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Tyrosinase biosynthesis in adult mammalian retinal pigment epithelial cells

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Abstract

Tyrosinase (EC 1.14.18.1) is the rate limiting enzyme of melanogenesis and it is unclear whether it is synthesized in postnatal retinal pigment epithelium (RPE). Cultured RPE cells from cattle were fed with isolated rod outer segments (ROS). After phagocytosis, RPE cells were tested for tyrosinase presence and activity with three independent methods: (1) ultrastructural DOPA (L-3,4-dihydroxyphenylalanine) histochemistry (2) immunocytochemistry with anti-tyrosinase antibodies (3) measuring tyrosine hydroxylase activity using [^{3}H]tyrosine. With all three methods tyrosinase was found in RPE cells after ROS-feeding but was absent without feeding. In contrast to the classical hypothesis, we demonstrated with three independent methods that the expression of tyrosinase and its enzymatic activity are induced in cultured adult RPE by phagocytosis of rod outer segments (ROS) in vitro.

Keywords: endosome; melanosome; oxidative stress; phagocytosis; phagosome

1. Introduction

Melanin pigment is produced in the neuroectodermic retinal pigment epithelium (RPE) and neural crest-derived melanocytes in mammals (Marks and Seabra, 2001). Melanin-synthesizing cells contain specific organelles, the premelanosomes, in which glycoproteinic transmembrane tyrosinase catalyzes melanin biosynthesis (Seiji et al., 1963).

The RPE is a functionally relevant monolayer of pigmented cells in the mammalian eye. Basic adult RPE functions are the formation of the outer blood-retina barrier, transepithelial transport, protection against reactive oxygen intermediates and light, storage of retinoids and turnover of the shed rod outer segment discs (Boulton and Dayhaw-Barker, 2001). Once fully differentiated, RPE cells do not divide and remain functional throughout the life of an individual. The RPE-choroid complex contains the highest melanin concentration of all human tissues. Disturbances in melanin biosynthesis have been implicated in genetic disorders, such as oculocutaneous albinism (OCA). Tyrosinase is the rate limiting enzyme of melanin biosynthesis and catalyses the first two steps of melanin synthesis: hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), and the oxidation of L-DOPA to DOPAquinone (Lerch, 1987). Furthermore, a third enzymatic reaction was assigned to tyrosinase: the oxidation of 5,6-dihydroxyindole to 5,6-dihydroxyquinone (Korner and Pawelek, 1982). It has been postulated that melanogenesis in the RPE is restricted to prenatal periods, since tyrosinase, the key enzyme in melanin biosynthesis, was detected in early stage human embryos only and was absent long before gestation ends (Carr and Siegel, 1979; Miyamoto and Fitzpatrick, 1957; Sarna, 1992; Smith-Thomas et al., 1996).

However, premelanosomes and early-stage-melanosomes have been found in adult RPE (Dorey et al., 1990; Schraermeyer, 1993; Schraermeyer and Heimann, 1999; Young, 1978) which led to the hypothesis questioned here. Tyrosinase activity has

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also been demonstrated in adult cultured bovine (Basu et al., 1983; Schraermeyer and Stieve, 1994), porcine (Dorey et al., 1990), mouse (Novikoff et al., 1979), rabbit (Varela et al., 1995), rat (Weisse, 1995) and human fetal RPE cells (Aronson, 1983). Additionally, tyrosinase activity was found in adult bovine RPE (Dryja et al., 1978).

Tyrosinase promoter activity was significantly up-regulated in cultured human RPE cells treated with basic fibroblast growth factor (bFGF), pigment epithelium derived factor (PEDF), verapamil, cholera toxin (CT) and tyrosine compared with control cells. In conclusion, the tyrosinase gene is not only expressed, but can be regulated in response to different chemicals in cultured human RPE cells. However, in that study tyrosinase enzymatic activity was not found (Abul-Hassan et al., 2000). In addition, recent studies show that phagocytosis of ROS induces expression of various genes in RPE cells.

Our study was performed to examine the presence of tyrosinase protein as well as its enzymatic activity in adult mammalian RPE. Phagocytosis of shed photoreceptor outer segment distal discs is one of the most important functions of the RPE. Cellular functions of RPE cells such as disc shedding and subsequent phagocytosis are controlled by the circadian rhythm (LaVail, 1980). Therefore we investigated whether phagocytosis of ROS can increase tyrosinase expression in vitro.

