Retinitis pigmentosa: rod photoreceptor rescue by a calcium-channel blocker in the *rd* mouse

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Retinitis pigmentosa is an inherited degenerative disease of photoreceptors leading to blindness. A well-characterized model for this disease is provided by the retinal degeneration mouse, in which the gene for the rod cGMP phosphodiesterase is mutated, as in some affected human families. We report that D-cis-diltiazem, a calcium-channel blocker that also acts at light-sensitive cGMP-gated channels, rescued photoreceptors and preserved visual function in the retinal degeneration mouse. The long record of diltiazem prescription in cardiology should facilitate the design of clinical trials for some forms of retinitis pigmentosa.

Mutations in several genes expressed in rod photoreceptors trigger the inherited degenerative diseases of photoreceptors called retinitis pigmentosa, which ultimately lead to blindness[1]. Available treatments are of limited benefit in retarding disease progression. In the last decade, several therapeutic approaches have been proposed to treat retinitis pigmentosa, including vitamin supplementation[2], gene therapy[3], growth factor application[4] and retinal cell transplantation[5,6]. The retinal degeneration (*rd*) mouse provides an adequate model[7] to evaluate these approaches, because the pattern of photoreceptor loss is similar to that in affected humans: rod cell death is followed by cone cell degeneration. Furthermore, the mutation is in the gene encoding for the β-subunit of rod cGMP phosphodiesterase[8], as in some affected human families[9]. In this model, rod cell death is correlated to an abnormal increase in cGMPconcentration[10] to a level that can become toxic to normal photoreceptors[11,12]. As cGMP gates cationic channels that are normally responsible for the light-sensitive current in photoreceptors[13,14], we sought to determine whether D-cis-diltiazem, a known calcium-channel blocker[15] that also acts at cGMP-gated channels[16], could rescue photoreceptors in the *rd* mouse.

Rod Rescue

We injected diltiazem intraperitoneally in *rd* mice beginning on postnatal day 9 at the onset of rod cell degeneration, with gradually increasing doses. We then immunolabeled rod cells in treated and untreated retinae on postnatal days 25 and 36 (Fig. 1). We quantified rod cell rescue in treated *rd* mice by counting immunopositive cells using a randomized procedure, only assessing right retinae in all our measurements to obtain independent data (Fig. 2). Surviving rod cells were 186% more numerous on day 25 in treated mice (38,613 ± 1,750; $n = 7$) than in control untreated *rd* mice (20,718 ± 594; $n = 7$). Although rods continued to decrease in both treated and control mice, the diltiazem-induced photoreceptor rescue was more prominent at 36 days (Fig. 2*b*). Rod numbers were

248% higher in treated mice (18,656 ± 1,160; $n = 10$) than in control *rd* mice (7,519 ± 751; $n = 9$). At both ages, the rescue was statistically significant ($P < 0.01$ at 25 days; $P < 0.001$ at 36 days; Student's *t*-test). Rod cell numbers in mice treated for 36 days were similar to those of control *rd* mice on day 25. Repeated injections of saline solution alone did not substantially affect rod cell survival at 36 days (7,416 ± 1,291; $n = 5$) compared with that of untreated mice (7,648 ± 774; $n = 4$). These results indicate that daily diltiazem treatment partially protected rod photoreceptors from degenerating.

