Role of PKA on photoreceptor degeneration in the rd1 mouse model

Stefan Kustermann[1], Theo van Veen[1,2], Wolfgang Raffelsberger[3], Per Ekström[2], Francois Paquet-Durand[1]
1Division of Experimental Ophthalmology, Centre for Ophthalmology, Tübingen, GERMANY.
2 Dept. Ophthalmology, Clinical Sciences, Lund, University of Lund, BMC-B13, SE-22184 Lund/SWEDEN
3 IGBMC, Strasbourg, FRANCE

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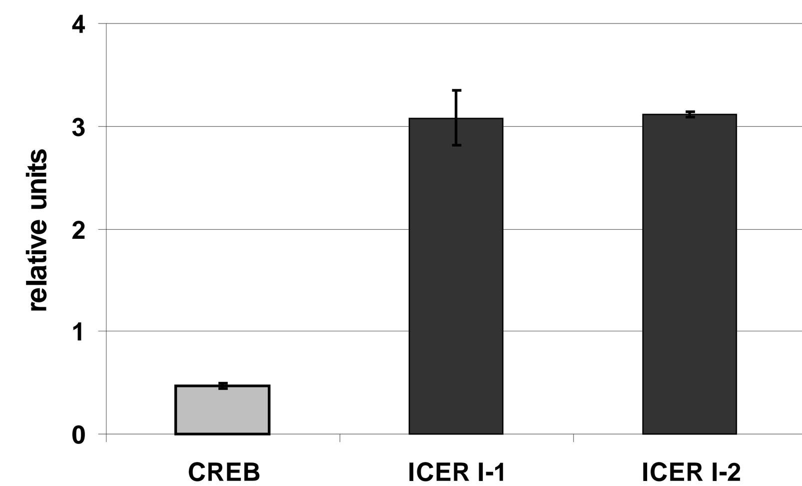
Purpose

PKA, a cAMP dependent protein kinase, is important in activating the transcription factor CREB (cAMP response element-binding protein) which is crucially involved in neuronal survival. Previously it has been demonstrated that in the rd1 mouse, a model for retinal degeneration, retinal CREB mRNA is significantly downregulated (Azadi et al., 2006). Furthermore, PKA bears the potential to stabilize ICER (inducible cAMP early repressor) which is the endogenous inhibitor of CREB. To investigate this bivalent role of PKA and the impact of PKA on photoreceptor cell survival we specifically activated or inhibited PKA activity in retinal explants of the rd1 mouse.



Gene transcription ratios (rd1/wt) for CREB and ICER. Values represent mean +/- SD (Azadi et al 2006; Paquet-Durand, 2006).

Methods

Organotypic retina culture:

rd1 mice were killed by decapitation at postnatal day 5 (PN 5) and retinal explants were generated with retinal pigment epithelium attached. Explants were maintained under serum-free conditions in R16 culture medium (Invitrogen, Paisley, Scotland) which was changed every two days. Explants were allowed to adjust to culture conditions for 2 days in vitro before being treated with PKA inhibitor (RP-8-CPT-cAMPS) or activator (SP-8-CPT-cAMPS) for another 4 days.

