Photosensitivity of the Frog Iris

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ABSTRACT The absorption of quanta by rhodopsin leads to the contraction of frog iris muscle. The contractions reach a maximum after about 8 sec. in the light. When the light is turned off the irises relax exponentially with a half-time of about 6 sec. Membrane polarization is not necessary for the response but calcium movement and membrane permeability changes probably are. The response is not mediated by acetylcholine or epinephrine. The curves of log *It vs.* log *t* for constant response amplitude bend progressively upward away from a unit slope line at short times as larger response criteria are used because (*a*) light influences tension development over longer times and (*b*) the higher intensity, shorter duration flashes are less effective.

Pupillary responses to light in frog (and in other amphibia as well as teleosts, especially eels) occur even when the eyes have been removed from the body. Arnold is said to have described this effect in 1841.[1] Brown-Sequard (1847 *a*,

[1] Arnold is cited by Steinach:

"Fr. Arnold, Physiologie II Bd. 1841"

and by Magnus:

"F. Arnold, Physiologie Bd. 2, 1841"

without further page references. According to Steinach, these experiments were carried out on isolated eels' eyes. We have not been able to document the reference. The most likely place is:

Friedrich Arnold, Lehrbuch der Physiologie des Menschen, Zweiter Theil, Zweite Abtheilung, Zürich Bei Orell, Füssli und Compagnie, 1841.

We have examined this book in some detail, but cannot find any reference to the experiments which Steinach describes. On the contrary, near the top of page 652 we find ". . . Da, wie diess mehrere Physiologen (Haller, Zinn, Fontana, Caldrani, Lambert) durch Versuch ermittelt haben, das unmittlebar auf die Iris durch die kleine Oeffnung eines Kartenblatts fallende, mehr oder weniger concentrirte Licht keine Veränderung in der Pupille hervorbringt, sondern diese sich erst dann verengert, wenn die Lichtstrahlen durch das Sehloch auf den Hintergrund des Auges gelangen; . . . "

Moreover we have not been able to find any evidence that Arnold had changed his mind on this question even as late as 1851. In his

Handbuch der Anatomie des Menschen, Band 2, 2 Abtheilung, Freiburg im Breisgau, Herder'sche Verlagshandlung, 1851

we note on page 1029 (in small print) the following:

"Ueber die Einflusse und Züstande, welche eine verschiedene Veränderung der Pupille bewirken, vergl. *meine* Phys. Th. II s. 643 ff. . . ."

1847 *b*, 1859) a few years later showed that the phenomenon was due to light which fell on the iris. Furthermore, he found that the response persisted after lethal doses of strychnine, ether, opium, or atropine and that it was not due to an increase in temperature in the iris.

Steinach (1892) reviewed and extended these earlier observations. He pointed out that only the internal edge of the iris must be illuminated and that the contraction spreads from one side of the iris to the other. He found the greatest response using the blue-green part of the spectrum but considered from the color of the iris granules that the blue-violet fraction was really the most effective. Finally, he found that the response was increased by storing the frogs for days or weeks in a dark cellar.

Using somewhat more reliable techniques, Magnus (1899) also found that the largest responses were elicited by the green light of a prism spectrum and suggested that Kühne's (1879) "*Sehpurpur*" (*i.e.*, rhodopsin) was the absorbing pigment. He found that atropine affected the response to photic stimulation more than the response to electric stimulation and concluded that the contraction of the iris sphincter was not due to a direct effect on muscle fibers but was by way of nervous elements within the iris. Both Steinach and Magnus eliminated the possibility of retinal involvement by observing that pupillary constriction still occurred when the iris was dissected free.

The possibility that the results described by Magnus were a consequence of an irreversible damage to the muscle due to the high concentrations of atropine employed was suggested by Guth (1901) who showed that the responses both to light and electricity diminish together.

The first modern attempt to define the action spectrum by Weale (1956) resulted in a sensitivity curve which decreased monotonically with wave length between 420 and 600 mμ, a fact which directly contradicts the results obtained in the last century (*i.e.*, that the wave length for maximum effect coincided with the absorption peak of rhodopsin). More recently Seliger (1962) observed the change in diameter of isolated eel irises in response to long flashes. He corroborated Steinach's and Magnus' result that the maximum response is obtained at about 500 mμ.

In view of the experiments just summarized, the characteristics of the response of the iris to light (action spectrum, time course, dependence on time in the dark, effect of variation of flash duration, and intensity) ought to be determined clearly and in detail.

The experiments discussed in this paper were designed to establish the photokinetics and a definitive action spectrum of the mechanical response. They also provide data which bear on the plausibility of events which might mediate the response, (*a*) changes of the state of the membrane, (*b*) release of cholinergic or adrenergic neurohumors, (*c*) movements of calcium.

METHODS

Frog iris preparations free of corneal, scleral, ciliary muscle components were made by cutting around the periphery of the iris. Most of the attachments to the ciliary body were removed, but some were left to avoid damage to the delicate iris. The irises were mounted between platinum hooks passed through the pupils. Sometimes two irises were mounted in parallel between double hooks. The tension developed was recorded on a Grass polygraph *via* a 3 inch balsam lever attached to a Grass tension transducer. Shortening was less than 2 per cent of initial length (about 5 mm) and identical records were obtained with much longer or heavier levers. The mechanical system was periodically calibrated with standard weights in the appropriate range.