Cone rescue

This diltiazem neuroprotection of photoreceptor was confirmed by examination of retinal sections (Fig. 3). The outer nuclear layer consisted of one to two cell layers at 25 days and was restricted to a single layer by postnatal day 36. Only very few pycnotic nuclei were found on each section. On such sections, immunolabeled rods were 165% and 278% more numerous on days 25 and 36, respectively, in treated mice (day 25, 49.6 ± 2.3, $n = 5$; day 36, 25 ± 1.6, $n = 5$) than in control *rd* mice (day 25, 30 ± 2, $n = 5$; day 36, 9 ± 0.7, $n = 5$) (Fig. 4*a* and *b*). At both ages, the rescue was statistically significant ($P < 0.001$; Student's *t*-test) and consistent with the results obtained in flat-mounted retinae. We estimated cone cell numbers indirectly by staining nuclei with the dye DAPI (4'-6-diaminodino-2-phenylindole) and subtracting immunolabeled rod cells from DAPI-stained photoreceptor nuclei in the outer nuclear layer. Cone cells were also 109% and 144% more numerous on days 25 and 36, respectively, in treated mice (day 25, 215.2 ± 1.5, $n = 5$; day 36, 160.2 ± 8.4, $n = 5$) than in control *rd* mice (day 25, 196.6 ± 4, $n = 5$; day 36, 111.2 ± 6, $n = 5$) (Fig. 4*a* and *b*). This cone rescue was also statistically significant ($P < 0.01$; student's *t*-test). By postnatal day 25, photoreceptors showed distinct inner segments sometimes connected to outer segment-like structures (Fig. 4*c* and *d*). These results indicate that diltiazem treatment prevented rods and cones from degenerating.
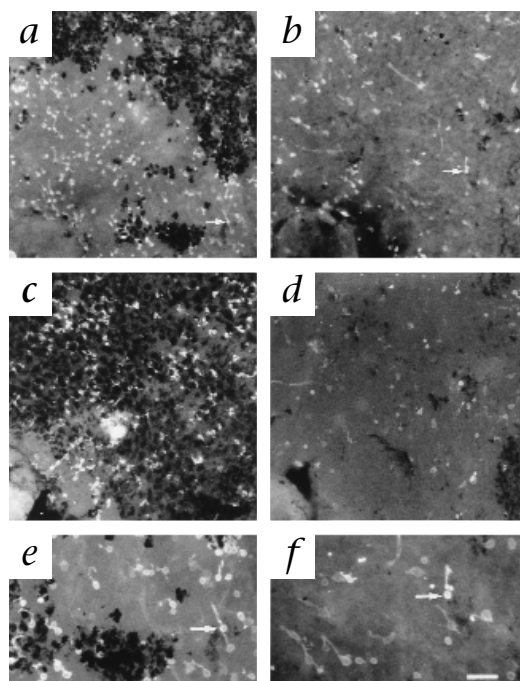
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Fig. 1 Rod cell rescue in diltiazem-treated *rd* mice. Flat-mounted retinae were immunolabeled with antibody against rhodopsin in diltiazem-treated (*a*,*c* and *e*) and untreated (*b*,*d* and *f*) *rd* mice on postnatal days 25 (*a*,*b*,*e*,*f*) and 36 (*c*,*d*). Immunopositive cells (arrows) from *a* and *b* are enlarged in *e* and *f* to demonstrate the morphology of surviving rods. Dark spots represent pigment epithelium cells that remained attached during retinal isolation. The scale bars represents 60 μm (*a*,*b*,*c*,*d*) and 30 μm (*e*,*f*).

Improved visual function

To determine whether this photoreceptor rescue could be correlated with improved physiological features, we recorded electroretinograms (ERGs) in treated and control *rd* mice (Fig. 5*a*). In *rd* mice, the amplitudes of ERG a- and b-waves decreased regularly from postnatal day 12 to extinction by day 24 (Fig. 5*b*), as reported[17]. These ERG signals seemed to be generated by cones, because they were not suppressed by an adapting background light but were simply reduced by 54% (*n* = 4), and they became measurable with the use of light stimuli of an intensity greater than 0.3 log cd/m^2 (*n* = 3). By postnatal day 25, all diltiazem-treated mice (*n* = 7) had measurable ERG signals (Fig. 5*a* and *b*), in contrast to control *rd* mice (*n* = 7). The amplitudes of these ERG responses (a-wave, 7.3 ± 1.3 μV; b-wave, 17.3 ± 1.9 μV; *n* = 7) were slightly larger than those of 19-day-old control *rd* mice (a-wave, 5.9 ± 0.7 μV; b-wave, 10.9 ± 2.0 μV; *n* = 4). At 36 days, despite having fewer rod cells than in 25-day-old control *rd* mice, 4 of 10 treated mice had ERG signals that could be measured in both eyes (Fig. 5*a* and *b*). These ERG measurements were slightly smaller (a-wave, 5.3 ± 1.6 μV; b-wave, 13.8 ± 1.7 μV; *n* = 4) than those of 25-day-old treated mice, but were still larger than those of 19-day-old control *rd* mice. These observations indicate that diltiazem treatment not only partially rescued photoreceptors but also protected visual function in *rd* mice.

