Relative Contributions of Epithelial Cells and Fibers to Rabbit Lens ATP Content and Glycolysis

Barry S. Winkler and Michael V. Riley

The adenosine triphosphate (ATP) content was measured independently in separated capsule–epithelium and fibers from whole rabbit lenses, both fresh and after incubation under various combinations of glucose and oxygen deprivation. Lactate production was also measured during aerobic and anaerobic incubations of whole lenses and of monolayers of cultured epithelial cells. The fresh capsule-epithelium contained 3.3 nmoles ATP, whereas the decapsulated lens contained 410 nmoles ATP, a value that was indistinguishable from that of the whole, intact lens. In the presence of glucose, the fibers and epithelium each maintained their respective ATP content under aerobic and anaerobic conditions. In the absence of glucose, the ATP content in each fraction declined with time, but only in the epithelium was the rate of decline of ATP significantly faster in nitrogen than in oxygen. In whole lens, the rates of anaerobic and aerobic lactate production were similar, whereas in the cultured epithelial monolayers, the anaerobic rate was two-fold greater than in oxygen. From this it is concluded that approximately 50% of the ATP of the epithelial cells is derived from oxidative metabolism. A Pasteur response shown here for the first time with the cultured epithelium allows these cells to compensate for the loss of ATP production when mitochondrial oxidation is curtailed. The epithelium does not contribute to the ATP content of the lens fibers under aerobic or anaerobic conditions. Invest Ophthalmol Vis Sci 32:2593–2598, 1991

Previous studies have used whole lenses incubated under various combinations of glucose and/or oxygen deprivation and have measured the accumulation of lactic acid in the medium, yield of CO₂ from ¹⁴C glucose, and the adenosine triphosphate (ATP) content in the whole lens at the end of the incubation period.¹–³ From such whole lens measurements, these studies have reached different conclusions regarding the extent of aerobic metabolism of the epithelial cells and their contribution to the production and maintenance of ATP in the entire lens, ranging from zero¹ to some³ to substantial.² Because the epithelial cells constitute less than 1% of the wet weight of the whole lens, this study seeks to determine whether reliable estimates of the contribution of the epithelial cells to energy production of the whole organ can be derived from measurements of glucose metabolism and ATP content in the whole lens. To determine this, we measured the respective ATP content of the separated lens epithelium and fibers after incubation of the whole tissue under aerobic and anaerobic conditions. Our results, which show aerobic metabolism to be an important part of the energy producing system of the epithelial cells, show that these cells do not provide ATP for the remainder of the lens.

Materials and Methods

Albino rabbits, about 12 weeks old and between 2.0–2.5 kg, were used in accordance with the ARVO Resolution on the Use of Animals in Research. The animals were killed with an overdose of sodium pentobarbital, the eyes were removed, and the lens (average wet weight of 308 mg) was isolated from other ocular structures and carefully cleaned of adherent vitreous. Experiments were performed in cylindrical, capped vials that contained 1 lens/5ml of bicarbonate-buffered Ringer media.⁴ The media were equilibrated with 7% O₂/5% CO₂/88% N₂ that is similar to the gas composition of the aqueous humor. The gas mixture was continuously bubbled into the small air space in the vial and pH was 7.4 at 37°C. The incubations lasted from 1/4–3 hr, and aliquots were removed at timed intervals for estimation of lactate production.³ When glucose was omitted from the media, it was replaced iso-osmotically with 2.5 mM NaCl. Respiration was inhibited with 1 mM KCN or 5

From the Eye Research Institute, Oakland University, Rochester, Michigan.

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Reprint requests: Barry S. Winkler, Eye Research Institute, Oakland University, Rochester, MI 48309.

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× 10^−3 M antimycin A or 95% N₂/5% CO₂; the results were identical regardless of the method used to inhibit respiration. Paired lenses from the same animal were used in the experiments that tested the effects of oxygen to minimize any differences in initial substrate reserves when aerobic and anaerobic conditions are compared.