Frogs were kept in dark, cool chambers and had access to running water. They were kept up to 2 weeks and fed strained liver every other day.

The chambers in which the irises were bathed were of Pyrex and Herasil quartz. The monochromatic stimulating light was provided by a mercury vapor light source or by a tungsten ribbon filament lamp (color temperature of about 3000°C) which then passed through a 1200 lines/mm Bausch and Lomb grating monochromator, with a 500 mm focal length. The exit slit was imaged with unit magnification by a quartz lens mounted about 10 cm away from the monochromator. The iris preparation was mounted in the plane of this image and was always smaller than the image of the slit. The energy of the monochromatic light at each wave band through the spectrum was determined using a lamp calibrated at the National Bureau of Standards and a thermopile. The intensity of the light was varied by varying the height of the entrance slit. White light was provided by a tungsten filament of about the same color temperature imaged on the preparation. A calibrated iris diaphragm immediately in front of the imaging lens served to vary the intensity, in this case.

The normal Ringer's solution comprised 112 mM NaCl, 1.87 mM KCl, 1.1 mM $CaCl_2$, 2.38 mM $NaHCO_3$, 0.072 mM NaH_2PO_4, and 11.1 mM glucose.

The K_2SO_4-substituted Ringer's solution was the same except that 74.7 mM K_2SO_4 was substituted for the 112 mM NaCl; in addition, $KHCO_3$ and KH_2PO_4 replaced $NaHCO_3$ and NaH_2PO_4 mole for mole. The solutions containing different amounts of $CaCl_2$, EDTA, or citrate were prepared to have the same osmolarity as the control solutions by varying the NaCl or K_2SO_4 concentrations. All solutions were at pH 7.3 and the bath was at room temperatures (22–25°C).

RESULTS

Individual Responses

When the isolated iris is exposed to light, it generates tension after a short latent period (Fig. 1 A). This is true even for exposures shorter than the latent period. Increases in the durations of short flashes result in larger responses, but the light becomes progressively less effective as it is prolonged. In fact, as flash duration is increased beyond about 8 sec. the rate of tension development

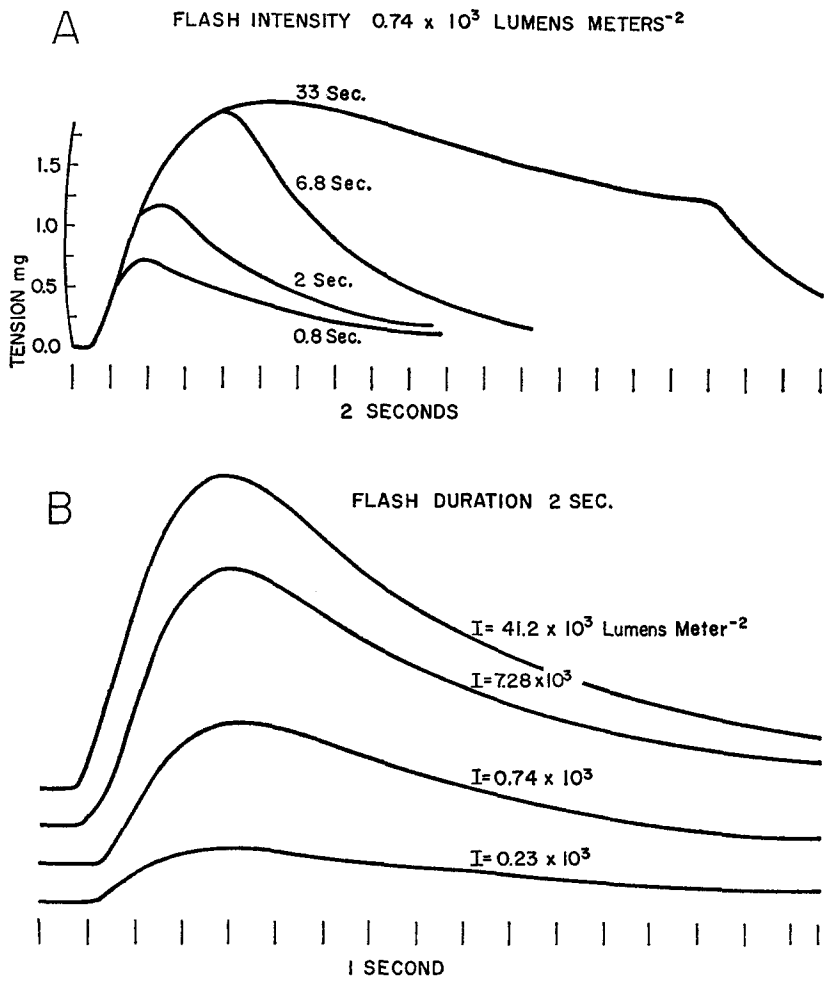

FIGURE 1. (A) Isometric contractions of the frog iris resulting from flashes of white light of the same intensity but of different durations. The number on each curve represents flash duration. The separation of the marks on the time scale is 2 sec. (B) Isometric contractions resulting from flashes of white light of the same duration but of different intensities. Same tension scale but different time scale. Note that the response to a 2 sec., 0.74×10^3 lumen m^{-2} flash appears in both (A) and (B).

during the response decreases until finally the iris actually begins to relax. Thus, for very long flashes the tension being developed by the iris passes through a maximum and falls off, approaching exponentially a value above the resting tension. When a flash ends the muscle continues to develop tension (short flashes) or maintain tension (long flashes) for about a second before relaxing.

