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HYPERSENSITIVITY TO LIGHT OF THE IRIS (SPHINCTER PUPILLAE) OF THE ALBINO AXOLOTL (*AMBYSTOMA MEXICANUM*)

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Summary

As is common for amphibians, the sphincter pupillae of the axolotl contracts *in vitro* in response to illumination with visible light.

1. In a comparison of photomechanical responses of albino and normally pigmented axolotls, similar time courses and maxima of force development were found.

2. The dependence of isometric active force development on the length of the sphincter pupillae is similar to that of other smooth muscles.

3. The action spectrum of the axolotl is similar to the absorption spectrum of frog rhodopsin.

4. At low stimulus strengths, the increase of normalized, isometric, active force with increasing stimulus strength is approximately seven times as great in albino axolotls as in normally pigmented ones.

5. Melanin appears to decrease the light sensitivity of the irises of normally pigmented animals by acting as a simple light shield.

Introduction

Although control of the aperture of the pupil in humans and many other mammals is due to a reflex triggered by light falling on the retina, contractions of isolated irises from a wide variety of vertebrates in response to stimulation by light have been reported by many investigators (for reviews see von Campenhausen, 1963; Glaus-Most, 1969). In the frog, the pupil is primarily controlled by the direct stimulation of the sphincter pupillae by light (Alpern *et al.* 1975), rather than by a reflex. The action spectrum of this response in the frog sphincter pupillae was found by Barr & Alpern (1963) to be very similar to the extinction curve of frog rhodopsin (Wald, 1949). In contrast to the threshold for vision, the amount of light required to elicit a measurable photomechanical response is quite large; the photomechanical response is not a sensitive response. However, the frog iris has a high but unexceptional sensitivity to acetylcholine, which is the normal excitatory neurotransmitter. Acetylcholine is primarily if not exclusively the motor transmit-

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ter for the better known retinal miotic reflex. In other words, iridial sphincter muscle is much less sensitive to light than is a rod, but it is just as sensitive to acetylcholine as is a skeletal muscle motor endplate. This study investigated whether any of the relative insensitivity to light is due to shielding of iridial rhodopsin by melanin. Other factors which may also be involved are the efficacy of the photomechanical coupling links and particularly the concentration of rhodopsin itself.

In the normally pigmented vertebrate animal, smooth muscle cells of the sphincter pupillae contain large numbers of melanin granules. The irises of albino hamsters which are photosensitive (Bito & Turansky, 1975) have been shown to be devoid not only of melanin but also of any form of rudimentary pigment granule (Zucker & Nolte, 1981). Thus, these granules do not contain the triggering rhodopsin.

The photomechanical response is highly developed in amphibians. To compare responses in a pigmented amphibian with those in an albino, the axolotl was chosen because it was easily obtainable in the albino form. In preliminary experiments it was found that the photomechanical responses of axolotl irises are similar to those of the more intensively studied frog. Thus it was possible to test the possibility that intracellular melanin acts as a simple shield of rhodopsin in the axolotl.

The light sensitivity of sphincter pupillae muscles from albino animals was found to be approximately seven times greater than that of normally pigmented animals. It was concluded that melanin does shield the rhodopsin of these iridial smooth muscle cells simply by preventing quanta from reaching rhodopsin independent of wavelength.

Materials and methods

Seven albino and six normally pigmented adult female axolotls were generously provided by Professor R. R. Humphrey, Indiana University. Their lengths ranged from 16 to 22 cm. Animals were kept individually in 2-l, glass fish bowls, half filled with dechlorinated, aerated tap water at about 20 °C and were fed strips of beef liver (2 mm × 5 mm × 15 mm) every other day. They were dark adapted for several days before use.

All dissections and experiments were performed in red light (i.e. light passed through Wratten II filters). Animals were killed by decapitation and then double pithed. The eyes were extirpated and pinned to a Sylgard layer on the bottom of a Petri dish filled with an amphibian physiological solution. The solution contained (in mmol l^{-1}) NaCl, 110; KCl, 2; $CaCl_2$, 1; Tris, 2; and glucose, 10. It was aerated using compressed air and the pH adjusted to 7·3. This solution was also used in the experimental chamber. After an eye had been fixed in place, the cornea was removed by cutting with iridectomy scissors around the intersection of the cornea and the sclera. An annular sphincter pupillae preparation was then made by cutting through the iris all the way around about 1 mm from the pupillary margin.