At the end of the incubation, the whole lens was removed from the solution, gently blotted on moistened filter paper, and the epithelium was isolated from the lens fibers by carefully peeling off the entire capsule with attached epithelial layer under direct microscopic viewing. The epithelium was rapidly transferred to 0.6 ml of ice-cold 5% perchloric acid (PCA) and homogenized. In parallel, and essentially at the same time, the decapsulated lens, comprised of the lens fibers, was transferred to 2.0 ml of 5% PCA and homogenized. ATP was measured with diluted aliquots of these two separate fractions by the luciferin-luciferase method in a luminometer (Turner Designs, Mountain View, CA). Appropriate standards of ATP were run simultaneously with the tissues each time.

Flat mounts were made of capsule-epithelium on Millipore filters, with the same technique for separation from the lens fibers. These were fixed in 3% glutaraldehyde in phosphate-buffered saline, stained with haematoxylin, washed with 0.5% HC1 in 70% ethanol and then with diluted ammonia. The filters were mounted in Cytoseal 60 (Stephens Scientific, Oak Ridge, NJ) for photography.

In a separate series of experiments, the capacity of monolayers of cultured lens epithelial cells to produce lactate was measured. We used a lens epithelial line (N/N1003A) that was established from a 4-day-old rabbit. Cells were grown in Eagle’s Minimal Essential Medium (MEM), as described previously. The population doubling level of the lens cells was 55. Confluent cells were removed from the plates 1 day before their use in the test incubation. Approximately 800,000 cells were put into individual culture dishes (60-mm diameter) that contained MEM plus 1% rabbit serum. The next day, the monolayer of cells was washed with serum-free MEM 30 min before the start of the experiment. The experiment was initiated by adding 5 ml of fresh MEM to the dishes and lactate production in each dish was measured over a 2-hr period.

Results

Figure 1 shows a typical capsule-epithelium preparation that is isolated from the intact lenses. Sections a–f show segments running from equator to equator via the anterior pole, as is shown by the changing density of the cells. There is no indication of remaining lens fibers in any segment. Sections g and h, from the equator and near the pole, respectively, show the different cell densities in the two regions, and the regular, undamaged appearance of the cells.

Figures 2 and 3 show the extent to which the normal levels of ATP in freshly isolated tissues (t = 0 values) are maintained in the lens fibers and epithelium, respectively, under varying conditions of incubation. For the fibers, the initial value was 410 ± 24 nmoles (mean: ± SD, n = 12), and for the epithelium was 3.3 ± 0.5 nmoles (mean: ± SD, n = 12). The value of ATP content in a freshly excised whole lens (415 ± 20 nmoles) was not significantly different from the value for the decapsulated lens. The incubation conditions chosen for study were similar to those used previously, with and without glucose in oxygen, and with and without glucose in nitrogen (or with inhibitors of respiration). In the presence of glucose, both the lens fibers and epithelium maintained ATP over a 3-hr period within 10–15% of their initial values, whether incubated aerobically or anaerobically. Antimycin A and KCN yielded the same results as did nitrogen in the anaerobic experiments.

When rabbit lenses were incubated without glucose, the ATP content of the lens fibers and epithelium declined. Comparison of the data in Figures 2 and 3 for the glucose-free conditions, however, shows two major differences between the behavior of the epithelium and lens fibers. First, the rate of decline in ATP content in the epithelium was considerably faster than in the fibers for both the aerobic and anaerobic conditions. In oxygen, a 50% loss in ATP content in the epithelium occurred in 60 min, whereas in the fibers, the t½ was about 160 min. This finding represents a near three-fold difference. In nitrogen, the t½ was less than 20 min in the epithelium and 130 min in the fibers, representing an approximate six-fold difference. Second, in glucose-free media, the effects of nitrogen on the rate of decline in ATP were much more pronounced in the epithelium than in the lens fibers. In the case of the fibers (Fig. 2), the rate of loss of ATP anaerobically was only slightly faster than the decline seen aerobically, whereas in the epithelium (Fig. 3), nitrogen increased the initial rate of loss of ATP by 300% and caused virtually total depletion of ATP within 60 min. With oxygen, this cell layer still retained almost 50% of its normal level of ATP at this time, and still had 25% after 3 hr.