In response to flashes of the same intensity but different durations, tension development always follows the same initial time course even though the

shortest flashes are over before the muscle contracts and the longest persist long after the maximum tension has been achieved (Fig. 1 A). In the complementary experiment, holding duration fixed and varying the intensity, the initial rate of tension development and the peak tension increases approxi-

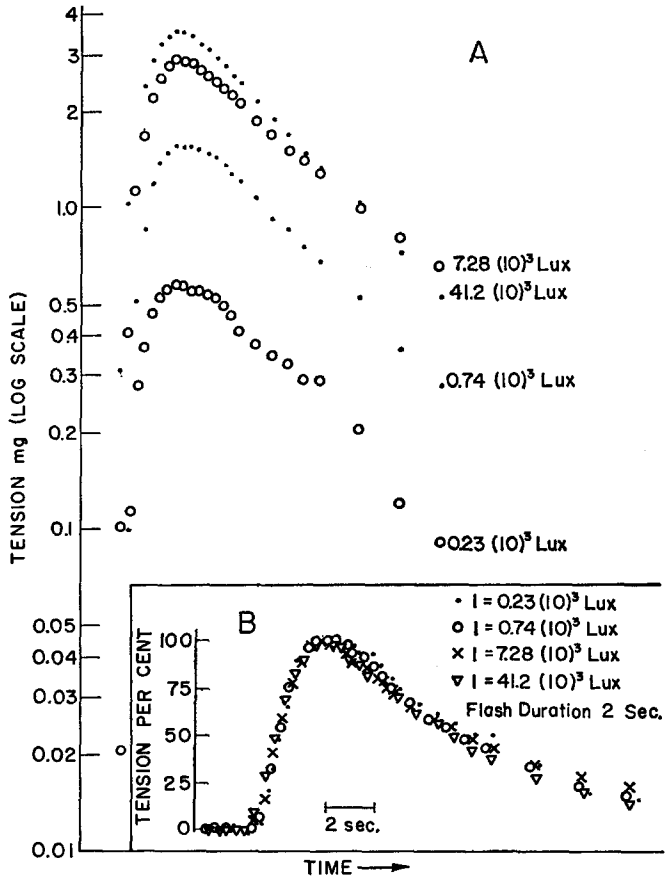

FIGURE 2. (A) The responses shown in Fig. 1B plotted with the logarithm of the tension developed as the ordinate. Both the initial rise and fall of tension follow simple exponential time courses with time constants independent of intensity. (B) The responses shown in Fig. 1B plotted with per cent of maximum tension developed during the twitch as the ordinate. The time course of the responses is independent of intensity.

mately linearly with the logarithm of the light intensity (Figs. 1 B and 4 A). If the tension time course curves in Fig. 1 B are plotted using a logarithmic tension scale (Fig. 2 A), both the initial rise of tension and the relaxation decay are seen as simple exponential functions of time. For these responses the time it takes to achieve any given fraction of the peak tension of the twitch is independent of intensity. The responses to flashes of different intensities, normalized for the peak tension, superimpose (Fig. 2 B).

Fig. 3 illustrates the maximum tension developed during a twitch as a function of flash duration and intensity. For any given intensity the peak tension increases monotonically with duration. From Fig. 1 A it is evident that the curve in Fig. 3 for any intensity will become a horizontal straight line as soon as the duration of the flash is longer than the minimum duration required for maximum peak tension (T_{max}) for that intensity; since at any given duration, the tension time course is independent of intensity (Fig. 2 B) the

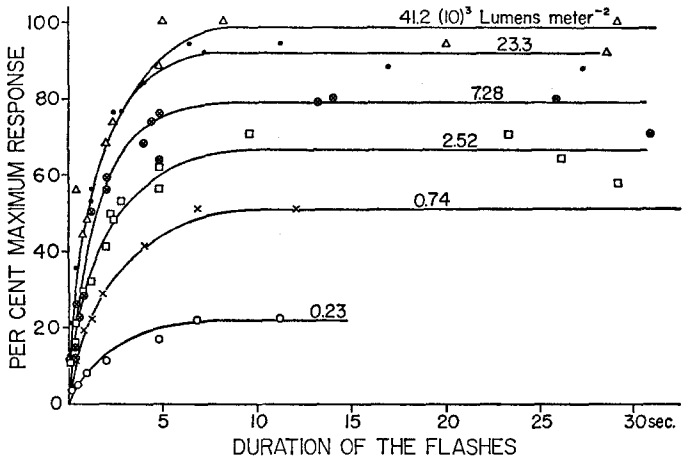

FIGURE 3. The maximum tensions of twitches of two preparations, as per cent of maximum tension developed by the muscle, plotted as a function of the durations of the flashes for several intensities. For each intensity there is a maximum attainable tension reacheP after about 8 sec.

minimum flash duration for T_{max} is always the same. The smooth curve for each intensity in Fig. 3 is drawn to indicate these trends of the data.