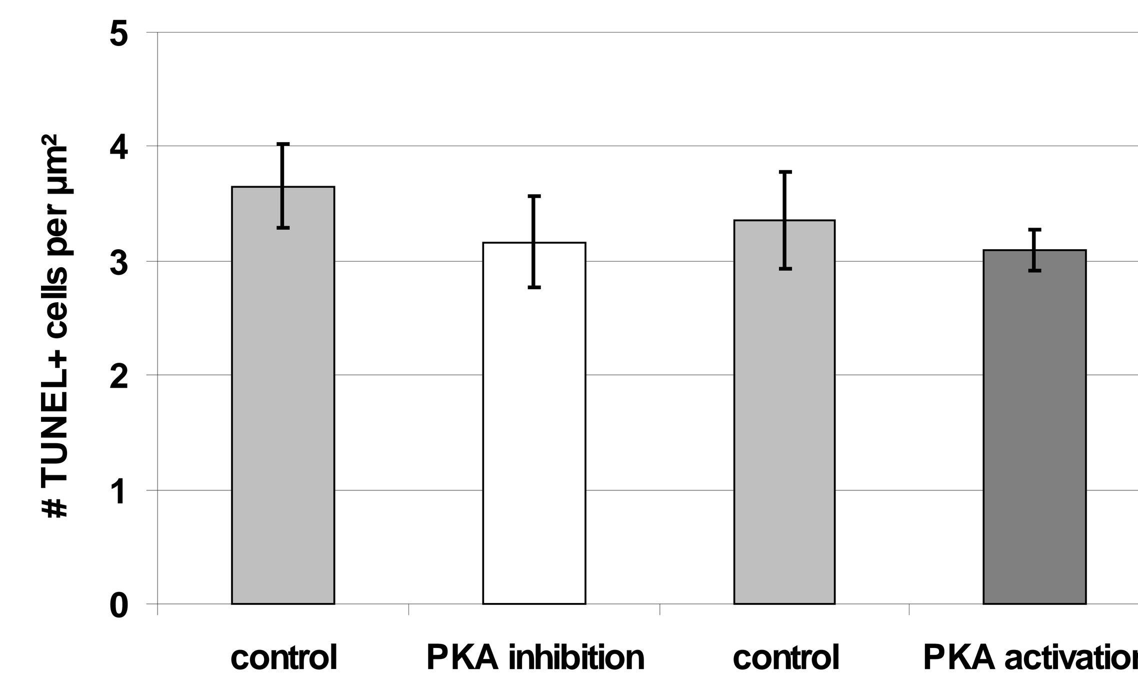
Cell death measurments:

Cell death rate was measured by perfoming TUNEL staining on retinal cryo-sections of organotypic retinal cultures which have been treated before with PKA activator or inhibitor for 4 days in vitro and their corresponding controls. Retinal cryosections adjacent to the optic nerve outlet were used for TUNEL staining by using in-situ cell death kit and the protocol of the manufacturer (Roche, Switzerland). For the calculation of the number of dying cell per µm² the number of TUNEL positive cells was counted in an defined area of the ONL. Afterwards the number of TUNEL positive cells was divided by the area (in µm²) and results were given as number of TUNEL positive cells per µm². The resultes were analysed for significance with student's t-test. Values in the graph are shown as average +/- standard error of the mean (SEM).

Immunohistochemistry:

Explant cultures or acutely disected retinas of rd1or wt mice were fixed in 4% PFA and afterwards cryoprotected in 30% sucrose over night . Specimen were embedded in tissue tec and immediately frozen in liqid nitrogen. Radial cryosection were prepared with 12µm thickness and mounted on silanized cover slips. Cryosections were allowed to dry for 1 hour at 37°C and afterwards washed 3 times with PBS. To block unspecific staining cryosections were treated for 1 hour with blocking solution containing 10% NGS and1% BSA in PBS with 0.1% Triton-X-100 added. Primary antibody was diluted in blocking solution and incubated over night at 4°C. Next day slides were washed 3 times with PBS and incubated with secondary antibody diluted in PBS with 0.1µg/ml DAPI added. After 3 final washes in PBS slides were coverslipped with Vectashield and photomicrographs were taken at a Zeiss ApoTome.