The capacity of the whole rabbit lens to form lactic acid in the presence and absence of glucose under aerobic and anaerobic conditions is shown in Figure 4. With glucose, lactate was produced at a nearly constant rate over the 3-hr incubation period. Aerobically, this rate was 2.5 μmoles/lens/hr, and anaerobi-
Fig. 1. (a–f) Montage of whole mount preparation of capsule–epithelium from one equatorial region to the other (bar = 50 μm). (g, h) Higher-power views (Bar = 50 μm) of epithelial cells from regions corresponding to (a) and (d), respectively.
Fig. 2. A graph of the time-course of change in the ATP content of the decapsulated rabbit lens as a function of various metabolic interferences. The decapsulation was performed at the end of the incubation of the intact lens, as described in the text. Results are expressed as means ± SD and each point represents the average of between 10 and 18 experiments. Conditions and symbols as follows: glucose with oxygen, •; glucose with nitrogen, □; no glucose with oxygen, ○; no glucose with nitrogen, □.

cally it amounted to 2.7 μmoles/lens/hr. The aerobic rate of lactate production was not significantly affected when the percentage of oxygen in the O₂/CO₂ mixture was increased from 7 to 95%. When glucose was omitted from the medium, the lactic acid production decreased to 0.8μmoles/lens/hr in the presence of oxygen or nitrogen. The appearance of lactate in the incubation medium was also measured in lenses maintained at 4°C to completely suppress the synthesis of lactate but only reduce its diffusional efflux. In the cold, the lactic acid that was produced amounted to nearly 1 μmole/lens at the end of the 3-hr incubation, most of which was released during the first 30 min of incubation.

Table 1 shows the rates of aerobic and anaerobic lactate production from monolayers of cultured epithelial cells. These rates were linear over the entire 2-hr incubation period. The anaerobic rate was 2.0-fold greater than the aerobic rate. Thus, a consider-

able Pasteur response was seen in these cultured cells. Iodoacetate virtually abolished lactic acid production in the cultured cells.

Discussion

The measurements of ATP content in freshly excised tissues in this study clearly show that a decapsulated lens comprised of only the lens fibers has essentially the same amount of ATP as found in the whole lens. In contrast, the lens epithelium contains approximately 1/100th of the amount of ATP measured in either the whole or decapsulated lens. It follows then, from this distribution, that from measurements of ATP in whole lenses, no estimates can be made of contributions of the epithelial cells to the maintenance of ATP in the whole organ. Moreover, the fact that the concentrations of ATP in the epithelial cells...
and the fibers are similar, ie, ATP and wet weight of epithelium are each about 1% of their values in whole lens, suggests, in contrast to previous conclusions, that the fibers are not supplied with ATP by the epithelium. Clearly, conclusions from such studies are only valid if the separation technique is reliable. The observation that negligible ATP was found in the epithelium after 1–3 hr in nitrogen without glucose, whereas the fibers from the same lenses contained a third or more of their initial concentration, shows that few fibers could have remained adherent to the capsule-epithelium preparation. This interpretation is strongly supported by the whole mount preparations of the capsule-epithelium that consistently showed negligible fiber contamination and virtually no loss of epithelial cells.