The data in Fig. 3 may be used to find a relation, for any given duration, between peak tension during a twitch (T) and intensity. To do this it is best to interpolate between the points to find the peak tensions at a constant duration. This can be facilitated by finding that transformation of coordinates for which the results most nearly fall on a straight line. This is a plot of logarithm $(1 - T/T_{max})$ *vs.* flash duration. Fig. 4 A shows the relation between T and log I for these interpolated flash durations. Linear extrapolation of these data to zero tension shows that the threshold intensity, even for 0.1 sec. flashes, is independent of duration. This means that the critical duration for threshold flashes is less than 0.1 sec.

Fig. 4 B demonstrates in the traditional way (Hartline, 1934) the amount of light required to produce a given criterion response as a function of flash duration. For each of the criteria illustrated in this figure, the peak tension is not influenced by prolonging the longer flashes. Data points for which this is true

plot on a straight line of unit positive slope (I = constant). Since all the flash durations used exceed the critical duration for a threshold response, the threshold criterion data all fall on such a time-independent unit slope line. As the response criterion is increased, however, light continues to be capable of influencing tension development, even though it has been shining as long as 8 sec. Furthermore, because light is less effective at higher intensities than at lower ones ($T \sim k \ln I$, see Fig. 4 A), more light is required in shorter flashes than in longer ones to evoke big contractions. For these reasons the curves in Fig. 4 B based on progressively larger response criteria bend progressively upward away from a unit slope line as the flash duration is reduced. The amount of light required to give a constant response in the neighborhood of three-quarters of a milligram is constant for flash durations up to 2 sec.

Effect of Prior Illumination

What is the time course of the recovery of iris photosensitivity following exposure to a strong light stimulus? To answer this, dark-adapted irises were bleached by an intense light (4.12×10^4 lumen m^{-2}) for about 20 mins. The irises contracted to a tension maximum in about 8 sec. and thereafter the tension exponentially decayed to about one-third its peak during the 20 min. in the light. When the bleach light was turned off, the tension was maintained for about a second and then decayed exponentially to the base level.

The recovery of photosensitivity was subsequently determined by obtaining responses to 10 sec. test flashes in the dark. In any given recovery experiment an iris was tested only three or four times, but it was possible to obtain responses at nine different intervals in the dark by repetition of the process with the test flashes interspersed at different times.

The means ($\pm$ SEM) of the ratios of test flash peak tensions to peak tension during the bleach, as a function of time in the dark, obtained from five irises are plotted in Fig. 5. It is evident that after about 2 min. in the dark the tension developed is 60 per cent of the maximum tension developed during the bleach. The time course of recovery of photoresponsivity was not influenced by using different test flashes (weak monochromatic or white light of different intensities and duration), suggesting that the procedure obviated possible distortions of the recovery curve due to the test flash.

The recovery of muscle photoresponsivity is similar to the recovery of the electroretinogram of the frog following strong bleaches. In Fig. 5 the lines show measurements of the latter process by Wald, Brown, and Kennedy (1957).

Latent Period

The latent periods of the responses to light were measured under all experimental conditions. The grand mean for control dark-adapted irises was 0.77

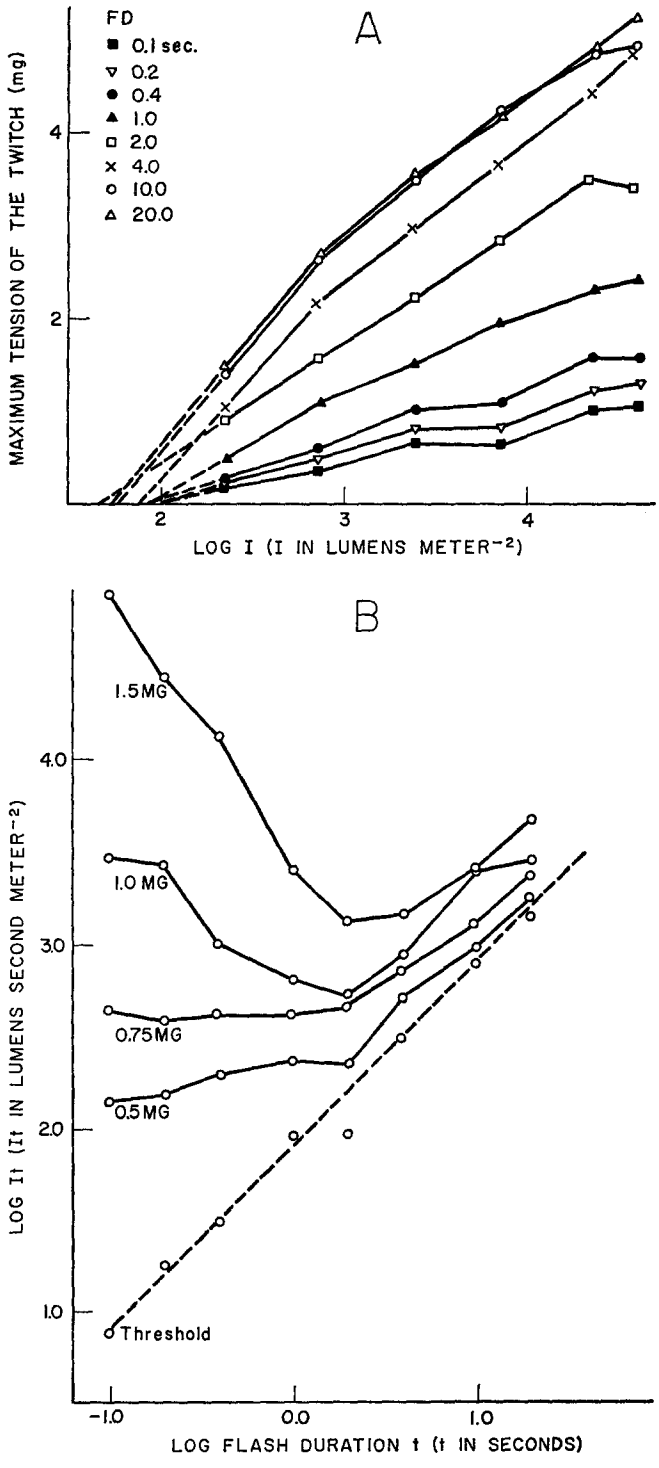
A
FD
0.1 sec.
0.2
0.4
1.0
2.0
4.0
10.0
20.0
MAXIMUM TENSION OF THE TWITCH (mg)
4
2
2
3
4
LOG I (I IN LUMENS METER^{-2})
B
1.5MG
1.0 MG
0.75MG
0.5MG
Threshold
LOG It (It IN LUMENS SECOND METER^{-2})
4.0
3.0
2.0
1.0
-1.0
0.0
1.0
LOG FLASH DURATION t (t IN SECONDS)