Diltiazem mechanism of action

To investigate the origin of this diltiazem neuroprotection, we measured ERG signals in wild-type mice after intraperitoneal injection of diltiazem (200 μl; 5 mM). The amplitude of the b-wave ERG decreased 48 ± 9% in these mice (*n* = 9)(Fig. 5*c* and *d*). The a-

wave was inconsistently decreased (85 ± 10%; *n* = 9). The ERG returned to its normal size after 4 days of diltiazem clearance after a single injection or 5 consecutive days of injection. No toxic effects were detected by the histology of retinal sections from these mice, stained with toluidine blue (data not shown). The considerable decrease of the ERG b-wave indicates that diltiazem can suppress calcium-dependent transmission between photoreceptors and secondary neurons.

Discussion

Our study indicates that diltiazem treatment rescues functional photoreceptors in the *rd* mouse. This diltiazem-induced photoreceptor rescue is reminiscent of that in rdgB *Drosophila* mutants[18], although the phototransduction cascades, visual information processing and the mutations are different in these two species. In the *rd* mouse, the occurrence of degenerative features has been correlated to an increase in cGMP concentration[10] that was found to be toxic by itself to normal photoreceptors[11,12]. Although this cGMP toxicity was described 20 years ago, its molecular mechanism is still unresolved. In physiological conditions, the nucleotide activates a channel permeable to cations (Na$^+$, Ca^{2+}) that mediates the depolarizing dark current sensitive to light in photoreceptors[13,14]. Diltiazem blocks cGMP-gated channels[16] and L-type calcium channels[15]. D-cis-diltiazem was used here; in contrast to L-cis-diltiazem, it has only a moderate activity on cGMP-gated channels[16,19], which is consistent with its lack of effect on the ERG a-wave in wild-type mice. The considerable diltiazem-induced decrease in the ERG b-wave is in agreement with its efficient block of L-type channels that mediate synaptic transmission between photoreceptors and secondary neurons[20]. The effect on the b-wave ERG also indicates that D-cis-diltiazem can enter the retina and permeate the hematopoietic barrier. Given its pharmacological properties, D-cis-diltiazem may therefore rescue photoreceptors by blocking L-type calcium channels rather than by suppressing cGMP-gated currents or by increasing blood flow[21], an alteration that could also interfere with photoreceptor degeneration. It is unclear why blocking L-type calcium channels could rescue photoreceptors, but it is possible that the constitutive activation of cGMP-gated channels leads to photoreceptor depolarization and a consecutive lethal calcium influx through L-type calcium channels. Evaluating the effect of specific blockers for either cGMP-gated or calcium channels should provide further insight about the molecular mechanisms responsible for cGMP toxicity
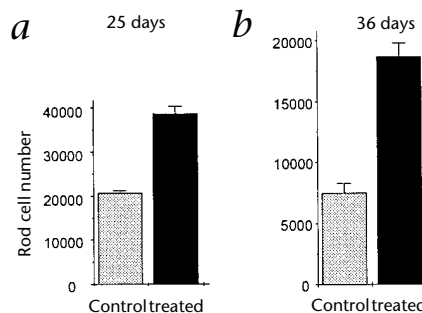

Fig. 2 Quantification of diltiazem-induced rod cell rescue in flat-mounted retinae of *rd* mice. Rod cell numbers were estimated on postnatal days 25 (*a*) and 36 (*b*) on flat-mounted retinae stained with an antibody against rhodopsin in treated and untreated (control) *rd* mice after daily diltiazem injections.

