Letter to the Editor

Photoreceptor Sensitivity and Kinetics in Light Adaptation

S. Nikonov, T.D. Lamb, and E.N. Pugh, Jr.

From the Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

In the preceding letter (Pepperberg, 2001), Dr. David Pepperberg offers an alternative interpretation to that given in our recent paper (Nikonov et al., 2000) for the nature of the changes underlying light adaptation in rod photoreceptors. We do not agree with Dr. Pepperberg's interpretation, and we set out our reasons below. From the outset, we should make it clear that we and Dr. Pepperberg are measuring different phenomena: our analysis concentrated primarily on the rising phase of the response (both to dim and bright flashes), whereas Dr. Pepperberg's analysis concentrates on the recovery phase of the response to bright flashes. This difference has important consequences for the interpretation of mechanisms.

Dr. Pepperberg refers to the "relative gain" g of transduction, estimated from the time during which the photoreceptor remains in saturation after exposure to an intense flash of light. In our view, the parameter so derived conflates factors that apply separately to the rise of the response and to the recovery of the response. Thus, when Pepperberg's g is found to be altered, it is not clear whether the amplification of the activation steps in the cascade has changed, or whether the lifetime of one or more active intermediates has changed (or both). For this reason, we sought, in our experiments and analysis, to separate the two possibilities, by concentrating on the rising phase. Furthermore, we concentrated for the most part on small-signal responses, rather than on saturating responses, because of our view that the adaptational state of the cell is more likely to be seriously changed by saturating responses than by dim flashes.

Our experimental measurements showed that (when expressed in fractional terms) the early rising phase of the response is invariant under different conditions of light adaptation. Accordingly, our experiments showed that the "amplification constant", A, (Lamb and Pugh, 1992; Pugh and Lamb, 1993) of transduction is unaltered during light adaptation, and that therefore there is no detectable change in the efficacy of the activation steps of the cascade, at least at early times in the response.

However, as numerous studies have shown over the years, the flash sensitivity (measured at the peak of the response) declines during light adaptation, even after correction for "response compression." This reduction occurs because, although the fractional response initially rises along an invariant time course, it reaches peak earlier and, therefore, the peak is smaller. As Dr. Pepperberg points out, the reduction in fractional sensitivity measured this way amounted to a factor of $\sim$12.7 for the brightest background in our Fig. 5 A (Nikonov et al., 2000), whereas the shortening of the saturated duration of the bright flash response corresponded to a reduction in g of $\sim$5 under the same conditions.

In our view, the most likely explanation of the lowered fractional sensitivity, in conjunction with the unaltered initial rise, is the shortening of the lifetime(s) of one or more active intermediates in the transduction cascade. This idea was proposed by Torre et al. (1986), who stated that "the importance of this common rise is that…it indicates that the difference between the responses involve differences of lifetime in one or more reactions stages, rather than differences in the gain of coupling between stages. For example, the data are consistent with effects of Ca_i on the lifetime of photoexcited rhodopsin (Rh*)…". In Nikonov et al. (2000), we used an analysis (of the "step/flash" effect) related to Dr. Pepperberg's analysis, and we concluded that the lifetime of R* shortened by three- to fivefold over the range of backgrounds we investigated (Figs. 10 and 11).

But furthermore, we showed that in addition to any calcium-induced change in the lifetime of rhodopsin, another lifetime that must be taken into account is that of cGMP. Our analysis of the dim-flash response showed that a given decrease in R* lifetime does not automatically convert to an equally large decrease in flash sensitivity. This is because the effect of a decreased R* lifetime on the dim-flash response amplitude is manifest through the subsequent steps of the cascade, which include the synthesis and hydrolysis of cGMP; hence, the sensitivity is also reduced by the in-

Address correspondence to Edward N. Pugh, Jr., Department of Opthalmology/Kirby Center, University of Pennsylvania School of Medicine, 422 Curie Boulevard, Stellar-Chance Building 309B, Philadelphia, PA 19104-6069. Fax: (215) 573-7155; E-mail: pugh@mail.med.upenn.edu

creased steady PDE activity. The exact manner in which shortened lifetimes of R* and cGMP contribute to the decrease in flash sensitivity is quite complicated, however, and requires theoretical analysis of the sort we provided. For the model of the "standard rod" in our paper, a background producing $I = 1000$ photoisomerizations s^{-1} was calculated to suppress 61% of the dark circulating current, to reduce the R* lifetime 2.7-fold, and to decrease the cGMP lifetime 9.2-fold, causing an overall decline of fractional sensitivity amounting to 12.3-fold. For a background of $I = 3000$ photoisomerizations s^{-1}, the corresponding calculations give a 76% suppression of current, a 3.4-fold reduction in R* lifetime, a 21-fold reduction in cGMP lifetime, and a 30-fold decline in fractional sensitivity. Finally, our analysis suggests that for these two backgrounds the reduction in R* lifetime contributes about 1/4 of the overall decrease in fractional sensitivity, with the remaining 3/4 due to the reduced cGMP lifetime (Fig. 14 C, dot-dashed, and dashed traces).

We agree with Dr. Pepperberg that the fivefold reduction in g measured with bright flashes does not arise from a change in steady-state PDE activity induced by the background. In our view it most likely arises from a shortening of the effective lifetime of activated rhodopsin (R*), though simulations with the model rod suggest that other factors arising from the relatively strong stimulation and consequent substantial decrease in Ca^{2+}_i could come into play to affect the precise magnitude of the change in g.

Another point where we differ from Dr. Pepperberg regards his suggestion that light adaptation leads to a delayed change in the amplification of the activation stages of transduction. As far as we can see, there is no evidence for such a scheme nor is there any need to invoke one. Instead, it appears to us that the experimental results are explicable on the basis of unaltered amplification in conjunction with altered lifetimes of transduction intermediates.

The key points can be summarized as follows. During light adaptation, the photoreceptor is desensitized and its response is accelerated, but the early rising phase of the fractional response is invariant, indicating that (at least at early times) the efficacy of the activation steps of transduction is unaltered. Many of the observed effects on the dim-flash response are explicable in terms of the combination of a reduced lifetime of activated rhodopsin and a reduced lifetime of cGMP. The adaptation-dependent shift in the duration of the response to a saturating flash is a further interesting manifestation of light adaptation, but one that is more difficult to analyze because of the large changes in parameters (such as Ca^{2+} concentration) elicited by the stimulus itself, rather than by the adapting background. Although we do not yet have a quantitative description for the change in saturation time measured during light adaptation, we think that it can largely be accounted for in terms of a reduced lifetime of R*.

Finally, we wish to add that, in preparing this response, we detected a regrettable error in panel C of Fig. 6 of our paper: three of the points in C were incorrectly plotted directly from B without dividing by the values in A. This error has no direct bearing on the issues dealt with in Dr. Pepperberg's letter, or in our response. The corrected Fig. 6 is published as an erratum on p. 367.

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