FIG. 4

± 0.31 (SD) sec. with a range of 0.43 to 2.4 sec. There was no statistically significant correlation between latent period and light intensity or amplitude of the response over the range of brief flashes used. There were no significant changes of latent period caused by high external potassium, scopolamine, atropine, physostigmine, carbachol, or acetylcholine.

However, as the contractions were attenuated to about 25 per cent normal in Ca-free citrate solutions, the latent period increased from 1.02 ± 0.05 (SEM) to 1.92 ± 0.06 (SEM) (t test $p < 0.01$).

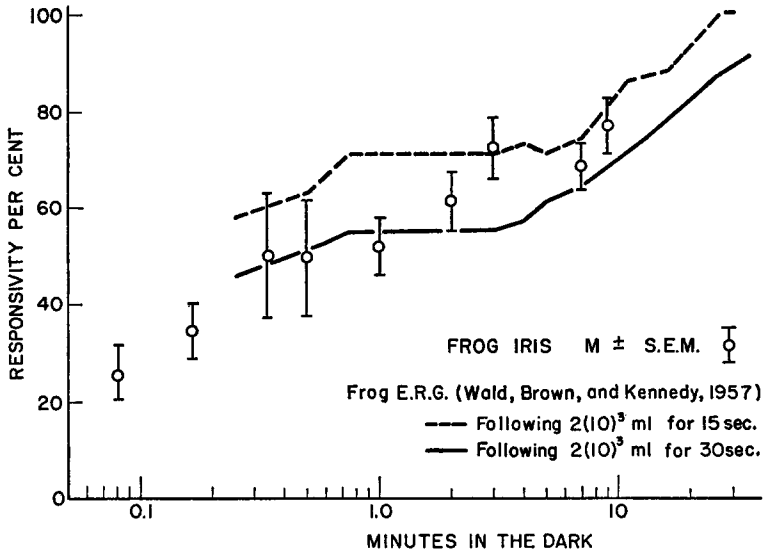

FIGURE 5. Recovery of sensitivity of five irises to light as a function of the time in the dark following exposure to 41.2 $(10)^3$ lumen m^{-2} for 20 min. The ordinate is the ratio of peak twitch tension in a response to the test flash to the peak tension developed during the light adaptation exposure. The test flash in these experiments was of the same intensity as the adapting exposures. The solid and dotted lines show the recovery of sensitivity of the frog retina following adaptation exposure of the indicated luminance (in millilamberts) and duration as measured by Wald, Brown, and Kennedy (1957) with the electroretinogram.

After 20 min. bleaches, as six irises recovered in the dark, the average latent period decreased progressively from 1.36 ± 0.42 (SEM) at 10 sec. to 0.74 ±

FIGURE 4. (A) The maximum tensions of twitches plotted as a function of the logarithm of the intensity of the flashes for different durations. The data are computed from Fig. 3 with the maximum tension of each preparation arbitrarily equated to 5.2 mg (*i.e.*, the maximum tension developed by either). That the curves for different flash durations all intersect the abscissa at nearly the same place indicates that there is an intensity threshold for the durations used. (B) The logarithm of the amount of light required to elicit a given amount of tension plotted as a function of flash durations. Note that the critical duration for threshold tension is less than 0.1 sec. but that there is a critical duration (It = constant) of about 2 sec. for 0.75 mg responses.

0.06 (SEM) at 9 min. The decrease was statistically significant. It had a Pearson product-moment coefficient $r = -0.31$ ($N = 77$; $p < 0.01$). However, there was no statistically significant change of latent period after 6 min. in the dark, even though the tension maxima of responses were still increasing at this time (see Fig. 5).

