Retinal Pigment Epithelial Cells of the Posterior Pole Have Fewer Na/K Adenosine Triphosphatase Pumps Than Peripheral Cells

Janice M. Burke,*† Brian S. McKay,* and Glenn J. Jaffe*

The density of Na/K adenosine triphosphatase (ATPase) pumps in retinal pigment epithelial (RPE) cells in different retinal regions was quantified by measuring the binding of 3H-ouabain to RPE in cow and human eyecups. In bovine eyes, pump density was estimated in RPE samples isolated from three retinal regions outlined with a 7-mm trephine: one from the posterior pole in the area centralis and two from the superior, equatorial retina representing unpigmented (in the tapetum) and pigmented zones. In human eyes, RPE samples were isolated from a posterior region centered around the macula and one superior region. Ouabain binding to RPE of the posterior pole of both species was approximately 40–60% lower than binding to RPE of more peripheral regions in the same eyes. For bovine eyes, ouabain binding did not differ between pigmented and unpigmented cells of the superior retina, suggesting that reduced binding in the relatively amelanotic posterior cells was not related to levels of pigmentation. For human RPE, binding to posterior cells was lower in eyes from donors of all ages (range, 17–90 yr). The data suggest that Na/K ATPase pump site density is lower in posterior RPE cells of both bovine and human eyes, perhaps due to a regional difference in requirements for ionic regulation.


Retinal pigment epithelial (RPE) cells maintain a gradient of Na⁺ and K⁺ across their apical plasma membranes by the action of ouabain-sensitive Na/K adenosine triphosphatase (ATPase) pumps.¹ It has been postulated that the pumps in RPE cells contribute to retinal adhesion² and maintenance and regulation of cell volume and intracellular and extracellular ionic environments.³ Because the structure and function of the sensory retina vary in different regions of the eye, we hypothesized that requirements for ionic regulation by RPE may also differ as reflected in topographic differences in the density of Na/K ATPase pumps.

We previously analyzed pump number and activity in RPE cells in vitro.⁴⁵ For this study, methods used for cultured cells were modified to measure the binding of ¹⁹H-ouabain to RPE cells in situ. The binding data were used to quantify Na/K ATPase pumps in different retinal regions, with emphasis on comparing pump site density in the area centralis (of bovine eyes) or macula (of human eyes) to more peripheral regions. Here we demonstrate that the density of Na/K ATPase pumps is lower in posterior cells of both species.

Materials and Methods

Eyes from 49 adult cows were transported on ice from a local slaughterhouse and assayed within 5 hr of death. The eyecups were prepared by dissecting the anterior segment about 5 mm posterior to the limbus and removing the vitreous and retina to expose the RPE layer. They were prepared similarly from both eyes of 31 human donors (age range, 17–90 yr) within 24 hr postmortem. For human eyes, the eyecups were oriented, and the macular region was identified, using the retinal vasculature before removal of the retina. The sclera was marked to retain the orientation as the dissection and incubation proceeded. For bovine eyes, the tapetum provides landmarks to locate the regions after removal of the retina (Fig. 1).

Ouabain is a cardiac glycoside which is used to quantify Na/K ATPase pumps because it binds essentially irreversibly with a 1:1 correspondence to the cat-

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alytic subunit of Na/K ATPase. The drug cannot penetrate the membrane and therefore binds to pumps on the cell surface, although during incubation the pumps may cycle temporarily through the endosomal compartment. Methods used here for measuring the binding of $^3$H-ouabain to RPE in situ were similar to those previously reported for cultured RPE cells except that incubations were conducted in the eyecups, and tissues were harvested by scraping specific regions. For these studies, the exposed RPE layer in eyecups was first rinsed with 37°C K+-free Ringer's solution (NaCl, 6.801 g/l; CaCl$_2$·H$_2$O, 0.153 g/l; MgCl$_2$·6H$_2$O, 0.158 g/l; NaH$_2$PO$_4$, 0.103 g/l; NaHCO$_3$, 2.453 g/l; and glucose, 0.903 g/l). The eyecups were then filled with labeling medium (K+-free Ringer's containing 5 × 10$^{-8}$ M $^3$H-ouabain [15.7 Ci/mmole; New England Nuclear, Boston, MA]) using volumes of 3.5 ml or 1.4 ml for bovine or human eyes, respectively. The tissues were incubated for 2 hr at 37°C, followed by three rinses of the RPE layer with 37°C K+-free Ringer's. Preliminary experiments were conducted in which the eyecups were incubated with varying concentrations of radiolabeled ouabain (1.5 × 10$^{-2}$–5 × 10$^{-7}$ M) to confirm that 5 × 10$^{-8}$ M was a saturating concentration.

