with the observation that calcium is not necessary for rhodopsin-mediated hydrolysis of cGMP (Yee and Liebman, 1978). Another alternative is that the transduction process in light-sensitive smooth muscles and vertebrate photoreceptors could be entirely dissimilar and expressed through different second messenger molecules. It may also be that in both cell types, photoisomerization of rhodopsin generates multiple second messenger signals that control several aspects of cell function besides those that have been most frequently monitored, i.e., membrane potential in photoreceptors and contractile tension in light-sensitive smooth muscle. This leaves open the possibility that both Ca^{2+} and cGMP play roles in phototransduction, but the nature of the role that each plays depends on the cell in question.

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