Metabolic constraints on the recovery of sensitivity after visual pigment bleaching in retinal rods

The shut-off of active intermediates in the phototransduction cascade and the reconstitution of the visual pigment play key roles in the recovery of sensitivity after the exposure to bright light in both rod and cone photoreceptors. Physiological evidence from bleached salamander rods suggests this recovery of sensitivity occurs faster at the outer segment base compared with the tip. Microfluorometric measurements of similarly bleached salamander rods demonstrate that the reduction of all-trans retinal to all-trans retinol also occurs more rapidly at the outer segment base than at the tip. The experiments reported here were designed to test the hypothesis that these two phenomena are linked, e.g., that slowed recovery of sensitivity at the tip of outer segments is rate limited by the reduction of all-trans retinal and results from a shortage of cytosolic nicotinamide adenine dinucleotide phosphate (NADPH), the reducing agent for all-trans retinal reduction. Extracellular measurements of membrane current and sensitivity were made from isolated salamander rods under dark-adapted and bleached conditions while intracellular NADPH concentration was varied by dialysis from a micropipette attached to the inner segment. Sensitivity at the base and tip of the outer segment was assessed before and after bleaching. After exposure to a light that photoactivates 50% of the visual pigment, rods were completely insensitive for nearly 10 minutes, after which the base recovered sensitivity and responsiveness with a time constant of approximately 200 seconds, but tip sensitivity recovered more slowly with a time constant of approximately 680 seconds. Dialysis of 5 mM NADPH into the rod promoted an earlier recovery and eliminated the previously observed tip/base difference. Dialysis of 1.66 mM NADPH failed to eliminate the tip/base recovery difference, suggesting the steady-state NADPH concentration in rods is approximately 1 mM. These results indicate the inner segment is the primary source of reducing equivalents after pigment bleaching, with the reduction of all-trans retinal to all-trans retinol playing a key step in the recovery of sensitivity.