Fig. 3 Diltiazem-induced photoreceptor rescue in retinal sections of *rd* mice. Retinal sections of diltiazem-treated (***a***) and untreated (***b***) *rd* mice on postnatal day 36 were stained with toluidine blue. Photoreceptors with outer segment-like structures are still detectable in the retina of the treated mouse (arrow). Scale bar (*b*) represents 10 μm.
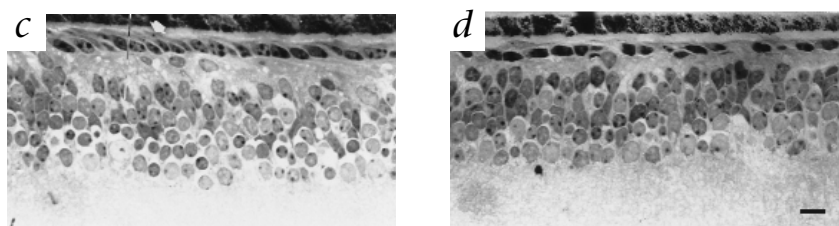
and photoreceptor degeneration.

Both rods and cones were found to be more numerous in diltiazem-treated mice than in control *rd* mice. Although some rods were rescued by the diltiazem treatment, their numbers were still considerably fewer than those of wild-type mice, in which rod numbers were estimated to 4.13×10^6 per retina (data not shown). As a consequence, the rod:photoreceptor ratio decreased from 97% in wild-type mice to 13.5% in treated and 7.5% in untreated *rd* mice at 36 days. However, despite its small amplitude, the rescue seemed to have important beneficial effects as demonstrated by ERG recordings in treated mice at 25 and 36 days. Given the considerable rod cell loss and the alteration of the phototransduction cascade by the phosphodiesterase mutation, these ERG signals measured in *rd* mice may emanate from cones. This was confirmed by the light intensity threshold for *rd* mouse ERGs and the persistence of ERGs in the presence of an adapting background light. Although these cone-mediated ERGs were not as large as those reported in wild-type mice (b-wave amplitude, about 250 μV; ref. 22), the diltiazem-induced preservation of an ERG signal is therefore consistent with cone rescue, which was confirmed by cell counting. This cone rescue could result from a paracrine stimulation of cones by rods, as described *in vivo*[23] and *in vitro*[24]. However, this paracrine effect seems insufficient to explain the measurable ERGs in treated mice on day 36, because cones were less numerous at this time than in control *rd* mice on day 25, for which ERGs were not measurable. Diltiazem may also affect calcium influx into cones although it is not yet clear whether it affects their cGMP-gated channels[25]. Investigating this diltiazem protection further should therefore provide new hypotheses on the mechanism of cone degeneration in the *rd* mouse.

Therapeutic approaches now being investigated for retinitis pigmentosa include vitamin supplementation[2], gene therapy[3], trophic factor application[4] and photoreceptor transplantation[5,6]. Administration of channel blockers therefore defines a new line of potential treatment for this disease. For diltiazem, its long

record in human therapy[21] would facilitate its approval for clinical trials in some identified forms of the disease. Given the genetic heterogeneity of the disease, initial clinical trials should be limited to patients with mutations in genes of the phosphodiesterase or possibly other proteins of the phototransduction cascade. However, care should be taken, as the daily dose used here (20–60 mg/kg) is greater than the usual human dose (5.14 mg/kg; ref. 26); nonetheless, it is less than the lethal dose in mice (260 mg/kg subcutaneously; ref. 27). We did not detect evidence of diltiazem toxicity on inner retinal cells at these doses; however, further studies should verify that a long-term treatment would not produce damage to these cells while rescuing photoreceptors. Although the rescue effect was seen for only a few weeks, which may seem limited compared with the human life span, this is very important given the time scale of photoreceptor degeneration in the *rd* mouse, in which this process is almost complete in a 2-week period. As the time scale of photoreceptor degeneration can extend over years and even decades in humans, this pharmacological approach might offer the possibility to postpone considerably the occurrence of blindness for people affected by retinitis pigmentosa.