The resulting ring of tissue was suspended between a hook attached to the experimental chamber and a hook attached by a thin thread to a lever of the force transducer (Grass FT03). Three-minute recovery periods were allowed between stimuli. White light stimuli were provided by passing light from a tungsten filament lamp (colour temperature about 2700°C) through an infrared-absorbing filter and a Wollensak shutter. Monochromatic light for stimulation was provided by passing light from a 150 W Zeiss xenon lamp through a 1200 lines mm^{-1}, 500 mm focal length, Bausch & Lomb grating monochromator. The preparations were uniformly illuminated by images of either the tungsten filament or the exit slit of the monochromator. Light intensity was attentuated by neutral-density filters. The energy of the monochromatic light at each waveband was determined using a calibrated NBS lamp generously provided by Professor Paul Liebman, University of Pennsylvania. Isometric force exerted on the lever by the sphincter pupillae preparations was recorded *via* a Grass 79 strip chart recorder. The system was calibrated using a range of standard weights.

Results

The time courses of the photomechanical responses of sphincter pupillae muscles from normal axolotls (Fig. 1A) were similar to those from albinos (Fig. 1B) and to those from frogs and other amphibians. The muscles developed force after a latent period of about 1 s (1·15 ± 0·05 s, S.E.M., $N = 37$). There was no

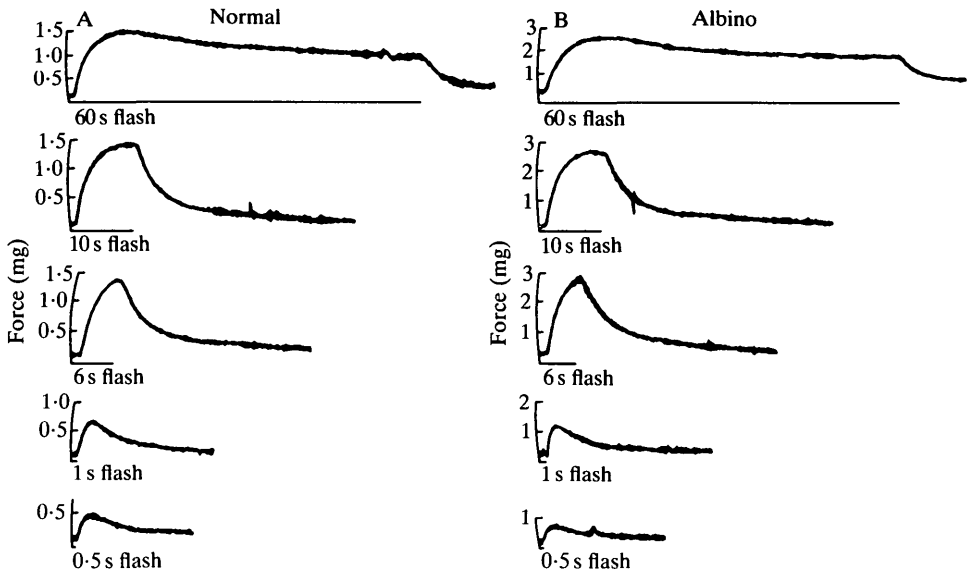

Fig. 1. Isometric contractions of sphincter pupillae muscles in response to a series of flashes of white light 60, 10, 6, 1 and 0·5 s in duration. (A) Responses of a sphincter from a normally pigmented animal (flash intensity, $0{\cdot}86\times10^3$ lx). (B) Responses of a sphincter from an albino animal (flash intensity, $0{\cdot}93\times10^3$ lx). Note difference in ordinate scale.

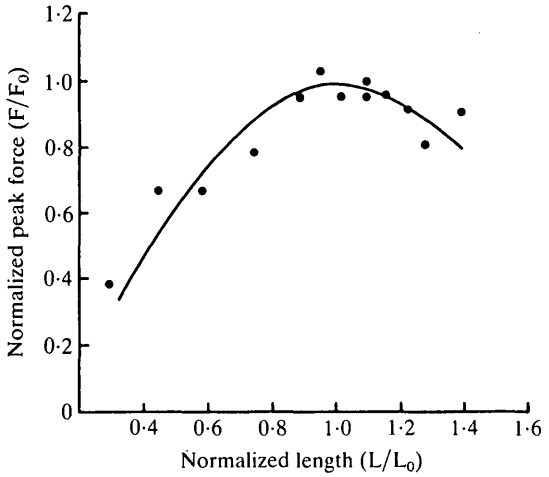

Fig. 2. A typical active force–length curve of an axolotl sphincter pupillae muscle. The data were normalized to the peak of an arbitrary smooth curve fitted by eye.

significant difference between the latent periods of preparations from normal and albino axolotls. The photomechanical responses of sphincter pupillae muscles of both normal and albino axolotls were prolonged similarly when the durations of light stimuli were increased (Fig. 1A,B). The muscles were capable of maintaining force for many minutes as one would expect from their *in vivo* function. However, the plateau of a tonic contraction was not reached monotonically after the initiation of a long flash, but instead the force achieved a maximum and then relaxed to a steady-state level after about 10 s of illumination. This may correspond to the pupillary overshoot sometimes observed *in vivo* in other animals.