The data in this study show that the epithelium and fibers maintain their ATP content at normal levels aerobic ally and anaerobically, provided that glucose is present. However, when glucose is absent, neither the fibers nor the lens epithelium maintain their levels of ATP. The rate of decline of ATP is faster in the epithelium than the fibers and is probably due to a higher rate of turnover in the epithelium, although the data on rates of synthesis and utilization necessary to confirm this are unavailable. Nevertheless, the rate of decline in ATP content is virtually unaltered by the absence of oxygen in the fibers, but is markedly accelerated in the epithelial cells. The former pattern is consistent with the interpretation that oxidative phosphorylation has a negligible role in ATP synthesis in the lens fibers, and the latter is the expected result for cells in which mitochondrial activity contributes to energy production. These dissimilar aerobic and anaerobic patterns of ATP decline in the epithelium of the glucose-deprived lenses over the same time period that the declines in the fibers are virtually equal strongly support the suggestion that the ATP of the fibers and epithelial cells are in metabolically distinct compartments. This does not mean that energy production and use in the fibers is independent of the metabolic status of the epithelial cells, because these cells contribute significantly to the maintenance of ion and substrate concentrations within the lens.

The difficulty in assessing the relative contributions of the epithelium and fibers to ATP content in a whole lens is seen also in the case of glycolysis. The whole lens data show only a small Pasteur response (approximately 1.1-fold), whereas lactate production increases two-fold, ie, from 0.3–0.6 μmoles/hr, when the cultured rabbit epithelial cells (800,000, equivalent to the number in a 300 mg rabbit lens) are made anaerobic. A comparison of the rates of lactate production between the whole lens and the cultured cells suggests that the epithelial cells produce lactate aerobically at about 12% of the rate in a whole lens, and

| Table 1. Lactate production from cultured lens epithelial monolayers* (cell line N/N1003A) |
|-----------------|-----------------|-----------------|
| Condition       | Aerobic         | Anaerobic       |
| MEM             | 0.30 ± 0.02 (8) | 0.61 ± 0.03 (8) |
| MEM + 1.0 mM IAA| 0.02 (4)        | 0.02 (4)        |

IAA = iodoacetate; MEM = minimal essential medium.
* Values are expressed as μmoles/800,000 cells/hr; means ± SD. This is considered equivalent to the number of cells present in the epithelial cell layer of a rabbit lens in situ (Giblin et al). The number of experiments, ie, individual culture dishes, is indicated in parentheses. Incubations were for 2 hr. Respiration was inhibited with Antimycin A.
that their two-fold Pasteur response would account for a 12% increase (from 2.5 to 2.8 μmoles/hr) in a whole lens, close to the anaerobic rate that was seen (2.7 μmoles/hr). Although this estimate of epithelial cell contribution to whole lens glycolysis may thus seem reasonable, it is still imprecise because the 800,000 cells contain about three times more protein than the natural epithelium and may, therefore, yield values for lactate that are too high. Thus, even on the basis of this upper limit for epithelial glycolysis, it is not surprising that there is only a slight increase in lactic acid production in response to anaerobic conditions when the whole lens was studied. Our results differ from earlier whole lens data on aged rabbit and bovine lenses where the Pasteur response was approximately 1.5-fold. One may calculate from these data, because such response can only be elicited in the mitochondria-containing epithelial cells, that these cells either show the same 1.5-fold response and contribute 100% of the lactate produced by the whole lens, or contribute a smaller fraction of total lactate but have an inversely greater Pasteur response (a 10% contribution requires a six-fold response and 5% an 11-fold response). Neither of these possibilities is supported by the present measurements.

Because ATP in the epithelial cells is maintained anaerobically by their two-fold Pasteur response, then under aerobic conditions, respiration must provide about 50% of the net ATP yield in these cells. Although this estimate may appear to be similar to the widely quoted value of 33% that was calculated from the Pasteur response of whole lens by Trayhurn and van Heyningen, their value refers to the aerobically derived fraction of ATP of the entire organ, whereas our conclusion regarding the contribution of aerobic metabolism to energy production applies solely to that of the epithelial cells and not the fibers.

**Key words:** rabbit lens, lens epithelial cells, lens fibers, glucose, oxygen, adenosine triphosphate, lactic acid production, Pasteur response

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