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Calcium Ion, a Putative Intracellular Messenger for Light-Adaptation in *Limulus* Ventral Photoreceptors*

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Abstract. Calcium ion fulfills several criteria for identifying an intracellular messenger for light-adaptation in *Limulus* photoreceptors. Direct injection of Ca^{++} mimicks two aspects of light-adaptation; sequestration of intracellular calcium tends to prevent light-adaptation; and light induces an increase in intracellular Ca^{++} as demonstrated by two independent techniques.

Key words: Calcium – *Limulus* – Photoreceptors – Adaptation.

Intracellular ionized calcium (Ca^{++}_{in}) has been proposed to be the natural intracellular messenger for light-adaptation in *Limulus* ventral photoreceptors (Lisman and Brown, 1971). Four lines of evidence support this hypothesis.

(1) Membrane currents evoked by light (light-induced currents) can be measured by a voltage-clamp technique. After bright illumination, the voltage-clamp currents induced by test flashes become attenuated and have shorter latency; that is, the sensitivity of the photoreceptor is reduced and the time-scale of the photoreceptor response is shortened. As the photoreceptor progressively dark adapts the sensitivity increases and the time-scale becomes longer. Direct iontophoretic injection of Ca^{++} into a voltage-clamped photoreceptor progressively both reduces the sensitivity and shortens the time-scale; after the injection, both the sensitivity and time-scale progressively recover. Therefore, direct injection of Ca^{++} mimicks these two aspects of light-adaptation (Brown and Lisman, 1975).

(2) The intracellular injection of the calcium-sequestering agent EGTA (ethyleneglycol *bis* [β aminoethyl ether] N,N,N′,N′ tetraacetic acid) tends to abolish several aspects of light-adaptation. First, the injection of EGTA tends to prevent reduction of sensitivity (measured by responses to test flashes). Second, the injection of EGTA affects the waveform of the light-induced current. The current induced by a prolonged stimulus normally falls from an initial transient peak to a plateau value

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maintained for the duration of the stimulus; that is, normally there is a delayed reduction of current/photon-flux during a prolonged stimulus. After injection of EGTA, the current induced by a prolonged stimulus tends to be square; the delayed reduction of current/photon-flux tends to be abolished. Third, intracellular EGTA changes the slope of the graph of light-induced current *versus* stimulus irradiance. Normally, an increment of irradiance superimposed on a low level of irradiance induces more photoreceptor current than does the same increment superimposed on a high level of irradiance. That is, the graph of light-induced current *versus* irradiance has higher slope with dimmer lights. The decrease of slope found with bright lights is another aspect of light-adaptation. After injection of EGTA, the slope of the graph of light-induced current *versus* irradiance tends not to decrease over a much wider range of irradiance. Thus, sequestering intracellular calcium tends to prevent light-adaptation (Lisman and Brown, 1975).

(3) Illumination of a photoreceptor causes an increase in Ca^{++}_{in}. This increase has been demonstrated directly by the stimulus-induced increase of luminescence of aequorin injected intracellularly (Brown and Blinks, 1974). The light-elicited increase of Ca^{++}_{in} reaches a maximum after the peak of the receptor response and falls to a lower level during prolonged, bright illumination.

(4) The transient increase of Ca^{++}_{in} elicited during a prolonged, bright stimulus can also be detected using the metallochromic indicator dye, arsenazo III (2, 7 *bis* [o-arsonophenyl] azo 1,8 dihydroxy, 3,6 naphthalene disulfonic acid). In a strongly light-adapted photoreceptor, Ca^{++}_{in} remains high for a long time. This has been shown by recording the absorption spectra of intracellular arsenazo III. The light-adapted minus dark-adapted difference spectrum of the intracellular dye has two maxima (at 610 nm and 660 nm) characteristic of dye-Ca binding. These double maxima are not seen with changes of either Mg^{++}_{in} or intracellular pH. By comparing difference spectra recorded from single cells with calibration curves for Ca-dye binding, Ca^{++}_{in} was found to exceed 10^{-4} M in strongly light-adapted cells (Brown et al., 1976).

Therefore, Ca^{++}_{in} satisfies several criteria for being the natural intracellular messenger for light-adaptation in *Limulus* ventral photoreceptors. Ca^{++} is normally present inside the photoreceptors; it normally increases after illumination; direct intracellular injection of Ca^{++} mimicks light-adaptation; and artificial sequestration of Ca^{++}_{in} tends to prevent light-adaptation. Nonetheless, at present there is neither sufficient quantitation of the above criteria nor sufficient evidence of biochemical machinery that normally sequesters or extrudes Ca^{++}_{in} to identify Ca^{++}_{in} as the intracellular messenger with certainty.

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