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P 424

TITLE : STUDY OF MYC MITOGENIC PATHWAY IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS IN CULTURE.

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PURPOSE
Migration, proliferation and metaplasia of retinal pigment epithelial cells have been implicated in the production of vitreoretinal proliferation in retinal detachment. The mitogenic intracellular pathways in these cells as a target for inhibiting cell proliferation was studied.

METHODS
Human Retinal Epithelial Cells in Primary Culture.
mRNA levels were assessed by Northern Blot.
Cell proliferation, for detection of 5-Br-2'-deoxyuridine incorporated into cellular DNA (BrdU Labeling and Detection KIT III by Boehringer Mannheim) was measured by ELISA.

RESULTS
By Northern Blot analysis we found that the myc mitogenic pathway is active in these cells. We found that both c-myc and proliferine alpha mRNA are expressed.
We used various inhibitors of cell proliferation, such as c-myc antisense oligo, PDGF-beta and ovastatin. We found that lovastatin was the strongest inhibitor, and that the results were similar to serum deprivation.

CONCLUSION
We have confirmed that the myc mitogenic pathway is active in human retinal pigment epithelial cells in culture and that specific inhibitors block this pathway.

P 425

BIOCHEMICAL AND IMMUNOHISTOCHEMICAL BASES FOR A RETINAL OSCILLATOR IN THE RAT RETINA

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Purpose: to demonstrate the occurence of an oscillator using dopamine (DA) and melatonin (MEL), that regulates the daily rhythm of light/dark adaptation in the rat retina, as exists in non mammalian species.
Methods: Biochemical assays for DA, di-hydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), N-acetyl-serotonin, and MEL by HPLC with amperometric detection, and immunocytochemistry of DA receptors in Wistar and Brown Norway (BN) adult male rats submitted for 10 days to different lighting cycles (LL=light-adapted, DD=dark-adapted, DL=12/12 cycle), or blockade of DA synthesis by alpha-methyl-paratyrosine (alpha-mpt), or blockade of DA-receptors by clozapine.
Results: A D2-like receptor was localized on photoreceptor inner segments with both an anti-D2 antiserum (directed against the 3rd intracytoplasmic loop) and an anti-idiotypic antibody, in DL rats. The expression of this receptor was enhanced in DD rats following dark-induced DA deprivation. MEL was quantifiable in DL BN rats sacrificed at night, while only N-acetyl-serotonin was detectable in Wistar rats. The retinal DA content was greatly decreased (80%) after blockade of tyrosine hydroxylase activity by alpha-mpt. In this condition, MEL was enhanced (about 40%) in DL BN rats. The MEL content also increased (about 30%) following blockade of the D2-like receptor by clozapine, in DL BN rats sacrificed at night.
Conclusions: The results confirm the implication of DA in the regulation of MEL synthesis by photoreceptor cells through a D2-like receptor in rats. The clozapine-induced increase in MEL synthesis indicates that the receptor involved is likely to be of the D4 subtype.

P 426

THE WEAVER MUTATION: EFFECT ON THE DOPAMINE SYSTEM OF THE RETINA.

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Purpose: While early degeneration of dopaminergic (DA) cells, correlated with 30% DA level depletion, occurs in mesencephalic nuclei of weaver mice, DA levels remain unchanged in the retina. So it was interesting to know whether the density and morphology of DA cells were also unchanged in that tissue.
Methods: DA cells were labelled by anti-Tyrosine hydroxylase in whole mounted retinas from 7, 10, 14 and 21 postnatal days (PND) normal and mutant mice. DA cell density and spatial distribution (analyzed by "Voronoï tesselation") as well as cell morphology were compared.
Results: In weaver retinas, the density of DA cells was increased (56 vs 40 cells/mm^2 at PND 14). Moreover, beyond the typical retinal DA cells (type 1) whose somata usually lay in the innermost sublayer of the inner nuclear layer (INL), another cell type (type 2) was observed: their somata were located in the middle of the INL and display a very particular morphology of immature neurons (round and clear nucleus, thick dendritic trunks); they sent horizontal processes which finally joined those of type 1 in the inner plexiform layer. Fusion between processes of neighboring cells could often be observed. Since they represent only a low percentage of total DA cells (max 12%), they cannot account for the increased density. It should be noted that they were mostly located in high density areas.
Conclusion: The meaning of type 2 cells can be questionned: they resemble to some accidentally displaced DA cells observed in other rodent retinas. The analysis of DA cells in older mice will permit to specify whether their number remain increase or become normal in adults. This observation confirms a specific sparing of the DA cell system in the retina, already reported in Parkinson patients.

P 427

DIFFERENCES IN HORIZONTAL-CELL NEMATOSOMES OF TWO TELEOST SPECIES DURING LIGHT AND DARK ADAPTATION

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Purpose: We have previously shown the existence of nucleolus-like or nematosomes in external horizontal cells (HCs) of White perch (*Roccus americana*) retinas. Nematosomes were larger and more numerous in dark-adapted retinas than in light-adapted ones. The number and size of the nematosomes were inversely correlated with the number of spinules. These data suggested that nematosomes could be the source of the electron-dense material observed in spinules. The aim of this work was to determine the changes in density and complexity of nematosome structure during light or dark adaptation, and study whether there are differences across species of teleost. **Methods:** Black bass (*Micropterus salmoides*) and White perch (*Roccus americana*) were adapted to dark or light conditions for a minimum of 2 hours. The fish were then sacrificed and their retinas prepared for EM. Electron micrographs of external HCs were printed and nematosomes were digitized to analyze the following parameters: cross-section area, form factor, fractal dimension, fibers thicknesses, distance between fibers, and area occupied by nematosome fibers. Statistical differences were checked by ANOVA. **Results:** In both species, nematosomes were larger, rounder, denser, and more complex in dark than in light-adapted retinas. However, the distance between nematosome fibers remained constant. When we compared Black bass to White perch nematosomes, the former appeared larger, rounder, and their fibers were thicker and less complex. ***Conclusions:*** *Light and dark adaptation produce changes in size,* form, and complexity of nematosomes, probably because of changes in the amount of fiber material. The invariable distance between fibers suggests a constant basic structure in nematosomes. Finally, we found some structural differences of nematosomes across species. Supported by DGICYT PM 92 0113 and GV-2521/94.