Action Spectrum

An action spectrum is properly compared with an absorption spectrum only when the action spectrum represents the reciprocal of the number of quanta

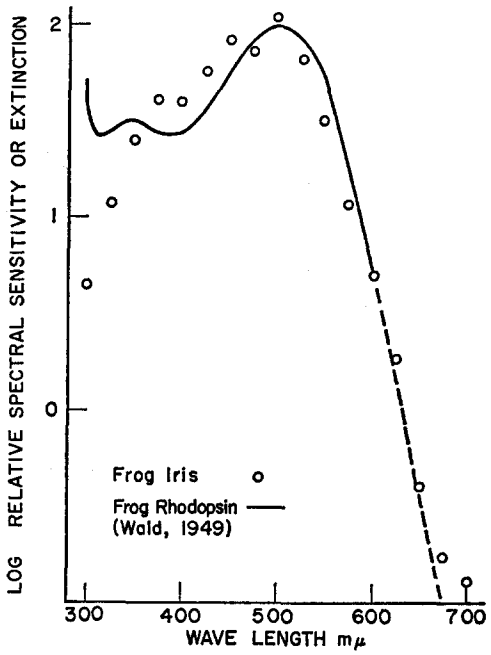

FIGURE 6. The action spectrum as measured by the reciprocal of the number of quanta required to produce a criterion tension level in three iris preparations. The points are the geometric means after the curves were vertically shifted for superimposition with minimum scatter. The smooth curve is the extinction spectrum of frog rhodopsin as measured *in vitro* by Wald (1949). The sensitivity measurements at 675 and 700 mμ are probably erroneously high because of stray light in the monochromator.

at each wave length required to produce some constant physiological response under conditions where the magnitude of the response is proportional to the number of incident quanta. The action spectrum of the frog iris determined in this way is illustrated in Fig. 6. The points are the means of measurements made on three preparations and the line is the *in vitro* absorption spectrum of frog rhodopsin measured by Wald (1949).

The agreement between the two spectra in Fig. 6 is not very good. However, it is close enough to leave little doubt that the excitation of the iris muscle by white light is due to the absorption of quanta by rhodopsin.

Dependence on Calcium

Calcium seems to be involved twice in the sequence of processes which is initiated by a stimulus to muscle and ends with contraction. First, excitable

membranes are stabilized by calcium (Shanes, 1958). Thus action potentials are elicited at lower stimulus strengths and indeed can be made to appear spontaneously in skeletal muscle, when external calcium is decreased (Shanes, 1958; Bülbring, Holman, and Lullman, 1956). In addition, however, the contraction itself seems to be dependent on an increase in intracellular calcium ions (Niedergerke, 1957; Bianchi, 1961). That is, the coupling between the membrane event and contraction involves calcium movement.

When external calcium was slowly washed away the strength of contraction of the iris in response to light increased before eventually declining. This result was obtained using simple calcium-free solutions or those which were calcium-

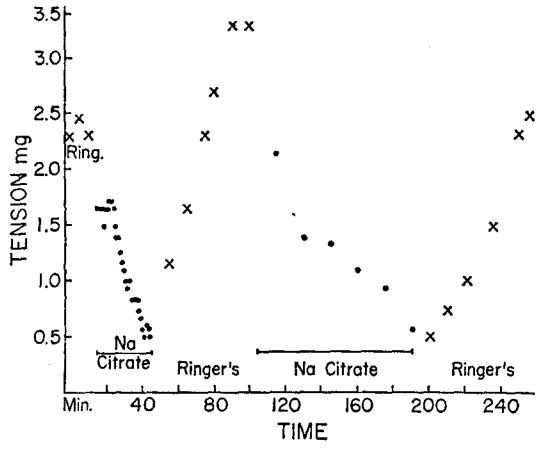

FIGURE 7. The maximum tension of twitches elicited by standard 10 sec. flashes of white light [I = 41.2 $(10)^3$ lumen m^{-2}] plotted against time when the incubating solution is either normal Ringer's or calcium-free, citrated Ringer's. The twitch amplitude decreases when calcium is washed out. Moreover, the decrease is much faster if the frequency of stimulation is greater.

free and contained in addition 2 mM EDTA or 2.67 mM citrate. The decline of contractility was too slow (time to half-amplitude when the iris was unstimulated was about an hour and a half) to be considered simply a function of sweeping calcium out of the extracellular space.

The possibility that calcium bound in some manner was released by the light stimulus and then was effective in causing contraction was investigated in the following way. If there is a bound store of calcium and external calcium is washed away, then the rate of loss of contraction strength should be determined by the rate of stimulation.

Fig. 7 shows the amplitudes of the contractions when an iris was (*a*) stimulated at a fast rate in a calcium-free bath, then (*b*) allowed to recover in a normal solution, then (*c*) stimulated at a slow rate in a calcium-free bath. The figure shows clearly that it was not simply the time of washing out of the calcium alone which decreased the strength of the contraction but that the frequency of stimuli (contractions) was a strong determinant of the rate of loss of contractile strength. When the order of treatment was reversed ((*a*) slow stimulus rate in Ca-free solutions; (*b*) recovery; (*c*) fast stimulus rate in

Ca-free solutions), the result was exactly the same; *i.e.*, the rate of loss of strength of contraction again increased with the frequency of contractions in the calcium-free solution.

