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LONG-TERM CULTURE OF THE WHOLE RAT RETINA

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Purpose: To establish a culture system for the whole immature rat retina as a convenient method for studying development and differentiation of retinal neurons *in vitro* as well as effects of various drugs on the establishment of intra-retinal connectivity.

Methods: Whole retinæ of newborn rats were prepared flat and co-cultured with slices of 2 to 4 days old rat Superior Colliculus. Tissue pieces placed onto a polycarbonate membrane insert were cultured within Costar Transwell culture chambers to allow easy exchange of O₂ and CO₂. Culture medium was placed in the lower compartment of the Costar's Transwell culture chamber in contact with the permeable polycarbonate membrane while tissue pieces lying on the membrane remained exposed to air. Retinæ were cultured in 5% CO₂ balanced air at 37°C and examined by conventional histological techniques at different intervals after explantation, up to two weeks.

Results: Explanted retinæ maintained their typical layering *in vitro* and underwent substantial maturation. Differences were noticed compared with the *in vivo* development however. Retinal thickness decreased to 50% of its original value during the culture period. On the other hand, retinal maturation was accelerated during the first days of *in vitro*. The three cellular and the two plexiform layers of the retina were clearly separated after 5 days, while at the corresponding time *in vivo*, separation of external and internal nuclear was just starting. After the first 5 days *in vitro* maturation was grossly completed. At 14 days the layering observed *in vivo* and *in vitro* was comparable, but the cultured retinæ showed reduced thickness, particularly in the inner plexiform layer, possibly as the result of axotomy-induced loss of retinal ganglion cells.

Conclusions: We have established a culture method to follow retinal maturation *in vitro* which provides a convenient system to investigate the action of pharmacological substances on retinal development.

ras MITOGENIC PATHWAY INHIBITORS IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS IN CULTUREPIÑEIRO A¹, SANCHEZ-SALORIO M¹, CAPEANS C¹, DOMINGUEZ F²

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Purpose: Searching for new inhibitors of Retinal Pigment Epithelial cells (RPE) proliferation. We have determined the action that two *ras* mitogenic pathway inhibitors (Lovastatin (LOV) and N-Acetyl-S-Farnesyl-L-Cysteine (AFC)) have in human RPE cells in culture.

Methods: RPE cells primary culture was initiated from adult human corneal donors. RPE cells were cultured in presence of different concentrations of LOV and AFC. Measurements of cell viability and proliferation were performed by using MTT and 5BrdU incorporation tests, respectively.

Results: We found that both inhibitors produce a diminution in proliferation rate. These effects were even greater than those obtained in serum-deprived cells. Moreover, we found that LOV did have no effect in RPE cells viability. Interestingly, LOV treatment changed cellular morphology to a neuron-like appearance.

Conclusions: The inhibitory effect that LOV and AFC have in human RPE cells suggests a role of *ras* pathway in these cells proliferation. On the other hand, these substances could constitute a new target for treatment of ocular diseases characterized by RPE proliferation (i.e. PVR).

IN-SITU HYBRIDIZATION WITH DIGOXIGENIN-LABELED RNA PROBES RECOGNIZING RETINAL PIGMENT EPITHELIAL-SPECIFIC mRNA.

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Purpose: Development and optimization of an *in-situ* hybridization (ISH) protocol for retinal pigment epithelial (RPE)-specific mRNA using digoxigenin (DIG)-labeled RNA probes.

Methods: A 209 basepair fragment from the bovine cDNA encoding RPE-specific 11-*cis* retinol dehydrogenase (RoiDH) was subcloned. Antisense and sense RNA probes were labeled with DIG. Specificity of the antisense probe was tested on Northern blot. An ISH protocol was developed for frozen and fixed sections. Different pretreatments of the tissue sections were tested. Suppression of background staining was tried to achieve with RNase and a dehydration series.

Results: On Northern blot the DIG-labeled 11-*cis* RoiDH antisense RNA probe recognized a single transcript of approximately 1.2 kb. No specific hybridization was obtained without pretreatments of tissue sections, although a control antisense RNA probe encoding opsin clearly recognized the photoreceptors. For the 11-*cis* RoiDH probe permeabilization by a proteinase K treatment was absolutely necessary. Suppression of background staining leads to a decrease of signal in the RPE.

Conclusions: The mRNA's in photoreceptors can be detected without pretreatments of the tissue sections. For detection of mRNA's in the RPE a proteinase K incubation can not be omitted. After a proteinase K pretreatment the morphology is better preserved in fixed tissues.

FAILURE OF ASCORBIC ACID TO LIMIT BLUE LIGHT INDUCED RETINAL INJURY IN RATS

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Purpose: Previous papers have demonstrated that natural and synthetic antioxidants ameliorate retinal photic injury by mid-visible light. Photosensitized oxidation of membrane lipids has been proposed as the most likely mechanism for rhodopsin-mediated retinal damage. The aim of the present study was to assess if ascorbic acid limits blue light retinal damage.

Methods: The narrow band light used for exposure peaked at 404 nm with a corneal irradiance of 70 kJ/m². Three days after approximately 10-min exposure, we studied retinal pathologic changes with morphology and morphometry in albino rats with or without ascorbic acid supplement.

Results: No distinct light microscopical difference in severity of blue light induced retinal damage was found between ascorbic acid treated rats and physiological saline injected control rats. The outer nuclear layer thickness of photoreceptor cells decreased by 56.5% and 58%, respectively.

Conclusions: Our findings suggest that the damaging mechanism for short wavelength light differs from that of mid-visible light.