Methods

Mice and treatments. The *rd* mice (Janvier, Le Genest Saint Isle, France) were maintained on a 12-hour light–dark cycle. Intraperitoneal injections of diltiazem (5 mM; 2.255 mg/ml in 0.9% NaCl) were started on postnatal day 9, and the doses were gradually increased from 50 μl (days 9–12) to 60 μl twice a day (days 13–16) and finally to 100 μl twice a day up to the end of the experiment. Mice weighed 5.33 ± 0.17 g ($n = 8$) on postnatal day 9 and 8.37 ± 0.16 ($n = 8$) on postnatal day 17; the treatment represents daily diltiazem doses of 21 mg/kg and 54 mg/kg, respectively, at these ages. It did not induce death, which is consistent with the higher lethal dose reported for mice (LD_{50} in mg/kg, subcutaneously: male, 260; female, 280; ref. 27). To allow clearance of diltiazem and prevent interactions with ERG measurements (Fig. 5*c* and *d*), the final injection was given 48 h before recordings were made. Each *rd* mouse litter was divided into two groups: treated and control mice. As repeated injections of saline solution alone did not substantially affect rod cell survival (rod cells per retina) by postnatal
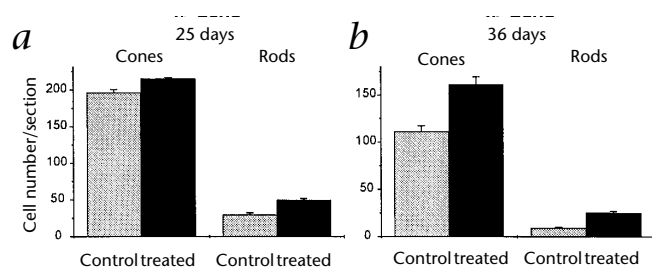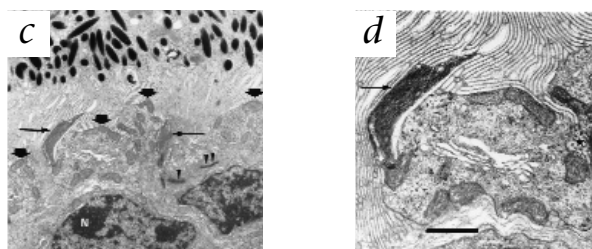
Fig. 4 Photoreceptor numbers and histology of retinal sections from diltiazem-treated *rd* mice. ***a*** and ***b***, Quantification of diltiazem-induced rod and cone rescue in retinal sections from *rd* mice. Immunolabeled rods were counted on postnatal days 25 and 36 in stained retinal sections from treated and untreated *rd* mice after daily diltiazem injections. Cone cell numbers were estimated by subtracting rod cell numbers from the number of DAPI-stained nuclei in the outer nuclear layer of the same sections. ***c*** and ***d***, Histology of diltiazem-treated photoreceptors in *rd* mice on postnatal day 25. *b*, In the outer retina, outer segment-like structures (long thin arrows) and inner segments (short thick arrows) are present between pigment epithelium cells and normal photoreceptor nuclei (N). Arrowheads, outer limiting membrane. *c*, Enlarged section of (*b*) showing a membrane stack resembling an outer segment (arrow), a connecting cilium (star) and an inner segment that contains a Golgi zone and many peripheral mitochondria. Scale bars represent 1 μm.
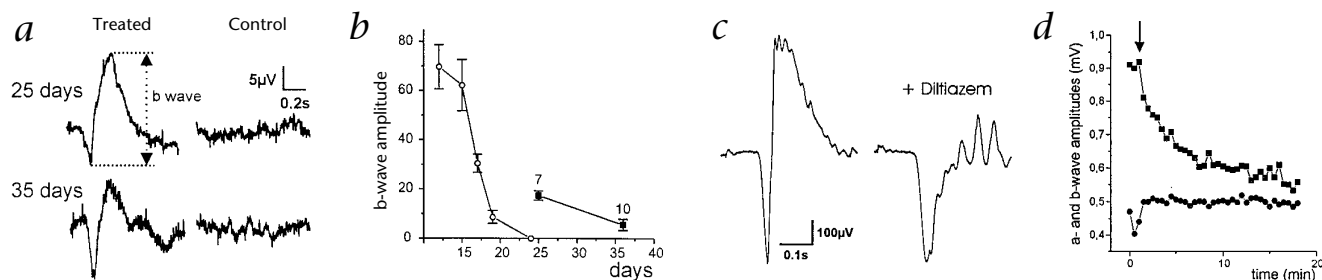
Fig. 5 ERGs after diltiazem treatment in *rd* mice and diltiazem injection in wild-type C57 mice. ***a***, ERG recordings of diltiazem-treated and control *rd* mice on postnatal days 25 and 36. ERG signals could be measured in 25- and 36-day-old treated mice (left), in contrast to control mice of the same age (right). In these experiments, the final injection was given 48 hours before recording to allow clearance of diltiazem and prevent its alteration of the ERG. ***b***, Diltiazem effects on the b-wave of the ERG. In all control untreated *rd* mice (open circles), b-waves disappeared by postnatal day 24, whereas an ERG signal could still be measured in all diltiazem-treated *rd* mice (filled squares) on postnatal day 25 (*n* = 7) and in some on postnatal day 36. ***c***, ERG recordings in wild-type C57 mouse before (left) and after (right) intraperitoneal injection of diltiazem. ***d***, Evolution of the a-wave (circles) and b-wave (squares) amplitudes after diltiazem injection.→

