of Ca^{2+} in mediating sensitivity changes that occur in rods during light and dark adaptation. We found that altering extracellular Ca^{2+} alters the amplitude of rod responses dramatically in the dark-adapted retina, but that the sensitivity of the rod (determined principally by σ, the position of the V–log I curve on the intensity axis) is virtually unchanged. As both amplitude and sensitivity vary substantially during light and dark adaptation, we concluded that changes in cytosol Ca^{2+} levels could not explain adaptation in photoreceptors. For example, we showed (Fig. 5 of ref. 2) that raising extracellular Ca^{2+} from 1.8 to 3.2 mM reduced V_{max} of the rod response by about 38%, but that in these conditions of high extracellular Ca^{2+} a significant shift in the position of the V–log I curve on the intensity axis could not be detected. There is, of course, some uncertainty in intracellular recording measurements, and therefore it is possible that within our error range (±0.2 log units) some small changes did occur. However, such changes are insignificant compared to the changes that occur when a rod is adapted by a light that reduces its maximum amplitude response by the same amount that 3.2 mM Ca^{2+} reduces V_{max}. For example, Fig. 6 of ref. 2 showed that an adapting light which reduced the rod response amplitude by 35% caused a shift of the V–log I curve along the intensity axis by at least 1.6 log units. In other words, in comparable conditions of V_{max} reduction, light causes a 65-fold change in rod sensitivity, but raised $[Ca^{2+}]_o$ changes sensitivity no more than 2–3 times. This was the main point on which our conclusion was based.

Flaming and Brown now report that if they raise or lower extracellular Ca^{2+} they can detect small changes in the position of the rod V–log I curve on the intensity axis and they suggest, therefore, that it is possible that alterations in cytosol Ca^{2+} levels can account for sensitivity changes in photoreceptors during light and dark adaptation. They report that raising extracellular Ca^{2+} from 1.8 to 6 mM causes the V–log I curve of the dark-adapted rod to shift by an average of 0.26 log units. In their Fig. 1 (ref. 1), they show that such an increase in $[Ca^{2+}]_o$ decreases a rod V_{max} by about 45%. If light is used to reduce V_{max} by this amount, however, the position of the V–log I curve alters not by 0.2–0.3 log units, but by about 2 log units. Thus, their data are in close agreement with our findings, and they support our conclusion that, "although cytosol Ca^{2+} concentrations are capable of regulating the membrane potential of the cell and its response amplitude, they affect only to a minor extent the sensitivity of the receptor cell in the dark-adapted or partially light-adapted state" (ref. 2).

Bastian and Fain[3] have recently carried out experiments similar to those of Flaming and Brown and ourselves. They found, in agreement with Flaming and Brown, that small shifts in the V–log I curve can be detected when extracellular Ca^{2+} is altered sufficiently, but their results also show clearly that the effects of increases in extracellular Ca^{2+} do not at all resemble the effects of background light on either rod sensitivity or response waveform. We may not have observed the small shifts of the V–log I curve in low and high Ca^{2+} because of the reasons outlined by Flaming and Brown. However, our failure to observe these changes in no way alters our conclusion that cytosol Ca^{2+} levels cannot be the major determinant of rod sensitivity during light and dark adaptation. Indeed, all three studies discussed here provide evidence in favour of this conclusion.

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FLAMING AND BROWN REPLY—in agreement with all other authors, Lipton *et al.*[1] found that increased extracellular Ca^{2+} markedly reduced V_{max}. Their new finding was that raised or lowered extracellular Ca^{2+} concentration exerted no detectable effect upon σ, the stimulus intensity eliciting a response of half the maximum amplitude, although σ was altered significantly by adaptation. They stated that "The experiments do indicate that in a partially light-adapted rod the membrane potential and cytosol Ca^{2+} levels do not regulate the major determinant of sensitivity (σ), ...". We recently[2] showed that when external Ca^{2+} was varied from 0.3 to 6.0 mM, σ shifted to higher light intensities by about 0.75 log units. Both increases and decreases of Ca^{2+} concentration from the control level of 1.8 mM elicited consistent σ shifts that were detected readily. Our conclusions were: "Thus, our results indicate that alteration of $[Ca^{2+}]_o$ can control the value of σ in the V–log I curve of toad rods. These results are consistent with a role for $[Ca^{2+}]_i$ in the control of sensitivity in vertebrate photoreceptors. Hence, changes of $[Ca^{2+}]_i$ may partially control the changes of sensitivity that occur during light and dark adaptation". Our conclusion that $[Ca^{2+}]_o$ can exert a controlling effect on σ differs from the conclusion of Lipton *et al.*[1] in an important respect, and we note that they now accept the validity of our findings on this point.

One remaining question concerns the degree to which $[Ca^{2+}]_i$ can control the value of σ. Taken at face value, our results suggest that $[Ca^{2+}]_i$ could only control a small portion of the adaptive σ shifts. However, we demonstrated that our Ca^{2+}-induced σ shifts depended on several improvements in experimental conditions, and one critical factor was a more normal perfusate containing 15 mM of bicarbonate, which was used for buffering[2] instead of HEPES[1]. It thus seems that the induction of σ shifts by altering $[Ca^{2+}]_o$ is more sensitive to subtle chemical factors in the perfusate than are the effects of $[Ca^{2+}]_o$ on V_{max}. This suggests that it is especially important to compare σ shifts induced by adaptation, and by alteration of $[Ca^{2+}]_o$, in physiologically normal experimental conditions. Although we have made some improvements in that direction, we are not convinced of having attained the requisite success, which may be very difficult with an isolated and perfused preparation. Also, even in a completely normal preparation, an elevated $[Ca^{2+}]_o$ may not adequately mimic the effects of increased $[Ca^{2+}]_i$. For reasons such as these, we deliberately drew no conclusion concerning the degree to which $[Ca^{2+}]_i$ may normally control adaptive σ shifts. Although Lipton *et al.*[1] failed to detect any of the controlling effect of $[Ca^{2+}]_o$ on the value of σ, their letter indicates great confidence concerning the quantitative control of σ by $[Ca^{2+}]_i$. As premature conclusions based on inadequate evidence can be 'misleading', it seems advisable to await more definitive evidence on this issue.

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