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Comparison of retinal pigment epithelium cell preparations from the bovine eye

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Summary

Retinal pigment epithelium (RPE) cells were collected from bovine eyes using a new method. The cells were harvested by vortexing the RPE and underlying choroid in 0.05 M citrate phosphate buffer, pH 5. RPE cells recovered by this method were compared to a standard method by microscopic examination of cell integrity, estimation of total protein, and assay of 11-*cis* and all-*trans* retinyl ester hydrolase (REH) activities. Results suggest that this method collects RPE cells of good integrity and with a significantly higher protein yield than the conventional method. Additionally, a much higher retinyl ester hydrolase activity was noted. Therefore we propose that this procedure offers a new and convenient method in the collection of RPE proteins for certain purposes such as enzyme purification.

Keywords

Retinal pigment epithelium; retinyl ester hydrolase; vitamin A; bovine

The retinal pigment epithelium (RPE) is a darkly pigmented single layer of hexagonally shaped cells situated between the choroid and the photoreceptor cells of the retina. RPE cells are joined together by tight intracellular junctions allowing little extracellular space¹. The RPE is one of the most important cell layers in the visual system and its anatomical arrangement is uniquely adapted to its many diverse functions such as the storage, transport, and metabolism of vitamin A, the nurturing and renewal of the photoreceptor cell layer, and the absorption by melanin granules of scattered light which improves the resolution of images². In addition, it possesses 'glia-like' functions to control water and metabolite fluxes via regulation of extracellular potassium³. Therefore it is important to study different methods in which to collect RPE cells.

In the present study, a new method was evaluated to collect RPE cells from the bovine eye. Quantitative recovery of RPE cells was monitored by a) microscopic examinations of cell integrity; b) estimation of recovered protein using the Lowry protein assay; and c) evaluation of enzyme activity by retinyl ester hydrolase (REH) assay. Our results show that

this new approach offers a convenient method to recover metabolically active RPE cell proteins in significantly larger amounts than the conventional method (brushing and dislodging RPE cells using a camel-hair brush).

Methods

Dissection of bovine eyes

Bovine eyes were obtained from a local meat packing company (Allstate Packing Co., San Antonio, Tx). The eyes were kept at 0 °C within 1–2 h of enucleation. The anterior portion (anterior to ora serrata) was removed and the vitreous was detached from the posterior segment by tilting the eye. The posterior eye cup consisted of a blue-green region (which was the amelanotic RPE overlying the tapetum lucidum) and a black region (which was the melanotic RPE). Overlying the entire region was the translucent retina consisting of branching blood vessels. With blunt forceps, the retina was teased and gently pulled away from the RPE in one piece. It was then collected at the optic disk and detached with iris scissors.

Collection of RPE cells

In the first method (Method 1), the surface of the exposed posterior eye cup was rinsed with a small volume of Tyrode (4 °C) saline, which was then discarded. About 1 ml of fresh saline was added and RPE cells were gently brushed loose from the surface of the eye cup with a 1/2" camel-hair brush. The detached cell suspension was removed by Pasteur pipette. The brushing and collecting of RPE cells was repeated three times. Before protein determination and enzymatic assay, RPE cells were centrifuged at $7000 \times g$ for 10 min, resuspended in 0.05 M citrate phosphate buffer, pH 5, (3 ml/eye), and homogenized with a Brinkman PT10/35 polytron (tissue grinder/homogenizer) for 30 s.

In the second method (Method 2), the RPE cell layer and underlying choroid were removed together from the eye cup. Before analyses, the RPE/choroid was vigorously vortexed for 1 min in 3 ml of citrate phosphate. The remaining RPE cells, together with the underlying choroidal layer, were subsequently removed. The cell suspension was then homogenized using a Polytron for 30 s.

RPE histology

RPE cells were placed on a microscope slide and examined under an Olympus Model BM compound microscope with an oil immersion lens (magnification 15×100).

Protein determination

Proteins were quantitated by the method of Lowry, using bovine serum albumin as standard⁴.

Retinyl ester hydrolase (REH) assay

Enzymatic activity was monitored by an REH assay. RPE cells collected from (1) and (2) were placed in 3 ml of sodium 0.05 M citrate-phosphate, pH 5. Tritiated retinyl palmitate was obtained by reacting 11-*cis* or all-*trans* retinol, with the symmetric anhydride of tritiated

palmitic acid⁵. 10 µl of substrate was dissolved in ethanol and added to 190 µl of sample and the mixture was incubated in a shaker bath for 1 h at 37 °C. 3 ml of extraction solvent (1.41 methanol: 1.25 chloroform: 1.00 heptane) and 1 ml of 0.05 M potassium carbonate buffer, pH 10, were added. The mixture was then vortexed and centrifuged at 1000 × g for 15 min. 1 ml of the upper aqueous phase was removed from each sample and radioactivity was measured in a liquid scintillation counter. All assays were carried out in triplicate. One unit of activity refers to the formation of 1 pmole product per s.