day 36 (7,416 ± 1,291; *n* = 5) compared with that in untreated mice (7,648 ± 774; *n* = 4) these mice were grouped together in the control group.

Electroretinogram recordings. Dark-adapted mice were anesthetized with an intraperitoneal injection (23 μl/g) of etomidate (0.2 mg/ml) and midazolan (1.25 mg/ml). Because this combination of anesthesia was incompatible with diltiazem, C57 wild-type mice were anesthetized using cotton soaked with forene (Abbott) when the diltiazem-induced effect on the electroretinogram (ERG) recording was evaluated.

Pupils were dilated with 0.5% tropicamide application, and the cornea was locally anesthetized with 0.5% topical proparacaine application. Upper and lower lids were retracted to keep the eye open and proptosed. A stainless steel reference electrode was inserted subcutaneously on the head of the mouse. ERGs were then measured with a saline-soaked cotton-wick placed at the apex of the cornea and connected to an Ag:AgCl electrode. Responses were amplified and filtered (low 0.1-Hz and high 1,000-Hz cut-off filters) with a Universal amplifier (Gould, Newburyport, Massachusetts). They were digitized using a data acquisition labmaster board (Scientific Solutions, Solon, Ohio) mounted on an IBM-compatible personal computer. A 150-watt xenon lamp bulb (Müller Instruments, Moosinning, Germany) provided the light stimulus of 2.9 log cd/m² as measured with a luxmeter at eye level. For measuring the threshold light intensity triggering a signal, the intensity of the light beam was reduced with neutral filters of various optical densities. The duration of the light stimulus (300 ms) was defined by a computer-controlled shutter. The cone ERG was measured in the presence of background lighting adjusted to 18 cd/m².

