Corneal amino acid supply and distribution

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The distribution of nonmetabolized carbon \(^{14}\)-labelled \(\alpha\)-aminoisobutyric acid (\(\alpha\)-AIB) in the anterior segment of the rabbit eye has been determined in vivo. In addition to confirming earlier observations of the active accumulation of the molecule by the lens, the distribution in the cornea was found to favor the hypothesis of passive diffusion through the corneal endothelial layer and active uptake by the epithelium. The passive role of the endothelium was shown also by measuring the flux of \(\alpha\)-AIB across the anterior stromal surface. This flux was substantially greater in the absence of endothelium. The active accumulation of \(\alpha\)-AIB by the corneal epithelium occurred only when the molecule was present on the stromal side. A significant barrier to diffusion as well as no evidence of active uptake was found when the molecule was present in the tears.

Key words: amino acid, \(\alpha\)-AIB, permeability, flux, stroma, epithelium, lens, aqueous.

Inadequate corneal epithelial nutrition has been shown to be an important element in the loss of integrity of corneal layers anterior to intralamellar membranes.\(^1\),\(^2\) Much experimental work has been devoted to assessing the relative roles of glucose and glycogen in energy supply to the epithelial cells as well as to determining availability of the glucose molecule at the anterior stromal surface.\(^3\)-\(^5\) Except in cases of anterior chamber blockade,\(^6\),\(^7\) or where there are marked changes in aqueous humor composition,\(^8\)-\(^11\) the glucose supply to the epithelial cells is more than adequate even under anaerobic conditions.\(^8\) It is possible, however, that defects in epithelial integrity under some circumstances might be related to other nutritional deficiencies. For example, there might be cases of inadequate protein synthesis as a result of insufficient amino acid availability.

The purpose of the work reported here is to estimate the amino acid supply available to the corneal epithelial layer by employing labelled \(\alpha\)-aminoisobutyric acid (\(\alpha\)-AIB) as a model, nonmetabolized amino acid. By quantitating the flux of \(\alpha\)-AIB across the cornea and determining its concentrations in various anterior segment tissues at steady-state equilibrium, the amount of amino acid available to the epithelium can be determined. By extrapolating from the data to the concentrations of amino acids normally found in the anterior cham-
ber\textsuperscript{12}-\textsuperscript{16} and epithelium,\textsuperscript{17} approximations of the amino acid supply to the corneal epithelium can be made and compared to estimates of amino acid requirements.\textsuperscript{15}

Materials and methods

\textit{Distribution and efflux of $\alpha$-AIB.} Immature albino rabbits weighing between 2 and 3 kilograms were injected parenterally with 5 to 10 millimoles of $\alpha$-AIB acid labelled with 25 to 30 $\mu$Ci of carbon\textsuperscript{14}-labelled $\alpha$-AIB. After 3 or 20 hours, anesthesia was induced with pentobarbital and maintained with ether inhalation. Aqueous humor, corneal epithelium, corneal stroma, lens, and a blood sample were taken, after which the animals were killed. $\alpha$-AIB levels in the aqueous humor and in aliquots of the injected material were counted directly by scintillation counting. Cornea, lens, and blood plasma were first extracted in 10 per cent trichloroacetic acid or 0.33 N perchloric acid, then aliquots were counted by scintillation counting. The ratio of labelled to unlabelled $\alpha$-AIB in the tissues was assumed to be equal to that in the injected material, so that a conversion to millimoles of $\alpha$-AIB per kilogram of tissue water could be made from the radioactivity assays.

\textit{Efflux of $\alpha$-AIB} was measured as described previously for glucose flux\textsuperscript{3,4} by measuring the appearance of $^{14}$C-$\alpha$-AIB into 0.5 ml of artificial tears present in an 11 mm diameter polycarbonate cylinder applied to the anterior surface of the cornea with cyanoacrylate glue. The tear solution was changed at 15 minute intervals. In all cases the epithelium was thoroughly removed with a No. 15 blade before the cylinder was applied. In some cases, aqueous humor was replaced with air immediately before the experiment. In others the endothelium had been scraped one day prior to the experiment. $^{14}$C-$\alpha$-AIB levels were measured by scintillation counting of the tear solutions, cornea, aqueous humor, and blood samples. Again, a constant ratio of labelled to unlabelled $\alpha$-AIB was assumed to convert the assay to molar concentrations of $\alpha$-AIB.

\textit{Penetration of $\alpha$-AIB.} After anesthesia of the rabbits, penetration of $\alpha$-AIB into the cornea and aqueous humor from the anterior surface was measured by applying the polycarbonate tube filled with artificial tear solution containing 1.4 $\mu$M $\alpha$-AIB with 1 $\mu$Ci $^{14}$C-$\alpha$-AIB per milliliter. In some cases the epithelium was first scraped off. After 20 minutes, 1 hour, and 3 hours, aqueous humor, corneal epithelium, and corneal stroma were taken, prepared and counted in a scintillation counter as described above, and the counts related to amounts of $\alpha$-AIB present.