Results

Microscopic examinations show that patches of RPE cells were recovered using both of these methods. Hexagonally shaped RPE cells were observed in single layers with intact melanin granules and other cell inclusions. Most cells remained unperturbed and small structures which resembled oil droplets were observed in the surrounding saline. Cell suspensions from (1), in comparison to those from (2), appeared to contain a larger number of intact cells. In addition, RPE cells in suspensions prepared by (1) tended to be aggregates with larger cell patches than those prepared by (2). Non-pigmented cells were not present in our preparations.

Table 1 indicates that RPE cells collected from (2) yielded more than eight times more protein (mg/RPE) than that of RPE cells collected by (1). The figure shows a significant level of REH activities in the recovered RPE cell homogenate. The highest hydrolytic activity was exhibited for 11-*cis* retinyl palmitate in both (1) and (2). Table 2 indicates that both 11-*cis* and all-*trans* REH specific activities were higher in proteins collected by (1) than those collected by (2). Taking into consideration the greater amount of protein collected by (2) (see table 1), the total enzyme activity recovered from the RPE was greater in proteins recovered from method 2.

Discussion

Microscopic evaluations of RPE cells collected by the two methods employed show both yielded intact, morphologically identical RPE cells. However, (1) collected a greater amount of intact RPE cells than (2) and larger cell patches were noted in cell suspensions prepared from (1). These results suggest that the vigorous vortexing of RPE/choroid using method 2 must have disrupted more RPE cells than the gentle brushing of method 1. Nevertheless, our results show that both methods yielded intact RPE cells, although a qualitative evaluation of these 'intact' RPE cells remains to be carried out.

Our results on protein yield from (1) was in agreement with those reported in the literature (6 mg per eye in the present study compared to 8 mg per eye in Berman⁶). Moreover, Lowry protein determination showed that (2) yielded more than eight times more total protein (mg protein/RPE) than (1). This suggests that (2) has dislodged more RPE cells into the buffer than (1) therefore resulting in a higher protein yield. This appears contrary to our histological examinations that (1) yields more 'intact' RPE cells. One possible explanation is that a significant number of RPE cells collected from (2) were disrupted (i.e. not 'intact' cells) as a result of vortexing thus lowering the number of intact RPE cells from (2). The

protein yield, nevertheless, suggests that (2) collected more proteins from the bovine RPE than (1).

The retinyl ester hydrolase activity has been reported in the retinal pigment epithelium of human cells⁷. In addition, the enzymatic activity was shown to be stereospecific, i.e. more active towards hydrolysis of 11-*cis* than all-*trans* substrate. In this study, our results agree with the literature that RPE protein contains active enzymes to hydrolyze both 11-*cis* and all-*trans* retinyl palmitate (table 2). Moreover, these enzymes were found to favor hydrolysis of 11-*cis* over the all-*trans* substrate. It is important to note that REH activity was found in RPE cells collected by both methods employed in this study and that RPE cells collected by (1) possessed higher specific activity for both 11-*cis* and all-*trans* retinyl palmitate yet (2) yielded more total enzyme activity.

In conclusion, both methods employed in the present study collected RPE cells of good quality. Although (1) resulted in relatively more pure RPE cell preparation, (2) yielded more RPE proteins and higher overall enzyme activity. Consequently, we suggest that the method tested in the present study (2), offers a new and convenient approach to collect more RPE protein appropriate for certain investigations such as enzyme purifications.

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Figure.
Retinyl ester hydrolase activities in methods 1 and 2.

Table 1

Total RPE proteins (mg/RPE) determined using the method of Lowry et al.⁴

Method 1	Method 2
1.0	15.7
2.8	14.3
2.6	17.3
1.6	13.4
1.2	14.0
1.9	18.2
$\bar{X} = 1.85 \pm 0.7$	15.48 ± 1.9

Table 2

11-*cis* and all-*trans* retinyl ester hydrolase activities (units/mg). The values were derived from the linear portion of the protein curves (see figure)

	Method 1	Method 2
11- <i>cis</i>	7.5	3.8
all- <i>trans</i>	5.5	2.0