Retinal immunostaining. For retinal immunostaining, mice were killed and their eyes were removed; retinae were then isolated and fixed in a paraformaldehyde solution (4% in phosphate-buffered saline, pH 7.4) overnight. After three washes in phosphate-buffered saline, rod cells were selectively labeled with an antibody against rhodopsin (rho-4D2; provided by D. Hicks; ref. 28). After the first incubation with rho-4D2, retinae were incubated in goat immunoglobulin G coupled to Texas Red and directed against mouse antibody (Jackson Laboratories, West Grove, Pennsylvania).

Fluorescent immunolabeled cells were viewed with an Nikon 2 Optiphot microscope under epifluorescence illumination with a plan 40/0.70 DIC (160/0.17) objective. They were estimated by unbiased stereology on flat-mounted retinae using a systematic random-sampling procedure that has been described[24]. For each retina, 180 images were randomly selected, digitized and analyzed with the BIOCOM software package (Biocom, Lyon, France). Labeled cells included in two square areas (900 μm²) defined by the program were counted in each image. Rod cells in the entire retina were then estimated by averaging and integrating these numbers to the retinal surface (13 mm²). The retinal surface, which was not affected by the treatment, was measured using the optiscan software and a camera coupled to a Macintosh computer.

Retinal sections. Immunolabeled retinae were embedded in cryomatrix (Shandon, UK), frozen and sectioned transversely on a cryostat (Leica, Nussloch, Germany). Retinal sections were stained with the nuclear dye DAPI (10 μg/ml 4'-6-diaminodino-2-phenylindole in PBS; Sigma) to count both immunopositive rods and DAPI-stained rod and cone photoreceptor nuclei in the outer nuclear layer.

Electron light and microscopy. For light microscopy of sections stained with toluidine blue and electron microscopy, eye cups were fixed in 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 mM cacodylate-buffered solution, pH 7.4, postfixed in 1% phosphate-buffered osmium tetroxide, dehydrated through alcohol and propylene oxide and finally embedded in Epon. 'Semi-thin' sections (0.5–1 μm) were stained with a toluidin blue solution (1% in distilled water) and ultrathin sections (0.05–0.1μm) with uranyl acetate and lead citrate.

1. Berson, E.L. Retinitis pigmentosa: Unfolding its mystery. *Proc. Natl Acad. Sci. USA* **93**, 4526–4528 (1996).
2. Berson, E.L. *et al.* A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch. Ophthalmol.* **111**, 761–772 (1993).
3. Bennett J. *et al.* Photoreceptor cell rescue in retinal degeneration (*rd*) mice by *in vitro* gene therapy. *Nature Med.* **2**, 649–654 (1996).
4. LaVail, M.M. *et al.* Protection of mouse photoreceptors by survival factors in retinal degenerations. *Invest. Ophthal. Vis. Sci.* **39**, 592–602 (1998).
5. Silverman, M.S. & Hughes, S.E. Tranplantation of photoreceptors to light-damaged retinas. *Invest. Ophththalmol. Vis. Sci.* **30**, 1684–1690 (1989).
6. Gouras, P., Du, J., Kjeldbye, H., Yamamoto, S. & Zack, D.J. Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest. Ophthalmol. Vis. Sci.* **35**, 3145–3153 (1994).
7. Farber, D.B., Flannery, J.G. & Bowes-Rickman, C. in *Progress in Retinal and Eye Research* vol. 13 (eds. Osborne, N.N. & Chader, G.J.) 31–62 (Pergamon, Oxford, 1994).
8. Bowes, C., Li, T., Danciger, M., Baxter, L.C., Applebury, M.L. & Farber, D. Retinal degeneration in the *rd* mouse is caused by a defect in the β-subunit of rod cGMP-phosphodiesterase. *Nature* **347**, 677–680 (1990).
9. McLaughlin, M.E., Sandberg, M.A., Berson, E.L. & Drya, T.P. Recessive mutations in the gene encoding the β-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. *Nature Genet.* **4**, 130–133 (1993).
10. Farber, D.B. & Lolley, R. N. Cyclic guanosine monophosphate : Elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science* **186**, 449–451 (1974).
11. Lolley, R.N., Farber, D.B., Rayborn, M.E. & Hollyfield, J.G. Cyclic GMP accumulation causes degeneration of photoreceptor cells: simulation of an inherited disease. *Science* **196**, 664–666 (1977).