The forces generated by sphincter pupillae muscles following 10 s maximal light stimuli were recorded isometrically as a function of preparation length. To compare the responsiveness of different muscles, the active length–force curves of each of the muscles were fitted by smooth curves and from them were determined the peak forces (F_0) and the lengths (L_0) at which the peak force was developed (Fig. 2). The resting length–force curves of different muscles appeared to be displaced upwards depending on the amount of the stromal component of the preparations, leading to large variability in the curves. In all preparations the resting force was at least as large as the active force at lengths in the neighbourhood of the standard length. This contrasts with the behaviour of skeletal muscles and various other smooth muscles. Although procaine (10^{-6} mmol l^{-1}), EDTA (5×10^{-3} mmol l^{-1}) or iodoacetic acid (1 mmol l^{-1}) increased compliance in this range of lengths, they did not increase it to that of skeletal muscle. Thus, it appears that the 'resting force' of the sphincter pupillae has at least two parallel components; a passive, connective tissue part and an active, contractile apparatus part. In contrast, the normalized active force was a relatively consistent function of normalized muscle length (Fig. 3). The data

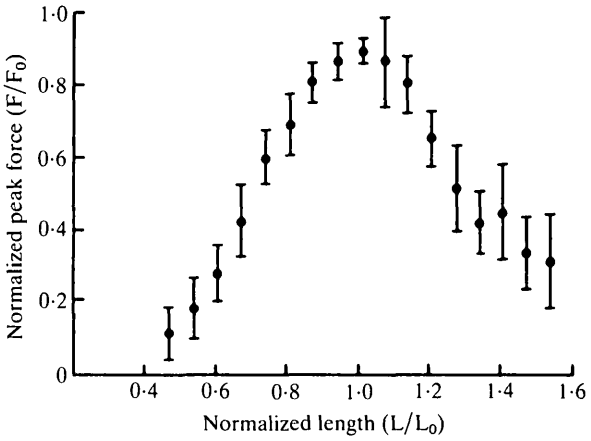

Fig. 3. Active force–length data from seven sphincter pupillae muscles (four albino and three normally pigmented preparations). The data from each muscle were normalized as in Fig. 2 before averaging. The vertical bars indicate the S.E.M.

normalized in this way are quite similar to similarly treated data from arterial (Murphy, 1980), intestinal (Meiss, 1971) and respiratory (Stephens, 1975) smooth muscles; in short, to smooth muscles in general (Barr, 1969; Murphy, 1980). When all the sodium ions of the physiological solution were replaced by potassium ions, the photomechanical responses were not blocked; they were attentuated but not changed appreciably in time course. If the extracellular calcium concentrations were reduced to very low levels (less than 10^{-8} mol l^{-1}) the photomechanical responsivity still persisted for as long as 90 min.

To test the participation of rhodopsin in the photomechanical response of the axolotl sphincter pupillae the action spectrum of the response was determined. Even though the absorption of light by rhodopsin is the first event in the light–contraction coupling sequence, the action spectrum of the photomechanical response will approximate the absorbance spectrum of rhodopsin only if certain conditions are met. First, photons once absorbed should contribute equally to the photomechanical response independent of their wavelength; that is, the 'univariance principle' (Nada & Rushton, 1966) should be obeyed. Second, there should be negligible wavelength-dependent screening of rhodopsin by other pigments. Third, to avoid saturation or other occlusive phenomena in the photomechanical coupling system, a response criterion should be established in a region where the magnitude of the response is proportional to the number of incident quanta. Finally, since the action spectrum is formally equivalent to an absorption spectrum, the number of incident quanta (or amount of energy) required to elicit the criterion response should be plotted against wavelength. If the pigment is very concentrated, the action spectrum will be broadened (Fein & Szuts, 1982). There were no significant differences between the action spectra of normal and albino animals. At wavelengths longer than 450 nm, the action spectrum of axolotl sphincter pupillae and the absorbance spectra of frog