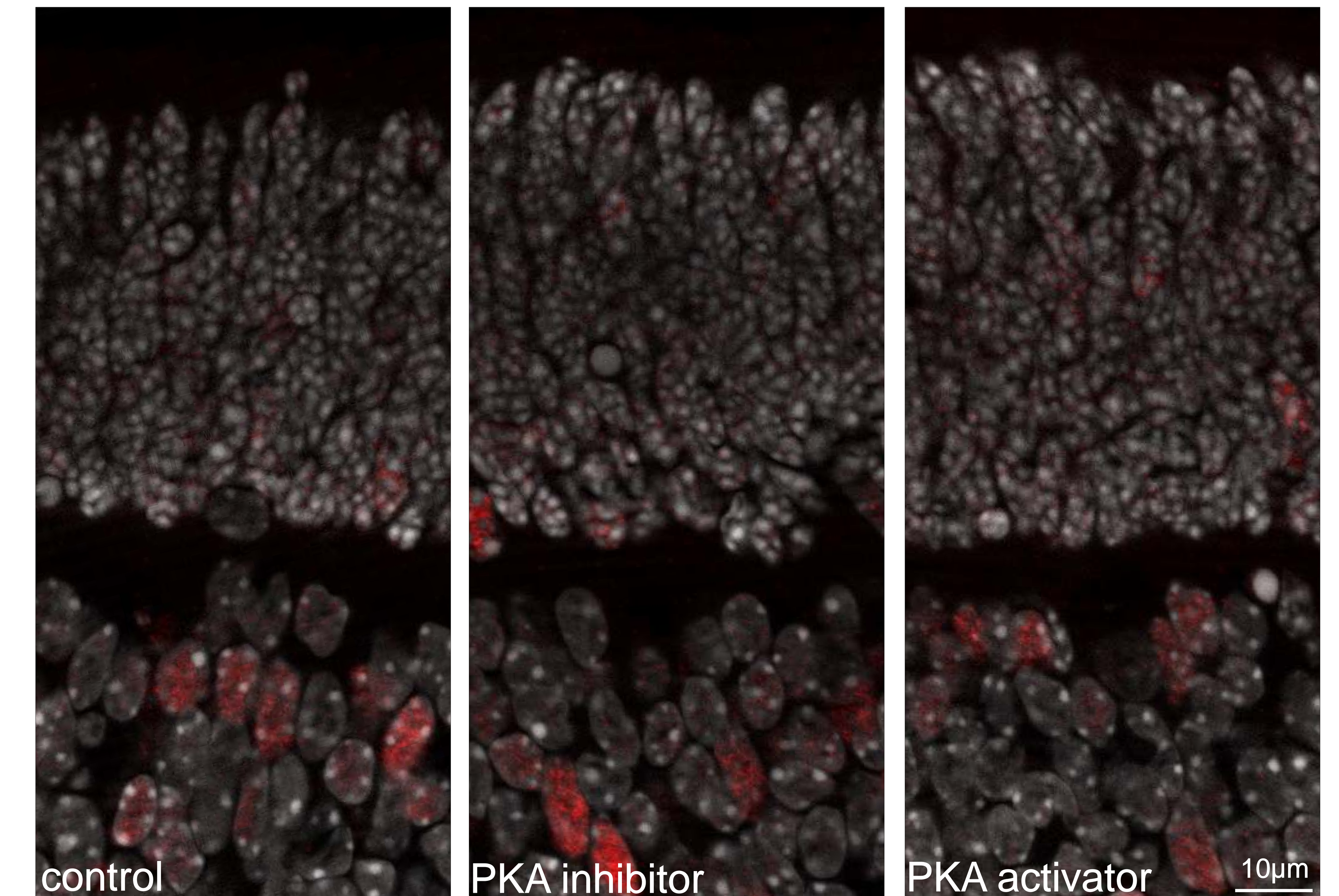
Impact of PKA activity on cell death



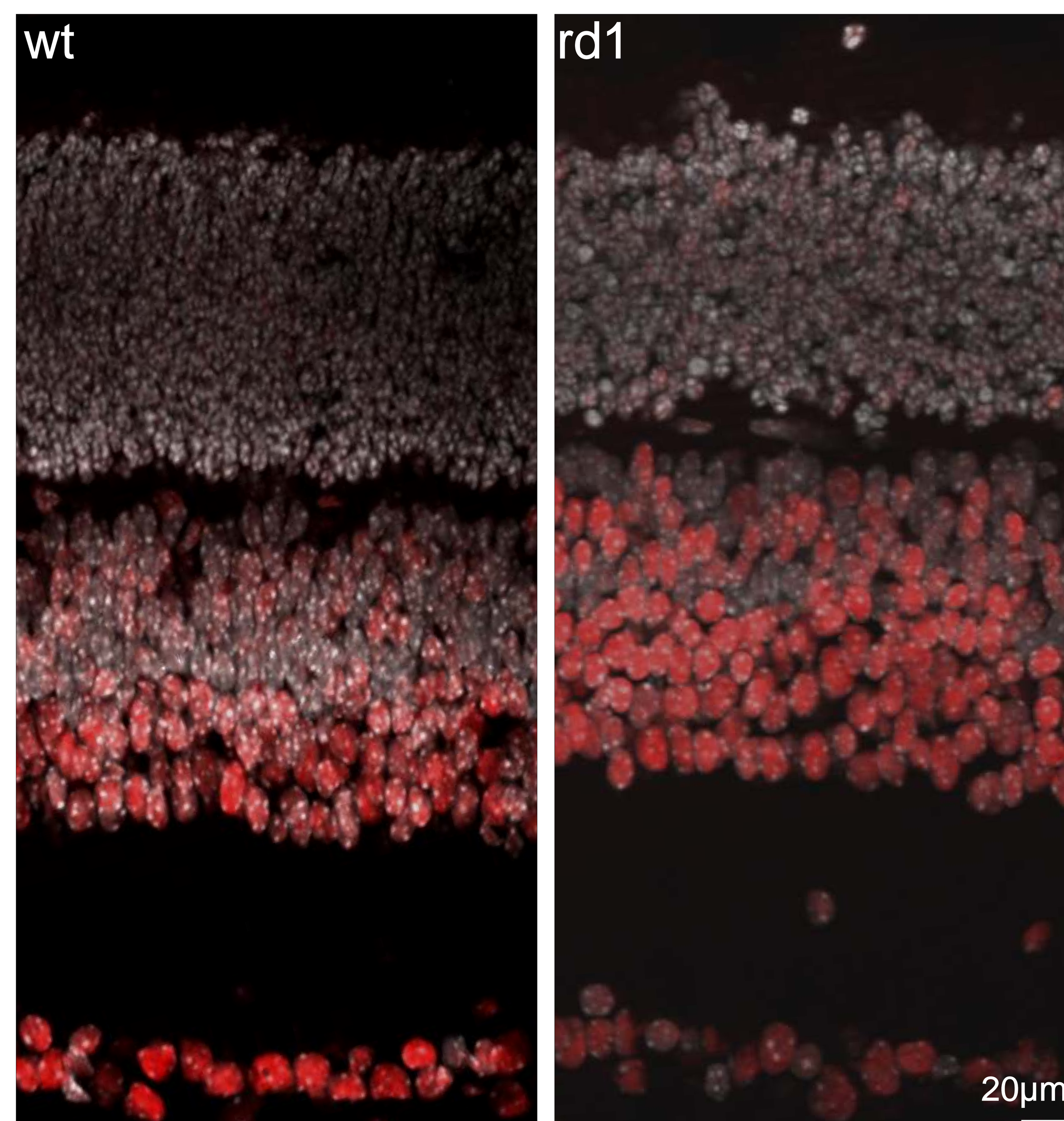
Impact of PKA activity on cell death:
rd1 retinas were treated in vitro for 4 days with PKA activator or inhibitor respectively. Afterwards cell death in the ONL was assessed by performing TUNEL staining on retinal cryosections. PKA inhibition as well as activation does not significantly alter the number of TUNEL positive cells in the rd1 retina.

Impact of PKA activity on pCREB expression:
pCREB immunohistochemistry was performed on rd1 mouse retinal cryosection of organotypic cultures whcih were treated before with PKA inhibitor or activator.
pCREB expression in the ONL of retinas treated with PKA activator or inhibitor is unchanged in comparison with control retina.