12. Ulshafer, R.J., Garcia, C.A. & Hollyfield, J.G. Sensitivity of photoreceptors to elevated levels of cGMP in the human retina. *Invest. Ophthalmol. Vis. Sci.* **19**, 1326–1241 (1980).
13. Cobbs, W.H. & Pugh, E.N.Jr. Cyclic GMP can increase rod outer-segment light sensitive current 10-fold without delay of excitation. *Nature* **313**, 585–587 (1985).
14. Fesenko, E.E., Kolesnikov, S.S. & Lyubarsky, A.L. Induction by cyclic GMP of a cationic conductance in plasma membrane of retinal rod outer segment. *Nature* **313**, 310–313 (1985).
15. Lee, K.S. & Tsien, R.W. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature* **302**, 790–794 (1983).
16. Koch, K.W. & Kaupp, U.B. Cyclic GMP directly regulates a cationic conductance in membranes of bovine rods by a cooperative mechanism. *J. Biol. Chem.* **260**, 6788–6800 (1985).
17. Noell, W.K. Differentiation, metabolic organization, and viability of the visual cells. *Arch. Ophthal.* **60**, 702–731 (1958).
18. Sahly, I. *et al*. Calcium channel blockers inhibit retinal degeneration in the retinal-degeneration-B mutant of *Drosophila. Proc. Natl Acad. Sci. USA* **89**, 435–439 (1992).
19. Stern, J.H., Kaupp, U.B. & MacLeish, P.R. Control of the light regulated current in rod photoreceptors by cyclic GMP, calcium, and l-cis-diltiazem. *Proc. Natl Acad. Sci. USA* **83**, 1163–1167 (1986).
20. Schmitz, Y. & Witkovsky, P. Dependence of photoreceptor glutamate release on a dihydropyridine-sensitive calcium channel. *Neuroscience* **78**, 1209–1216 (1997).
21. Buckley, M.M.T., Grant, S.M., Goa, K.L., McTavish, D. & Sorkin, E.M. Diltiazem A reappraisal of its pharmacological properties and therapeutic use. *Drugs* **39**, 757–806 (1990).
22. Peachley, N.S., Goto, Y., Al-Ubaidi, M.R. & Naash, M.I. Properties of the mouse cone-mediated electroretinogram during light adaptation. *Neurosci. Lett.* **162**, 9–11 (1993).
23. Mohand-Said, S. *et al*. Photoreceptor transplants increase host cone survival in the retinal degeneration (rd) mouse. *Ophthalmic Res.* **29**, 290–297 (1997).
24. Mohand-Said, S. *et al*. Normal retina releases a diffusible factor stimulating cone survival in the retinal degeneration mouse. *Proc. Natl. Acad. Sci. USA* **95**, 8357–8362 (1998).
25. Yu, W. P., Grunwald, M. & Yau, K. W. Molecular cloning, functional expression and chromosomal localization of a human homolog of the cyclic nucleotide-gated ion channel of retinal cone photoreceptors. *FEBS Lett.* **393**, 211–215 (1996).
26. Harman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. & Gilman, A.G. in *Goodman & Gilman's Pharmacological Basis of Therapeutics* 9th edn. (MacGraw–Hill, New York, 1996).
27. Budavari, S., O'Neil M.J., Smith A., Heckelman, P.E. & Kinneary, J.F. in *The Merck Index* 12th edn. 451(Merck, Whitehouse Station, New Jersey, 1996).
28. Hicks, D. & Molday, R.S. Differential immunogold-dextran labeling of bovine and frog rod and cone cells using monoclonal antibodies against bovine rhodopsin. *Exp. Eye Res.* **42**, 55–71 (1986).