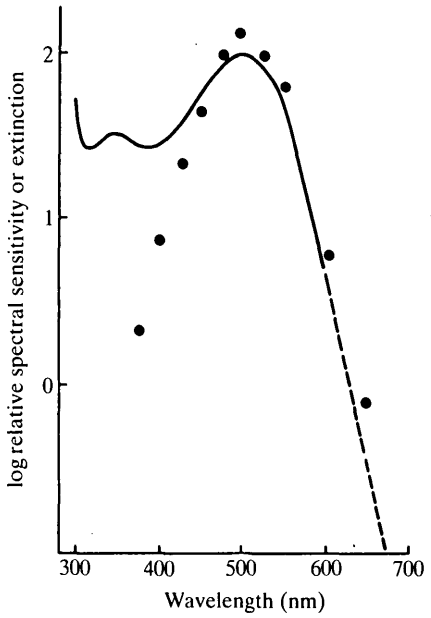

Fig. 4. Action spectrum of the axolotl sphincter pupillae muscle as measured by the number of quanta required to produce a criterion response. Data (filled circles) from four preparations were averaged geometrically by shifting individual action spectrum curves vertically to minimize scatter. The smooth curve is the absorption spectrum of frog rhodopsin determined by Wald (1949).

rhodopsin (Wald, 1949) are fairly similar (Fig. 4). However, sphincter pupillae muscles show a severe decrease of sensitivity to shorter wavelengths. This may be due to several causes, for example screening by a blue-absorbing pigment or a decrease in the coupling effectiveness in the blue region. Of these two possible causes the former seems rather more likely on the basis of photochemistry. However, although there are 'yellow albino' axolotls, the true albino axolotl appears to be devoid of all epithelial pigments and the sphincter pupillae does not contain pterinosome-containing cells. In any case, the agreement between the two spectra from about 450 nm through the peak and on through the rest of the visible spectrum is close enough to allow acceptance of the hypothesis that the muscle is excited by rhodopsin bleaching.

With strong enough light stimuli, normal pigmented sphincter preparations generated maximal forces not statistically different from the maximal forces generated by preparations from albinos. Likewise, the maximal forces elicited by stimulation with maximal carbachol stimulation (10^{-5} mol l^{-1}) were not different in the two kinds of sphincters. However, when dimmer, short flashes were given, such that the responses were proportional to the quantal content of the flashes, the forces generated by the pigmented sphincters were increased less per unit stimulus than were those of the sphincters from the albino animals. For the same increase in stimulus strength, the response peak amplitude of albino sphincters increased

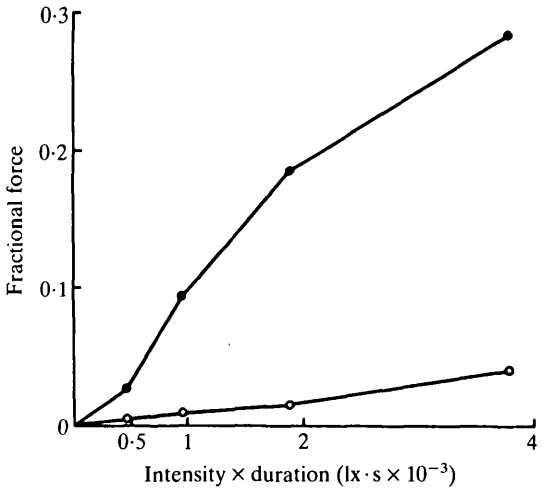

Fig. 5. Response amplitudes of axolotl sphincter pupillae muscles as a function of stimulus strength, following stimulation by 10·0 s flashes of different intensities; (○) normally pigmented, (●) from an albino animal. The photomechanical responses were normalized to the maximum force developed in response to 10^{-6} mol l^{-1} carbamylcholine.

approximately seven times as much as the response amplitude of normally pigmented sphincters (Fig. 5).

Discussion

Previous studies (Barr & Alpern, 1963; Kargacin & Detwiler, 1985) have provided considerable evidence supporting the hypothesis that the frog sphincter pupillae myocyte is triggered to contract in response to light by a release of calcium ions, from an intracellular store, caused by the absorption of light by rhodopsin resident in the plasma membrane. On the basis of the results reported here, it seems likely that the photomechanical response of the axolotl sphincter pupillae muscle is triggered in a similar way. This tentative conclusion is based on the similarities of the action spectra, kinetics and sensitivities to extracellular concentrations of potassium and calcium ions of the photomechanical responses of the frog and axolotl muscles.

This is not to say that the two are identical. In particular, the axolotl muscle is a little slower in both latent period (1·15 *versus* 0·77 s) and time to peak during a long flash of constant intensity (10 *versus* 6 s). Also, in the blue region of the spectrum, the action spectrum of frog muscle is closer to the absorption spectrum of rhodopsin than is the action spectrum of axolotl muscle.

Finally, from the results of the present study, it seems necessary to consider that the melanin in the smooth muscle cell organelles and elsewhere in the iris acts to shield the smooth muscle rhodopsin to a significant degree. This screening occurs

without a change in the kinetics of the light–contraction coupling sequence or a change in the action spectrum.

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