At the end of the incubation period, RPE cells were harvested by outlining specific regions with a 7.0-mm trephine using light pressure, then scraping the cells with a scalpel blade. For bovine eyes, the RPE cells were harvested from three retinal regions: the posterior pole in the unpigmented area centralis (P region); a superior, unpigmented region inside the tapetum (S); and a superior, pigmented region outside the tapetum (S') (Fig. 1). For human eyes, a posterior region centered around the macula (P region) and a superior region (S) at the level of the equator were harvested. The order of retrieving the regional RPE samples in the eyes was randomized. Scraped tissue was homogenized in 1.0 ml of 0.1 N NaOH. Then 10 min later, 200 µl of the homogenate was removed for protein determination by the method of Lowry et al. using bovine serum albumin as a standard. The rest of the sample was counted in a liquid scintillation counter. For each sample, the data were expressed as fmol of $^3$H-ouabain bound/mg protein. Binding data for different regions in the eyes were analyzed using a Friedman two-way analysis of variance (bovine eyes) or a Wilcoxon signed-rank test (human eyes).

In preliminary experiments, nonspecific binding was determined in ten bovine eyes by incubations in labeling medium which contained 10$^{-4}$ M unlabeled ouabain. Nonspecific binding was equivalent in all regions of bovine eyes and did not exceed 480 fmol/mg protein, less than 15% of the total binding observed in any region in the absence of excess unlabeled ouabain. Nonspecific binding in human eyecups was analyzed using nine pairs of donor eyes. For three of the pairs, one eye was incubated in the presence of 10$^{-4}$ M unlabeled ouabain, and the fellow eye was incubated in the absence of the excess unlabeled ouabain. For the other six pairs, nonspecific binding was analyzed in both eyes. Nonspecific binding did not differ between regions or exceed 20% of the total binding observed in either region of any human eye.

The number of RPE cells per unit area was counted in wet mounts prepared from the three regions of four bovine eyes. To prepare the wet mounts, the RPE cells were scraped gently with a scalpel blade, by methods which have been used to produce RPE cell cultures, and the resulting sheets of cells were placed in culture medium on microscope slides. The preparations were cover slipped, and RPE cells were immediately counted at 200 × using a phase-contrast microscope, outfitted with an ocular grid. For each region, RPE cells in approximately 40,000 µm$^2$ of epithelium were counted.

### Results

Measurements of protein in the regional RPE isolates retrieved from bovine and human eyes are shown in Table 1. The amount of sample protein differed between bovine and human eye samples, but it was equivalent among regions for each species. The
Table 1. Comparisons of sample protein in regional RPE isolates from bovine and human eyes

<table>
<thead>
<tr>
<th>Sample protein (mg)*</th>
<th>Bovine eyes</th>
<th>Human eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>0.099 ± 0.006</td>
<td>0.128 ± 0.007</td>
</tr>
<tr>
<td>S'</td>
<td>0.095 ± 0.007</td>
<td>NA</td>
</tr>
<tr>
<td>P</td>
<td>0.103 ± 0.006</td>
<td>0.112 ± 0.006</td>
</tr>
</tbody>
</table>

*Data are means of analyses of 49 bovine eyes and 62 human eyes (± SEM).

\(^3\)H-ouabain binding correlated with the amount of sample protein for all retinal regions of both species; data for the S' region of bovine eyes are shown in Figure 2 which also illustrates data variability for a given region.

Microscopic examination of freshly isolated bovine RPE cells indicated that posterior pole cells were smaller; there were more cells per unit area in the posterior region than in the S and S' regions of the equatorial retina (Fig. 3). The mean RPE cell size (in \(\mu m^2\) ± standard deviation) calculated from counts of cells per unit area were: P region, 234 ± 26; S region, 333 ± 75; and S' region, 342 ± 47.

The \(^3\)H-ouabain binding to RPE in different retinal regions of bovine eyes is shown in Figure 4. Binding did not differ between hypopigmented (S region) and pigmented (S' region) RPE cells of the superior retina. However, ouabain binding in the area centralis (P region) was significantly lower than in the superior regions. When binding data for the superior regions in the eyes were expressed as a ratio of binding in the P region, P region binding was approximately 40% less than in the superior zones (Fig. 4).

Similar results were obtained for the RPE of human eyes; \(^3\)H-ouabain binding in the P region was significantly lower (approximately 60%) than in the more peripheral S region (Fig. 5). Figure 6 illustrates the ouabain-binding data for human RPE as a function of donor age. Binding was lower in posterior pole cells of all eyes at all donor ages, and no age-dependent changes in binding to either the P or the S region were apparent.