The rates of decrease of contractile force in calcium-free EDTA solutions were similar when either carbachol (10^{-5} and 10^{-8} w/v) or acetylcholine (10^{-6} and 10^{-9} w/v) was used as the stimulus.

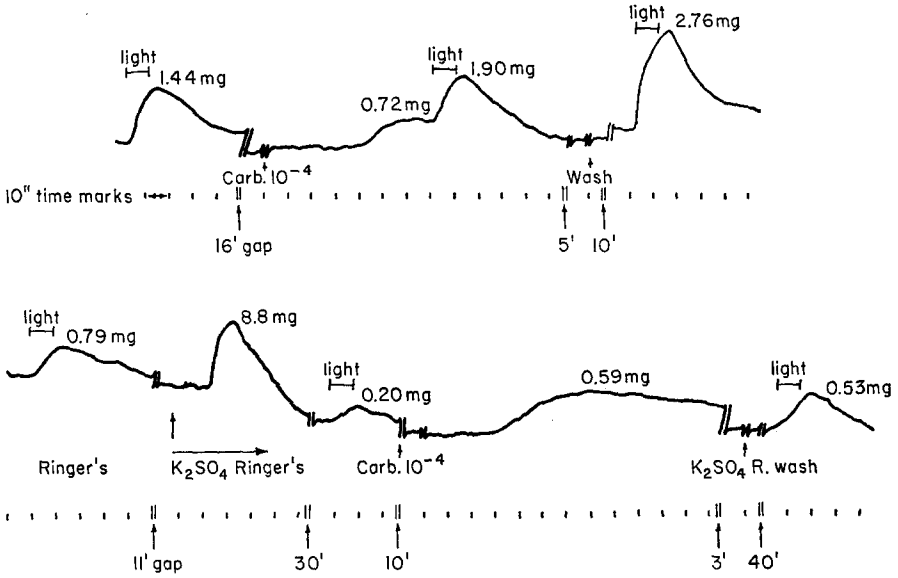

FIGURE 8. Record of isometric tension of isolated frog iris. Upper tracing shows the response to 10 sec. flashes before, during, and after exposure of the iris to 10^{-4} w/v carbachol in normal Ringer's. Lower tracing shows the contraction caused by switching from a normal Ringer bath to a K_2SO_4-substituted Ringer bath and also the iris response to light and carbachol in the presence of the K_2SO_4-Ringer's solution. The amplifier gain was turned down to record all of the large initial contraction to the K_2SO_4-Ringer's. On the time scales, the single prime marks refer to minutes and the double prime marks refer to seconds.

High External Potassium

Is a change in membrane potential involved in photoexcitation? Is a change necessary? The results of the following experiments suggest that the answers are yes to the first question, but no to the second. When an isolated iris is exposed to depolarizing KCL-Ringer's or K_2SO_4-Ringer's it contracts strongly. This contraction is not maintained indefinitely in the presence of high external potassium, but decays away (see lower trace of Fig. 8). The iris still responds to light and drug stimuli in high potassium solution even though it has completely relaxed from the initial potassium contraction.

Although the responses to light and drugs are decreased in high potassium

solutions, the fact that the muscle still responds after as long as a hundred minutes in K_2SO_4-Ringer's indicates that an effective stimulus need not cause an appreciable change of membrane potential. This in fact has been previously established for guinea pig intestinal muscle by Evans, Schild, and Thesleff (1958) using drug stimulation in high external potassium solutions and measuring the transmembrane potential. Since depolarizing a membrane decreases the response to light, however, there still seems to be a membrane permeability change in the stimulus-response sequence. One might infer, therefore, that in a high sodium solution, generator potentials and action potentials are results of photic excitation. Preliminary experiments have shown that the rate of loss of radioactive potassium from irises is increased as a result of photic excitation in both high and lower external potassium solutions (Barr, unpublished data).

Action of Drugs

The iris is said to comprise two histologically distinct smooth muscles, the radial dilator muscle and the circular constrictor muscle which forms a sphincter at the internal edge of the iris. The response to light reported here appeared from the mechanics of recording to be due to the contraction of the circular muscle. Certainly this is true, when, as in some preparations, the dilator muscle was dissected almost completely away. The response of the iris preparations to autonomic drugs verifies this.

The addition of l-epinephrine (up to 10^{-6} w/v) or the less potent phenylephrine (up to 10^{-5} w/v) to the muscle bath caused a very small decrease in tension. The presence of the highest concentrations of these drugs did not affect the response of the iris preparations to light. The addition of acetylcholine to the muscle bath caused the iris to contract. Contractions due to concentrations of 10^{-10} w/v acetylcholine were easily detected. The maximum response to acetylcholine was obtained at concentrations about 10^{-7} w/v. However, the contractions due to the larger concentrations were quite variable. This is probably due to variability in the onset of the blocking action of the acetylcholine. The sensitivity of the preparations to carbachol was an order of magnitude less than to acetylcholine.

However, when the iris was contracting maximally in response to 10^{-5} w/v acetylcholine or 10^{-4} carbachol a flash of light still elicited a further contraction (Fig. 8). The response to light still occurred after initial contraction to 10^{-5} w/v acetylcholine was over. The tension development caused by light in the presence of concentrations of acetylcholine where additional acetylcholine is ineffective is evidence against the possibility of mediation of the light response by acetylcholine. Furthermore, the iris developed a tachyphylaxis to acetylcholine and carbachol, but not to light.