Impact of PKA activity on pCREB expression



pCREB expression at PN11



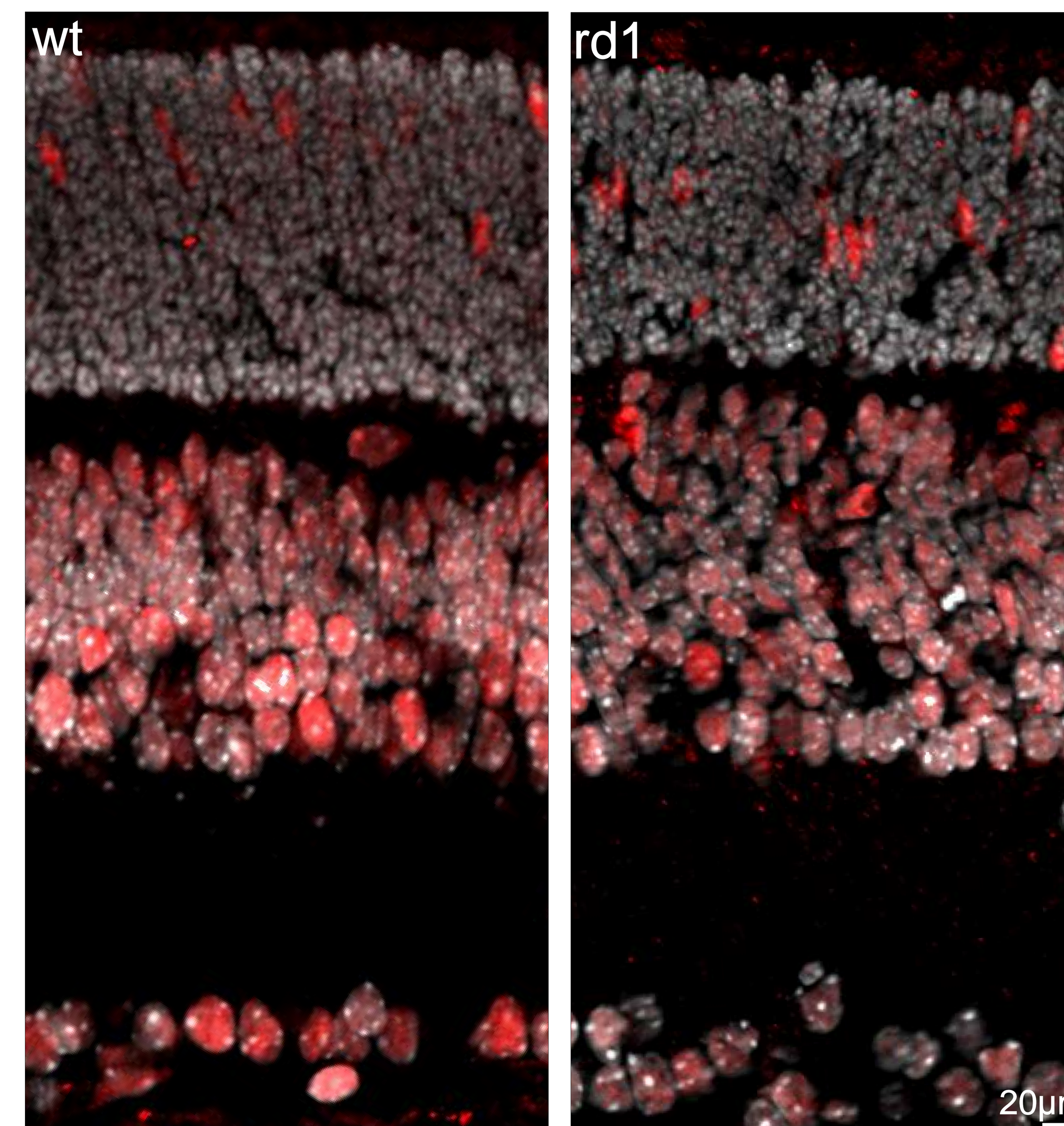
pCREB expression at Pn11:

At PN 11 pCREB was analyzed with an antibody specific for CREB, phosphorylated at Serin 133 in rd1 and wt retinas.
Main pCREB expression is present in the INL and GCL of the retina, whereas retinal fiber layers are devoid of pCREB expression. The ONL of both retinas exhibits a faint pCREB staining in scattered cells however pCREB immunoreactivity seems to be slightly stronger in the rd1 retina

ICER expression at PN 11:

ICER expression was analysed with an antibody specific for ICER (kindly provided by Carlos Molina), the endogenous CREB supressor, in wt and rd1 retinas at PN 11.
Most ICER positive cells are present in the retinal INL and GCL of wt and rd1 mice. The retinal fiber layers are completely lacking ICER immunoreactivity. In the ONL scattered cell are strongly expressing ICER however there are no obvious differences in the distribution pattern between wt and rd1 mice.

ICER expression at PN 11



Conclusion

- Photoreceptor degeneration in the rd1 mouse seems not to depend strongly on PKA activity
- Changes in PKA activity seem not to alter pCREB expression in the rd1 mouse retina
- pCREB expression in the ONL is similar in rd1 and wt mice
- ICER expression pattern in the ONL of rd1 and wt mice is similar

Acknowledgements

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Contact: stefan.kustermann@uni-tuebingen.de