Discussion

Using \(^3\)H-ouabain binding to quantify the number of Na/K ATPase pump sites in RPE cells from different retinal regions of bovine eyes, it was observed that pump density was lower in the tissue taken from the
area centralis than in more peripheral tissue isolated from superior regions. The RPE cells of the area centralis, which overlies the bovine tapetum lucidum, are amelanotic relative to cells from nontapetal zones, but differences in pigmentation did not account for differences in ouabain binding. A comparison of binding to peripheral RPE cells which varied in pigmentation (S versus S' regions) showed no differences.

Similar results were obtained for human eyes; posterior (macular) RPE cells showed a lower apparent Na/K ATPase pump density than equatorial cells from the superior retina. For human eyes there were no significant changes in pump site density in either region which correlated with donor age. Because of individual variation, it would be difficult to identify small aging changes (if any). These might be revealed by analyzing additional specimens, especially from middle-aged donors and donors at the extremes of the age range.

Since ouabain binding was used to estimate pump number, it should be noted that the α subunit of Na/K ATPase exists in isoforms which have different ouabain-binding affinities. It is therefore possible that the regional difference in ouabain binding to RPE cells observed here resulted from differences in subunit isoforms rather than differences in Na/K ATPase pump density. Although this possibility cannot be excluded, only the α1 subunit has been detected in rat RPE by immunofluorescence, and our studies of ouabain binding to cultured human and bovine RPE suggest a single isoform (unpublished data). Furthermore, if there were regional differences in isoforms we would have expected nonspecific binding also to vary with retinal region, but this was not observed.

The topographic differences in ouabain binding which were observed did not appear to relate to sampling differences. The amount of cellular protein retrieved was equivalent among regions, and there was a high correlation between sample protein and the amount of ouabain bound which suggested that protein is a reliable measure of the volume of tissue which was actually harvested. It is likely that the number of cells in the isolates from the posterior pole was greater than in isolates from the periphery. Our counts of RPE cell numbers in different regions of the bovine eyes, and those of others on canine eyes, indicate that the posterior pole has more cells per unit area than the equator. According to the work of others, the density of RPE cells in the posterior pole is also greater in human eyes, despite changes in cell number with age. Therefore, if our ouabain-binding data were standardized to cell number it is likely that the differences between the posterior and peripheral regions would be even greater.

In vitro, RPE cells show declining numbers of Na/K ATPase pumps as cultures become confluent and cell density increases. This observation suggests that pump number in RPE may be related to cell packing, and further suggests that the lower pump number in posterior RPE cells observed here may be due to the higher cell density in that region compared with the periphery. However, decreasing pump number with increasing culture confluence is not peculiar to RPE
or to transporting epithelia, but rather it is a general property of cultured cells, including fibroblasts. It is therefore unclear if the factors which control density-dependent pump expression in vitro also contribute to the control of pump number in RPE in situ.

We,15,16 and others,11,17 have also observed topographic variations in RPE enzymes other than Na/K ATPase. All enzyme systems were not, however, consistently higher in one region relative to another. Posterior pole RPE cells of bovine,15 canine,11 and human17 eyes have a higher activity of the lysosomal enzyme cathepsin D than peripheral RPE cells in the same eyes. However, posterior pole cells have a lower activity of some other lysosomal enzymes,11 a lower activity of the mitochondrial enzyme complex cytochrome oxidase,16 and fewer Na/K ATPase pumps. In two studies, enzyme activities were also examined in cultures of RPE cells grown from the different regions of eyes.11,15 Since topographic variations were not retained in the cultured cells, it was suggested that regional variations in RPE functions measured in freshly isolated samples resulted from local in vivo tissue influences which were not present in vitro. It is unknown if local tissue interactions led to the regional differences in RPE Na/K ATPase pump number we found. Since the RPE pump contributes to the maintenance of the ionic composition of the subretinal space,3 and to the active transport of most metabolites,18 we might speculate that requirements for these functions differ in the macular region relative to the periphery. What may be surprising is that posterior RPE cells have fewer ion transporters than peripheral cells, although macular RPE in humans are associated with more photoreceptors than peripheral cells, especially with aging.14 These observations suggest that the transport capacity of the macular RPE, with its reduced pump number, may become insufficient to accommodate the needs of the photoreceptors in the aged eye.

The accumulated data on topographic variations in RPE enzymes, and in the potential for RPE to be grown in vitro,8 support the view that the RPE monolayer is a functionally heterogeneous population of cells, even in normal eyes of young individuals. Our studies of regional differences in the RPE are aimed at identifying ways in which macular tissues differ from those in the periphery in an attempt to determine why the macula is predisposed to aging pathologies. One view is that macular tissues might be at risk for pathology change because, due to their position in the eye, they are exposed to different or more severe aging influences over the lifetime of an individual. Another view, based on the observation that macular RPE cells may function differently even in normal, young eyes, is that the same aging changes may have a regionally different impact.

Key words: pigment epithelium, Na/K ATPase, area centralis, ouabain binding, macula

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References