Further evidence against the possibility of acetylcholine mediating the re-

sponse was obtained using other drugs (Table I). The light response was not potentiated by 10^{-7} w/v physostigmine whereas the response to 10^{-7} w/v acetylcholine was increased about 25 per cent. Concentrations of scopolamine (10^{-7} w/v) which completely blocked the iris response to 10^{-6} w/v acetylcholine had no effect on the response to light. Although 10^{-7} w/v atropine produced a 90 per cent block of a 10^{-6} w/v acetylcholine response, it did not affect the light response. Procaine (10^{-5} w/v) also did not affect the light response at the same time that it decreased the responses to acetylcholine to almost half control values.

TABLE I
EFFECT OF DRUGS ON IRIS RESPONSE

Conditioning drug	Test stimulus	Control tension*
		per cent
10^{-6} w/v epinephrine	Light	100
10^{-5} w/v phenylephrine	Light	100
10^{-7} w/v physostigmine	10^{-7} acetylcholine	125
10^{-7} w/v physostigmine	Light	100
10^{-7} w/v scopolamine	10^{-6} acetylcholine	0
10^{-7} w/v scopolamine	Light	100
10^{-7} w/v atropine	10^{-6} acetylcholine	10
10^{-7} w/v atropine	Light	100
10^{-5} w/v procaine	10^{-6} acetylcholine	60
10^{-5} w/v procaine	Light	100

* The figures in this column were computed as the ratio (in per cent) of the amplitude of the response to the test stimulus in the presence of the conditioning drug to the amplitude of the response to the test stimulus alone.

The response to light is potentiated by prior stimulation with cholinergic drugs and very low continuing stimulation with them. This is true even when the membrane is depolarized. Thus while it appears certain that the photoresponse is not mediated by acetylcholine, it is probable that cholinergic drugs and light share a common locus of action, the membrane.

DISCUSSION

The deviation of the action spectrum points from the rhodopsin absorption spectrum in Fig. 6 is too great to be accounted for simply by experimental error. It is, of course, conceivable that this difference between the iris action spectrum and the *in vitro* absorption spectrum is due to slight differences in the apoproteins or to some component of the intracellular environment.

Another explanation is that a second pigment contributes to the iris action spectrum. This could occur in at least two ways. First, a pigment whose excitation does not lead to contraction is screening rhodopsin. Second, activation of a pigment besides rhodopsin is contributing to the iris contraction.

In this regard it should be kept in mind that some retinal rods of the frog do not contain rhodopsin. Since the discovery of rhodopsin (Boll, 1876) it has been realized that some retinal rods contain a different pigment which absorbs strongly in blue light, is transparent in the green, but absorbs again in the yellow and red parts of the spectrum. Denton and Wyllie (1955) showed that the pigment (or pigments) in these "grass green" rods is photolabile in the blue but photostable in the yellow and red regions of the spectrum. Donner and Rushton (1959) proved that the absorption of light in the blue region of the spectrum by this pigment contributed to the physiological response eventually detectable in the discharge of retinal ganglion cells.

Too little is known about the absorption spectrum of this green pigment(s) to make any predictions which are testable by the extent to which the iris action spectrum deviates from the rhodopsin absorption spectrum. However, the fact that the agreement in Fig. 6 is much poorer in the blue than in the red region of the spectrum seems to support the idea that this additional pigment acts in the same way that rhodopsin does in the initiation of the iris photoresponse.

The question of the chemical pathways between the photoactivated pigment and the excitation of the contractile apparatus must be considered entirely open. However, it seems certain that a membrane event and a movement of calcium ions occur.

The importance of calcium as the coupling agent is emphasized both by the dependence of tension on its presence and by the increase of the latent period when calcium is taken out of the system. Since the latent period is not affected by intensity or flash duration (> 0.1 sec.), it is concluded that the major portion of the time lag between the absorption of quanta and the onset of contraction is due to calcium-mediated "coupling" processes.

The results of the drug experiments rule out the possibility of adrenergic or cholinergic transmitters mediating the photoresponse. Nonetheless, the only pigment granules in the iris muscle cells look exactly like the melanin granules in the pigment epithelial cells (personal communication from M. M. Dewey). Thus the possibility remains that the rhodopsin resides in non-muscle cells which activate the muscle cells by way of irin, 5-hydroxytryptamine, or some other chemical transmitter. There are, however, certain difficulties with this hypothesis. If the response were mediated by some transmitter not so far investigated it might be expected that the time course of the responses evoked by light would be appreciably slowed in the presence of depolarizing potassium solutions. In fact, it is found that although the peak twitch tension is reduced by a factor of about 2.5 in such solutions the latent periods and time courses of development and decay of tension remain unaffected by the depolarization of the membrane.

By suitable calibration with the standard lamp it was possible to determine

the amount of energy required to evoke a response. One of the most sensitive iris preparations generated about 4 mg tension when it was exposed to 12.5 ergs of blue-green light ($\lambda = 500$ mμ). This is a considerably larger amount of energy than any reasonable estimate of mechanical work done. Thus the question of whether or not light energy is converted into mechanical work must remain open.

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