2. Materials and methods

2.1. Organ culture of bovine RPE-choroid complexes

Pigmented RPE-choroid complexes were isolated according to Schraermeyer and Stieve (1994). In brief, cattle eyes from two year-old animals were transported on ice from a slaughter house to the laboratory. The eyes were washed once with Hanks' balanced salt solution (HBSS) containing penicillin (100 U/ml) and streptomycin (0.1 mg/ml). All tissue culture media or solutions were purchased from Sigma (Deisenhofen, Germany). The anterior half of the eye was removed and discarded. The vitreous was removed, and the retina was gently floated off the RPE by pipetting HBSS into the subretinal space. The retinae were removed after cutting the optic nerve and used for isolation of the rod outer segments (ROS), as described below. Specimens (1 mm^3) of the RPE-choroid complex were kept in 24-well tissue culture plates (Cluster^{24}, Costar, Cambridge, UK). The complete tissue culture medium consisted of Dulbecco's Modified Eagle Medium with 4500 mg l^{-1} glucose supplemented with 10% fetal calf serum, 25 mM HEPES, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 3.7 g/l sodium bicarbonate and 4 mM L-glutamine. The cultures were gassed with 5% CO_2 and the medium was changed completely once a week. In a separate set of experiments, immunodetection of tyrosinase RPE cells were cultured as monolayers, as described earlier (Dintelmann et al., 1999).

2.2. Isolation of rod outer segments

The method was performed according to Schraermeyer and Stieve (1994). In brief, isolated retinae were agitated for 2 min in KCl buffer (0.3 M KCl, 10 mM HEPES, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 48% sucrose) at pH 7.0 and then centrifuged at 2000 rpm in a tabletop centrifuge (type UJ 1, Christ, Germany) for 5 min. The supernatant was filtered through a tube gauze fingerling and then diluted with KCl buffer (1:1) without sucrose and centrifuged at 2500 rpm for 10 min. The pellet, containing the rod outer segments, was washed and centrifuged in complete culture medium without serum before feeding to the RPE.

2.3. Assay for phagocytosis

RPE cell monolayers or RPE-choroid complexes were cultured for 2 weeks in order to obtain a confluent cell monolayer. This period of time was necessary because the explants needed at least a week to attach to the dish. The cells were then exposed to rod outer segments (rods from one eye/4 wells), which had been isolated as described above, in full culture medium containing serum. After 4 h the non-phagocytosed rod fragments were washed off and fresh medium was added. The medium was changed daily during the experiments. Cells and explants without ROS feeding were used as controls.

2.4. Electronmicroscopical localization of tyrosinase

The enzyme tyrosinase was localized by electron-microscopical histochemistry using the DOPA reaction (Schraermeyer, 1992a,b). In brief, to localise tyrosinase, RPE cells from the RPE-choroid complex, which had been exposed to fragments of rod outer segments as described above, were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 6.8) for 1 h. RPE cells without feeding were treated in the same manner. Specimens were washed twice in sodium cacodylate buffer and kept at 4 °C overnight in this buffer, containing 5 mM L-dihydroxphenylalanine (L-DOPA) or, as a control, 5 mM D-DOPA (Sigma, Deisenhofen, Germany). Thereafter, these solutions were renewed, and the tissue pieces were incubated for a further 5 h at 37 °C. The reacted RPE cells were washed in sodium cacodylate buffer and immersed for 1 h at room temperature in the same buffer containing a mixture of osmium tetroxide (1%) and potassium ferrocyanide (1.5%). Finally, the tissue pieces were dehydrated and embedded in Spurr's resin for routine electron microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 902A electron microscope.

2.5. Immunodetection of tyrosinase

The mouse anti-tyrosinase monoclonal antibody 2G10 was purchased from Chemicon Int. Inc. (Temecula, CA, USA) and is described by the manufacturer as an antibody to human tyrosinase (Cuomo et al., 1991). The monoclonal anti-tyrosine hydroxylase antibody, clone TH-2 and IgG1 isotype, (Sigma, Deisenhofen, Germany) was used as a control. This antibody was raised against an epitope of rat tyrosine hydroxylase, also present in human tyrosine hydroxylase. The secondary purified Cy3-conjugated goat anti-mouse IgG was obtained