Results

Reference to Fig. 1 shows the relative concentration of $\alpha$-AIB in various tissues of the anterior segment 3 and 20 hours following injection of labelled $\alpha$-AIB. For convenience, the plasma level has been indicated on the iris, but iris tissue itself was not analyzed. After 3 hours, using the student $t$ test, the $\alpha$-AIB levels are not significantly different in any of the tissues measured.

At 20 hours there is a significant difference between epithelial and stromal levels of $\alpha$-AIB ($p < 0.001$). In addition, the lens at 20 hours maintains a concentration of $\alpha$-AIB equal to that found at 3 hours, while the aqueous level falls substantially.

The quantity of amino acid available at the anterior stromal surface, as measured by the efflux experiments, is shown in Fig.
2. The \( \alpha \)-AIB flux to the anterior surface, measured in the absence of epithelium, averages about 300 nM per square centimeter per hour, after steady state is achieved. When anterior chamber blockade is produced by the introduction of air, the flux is reduced to one third its previous value, indicating that the source of most of the amino acid at the anterior stromal surface is the aqueous humor. Scraping of the endothelium, however, causes at least a fivefold increase in the amount of \( \alpha \)-AIB crossing the anterior stromal surface. While this difference appears considerably greater

in the figure, the aqueous humor concentration of \( \alpha \)-AIB in eyes having had the endothelium scraped is 1.6 times higher than that of those in which the endothelium was undamaged.

The accumulation of \( \alpha \)-AIB when it is present at the epithelial surface alone is shown in Fig. 3. When the epithelium is present, the stromal water is less than 10 per cent equilibrated with the \( \alpha \)-AIB in the tears after 3 hours. Without the epithelium, there is prompt entry of amino acid into the stromal water. The penetration of labelled molecule into the aqueous (not
Table I. Epithelial uptake of α-AIB from adjacent layer

<table>
<thead>
<tr>
<th>Time</th>
<th>E/S</th>
<th>E/T</th>
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</thead>
<tbody>
<tr>
<td>20 (min.)</td>
<td>—</td>
<td>0.066 ± 0.01 (4)</td>
</tr>
<tr>
<td>60 (min.)</td>
<td>—</td>
<td>0.41 ± 0.04 (4)</td>
</tr>
<tr>
<td>3 (hrs.)</td>
<td>1.56 ± 0.14</td>
<td>0.52 ± 0.08 (6)</td>
</tr>
<tr>
<td>20 (hrs.)</td>
<td>3.35 ± 0.43 (9)</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as a ratio of α-AIB in epithelial water (E) compared to that in the adjacent fluid (S = stroma; T = tears). The stromal and tear solution concentrations of α-AIB were the same. The values are expressed as averages plus or minus the standard errors of the mean. The number of determinations is shown in parentheses.

Table I shows the uptake of α-AIB by the epithelium from the stromal water as well as from the tears. The 3 hour value represents a direct comparison between uptake by the epithelium from its front vs. its back surface, and indicates that 3 times more amino acid is taken up by the epithelial layer when the molecule approaches its posterior surface.

Discussion

The distribution of α-AIB in the anterior segment of the rabbit eye in vivo confirms data obtained by Reddy and Kinsey with respect to uptake by the lens. While there is no concentration of α-AIB in lens as compared to aqueous humor after 3 hours in the present experiments, the lens to aqueous ratio (L/A) is 7 after 20 hours. This compares to a lens/medium (L/M) ratio of 10 obtained in vitro at 24 hours by those workers when only tracer quantities of radioactive α-AIB were used, and a L/M ratio of 6.5 found when a total concentration of 1.7 mM per liter (equal to the aqueous humor concentration in these experiments) of α-AIB surrounds the lens.

As reference to Fig. 1 shows, the corneal stroma contained α-AIB at about the same concentration as does the aqueous. As the aqueous humor concentration of α-AIB falls over the equilibration period of 20 hours, the stromal concentration decreases to the same extent. The data do not indicate a significant endothelial permeability barrier to α-AIB at the levels of flux required for this slow change in concentration, as opposed to the apparent barrier due to the endothelium at higher rates of flux.

Concentration of α-AIB by the corneal epithelium after 20 hours of equilibration is also demonstrated by the distribution data. It could be argued that the concentration is really the result of a lag of diffusion of the molecule out of the epithelial cells, after initial loading. No such lag of diffusion into the cells at the 3 hour period is evident, however. This ability of epithelial cells to concentrate amino acid from the surrounding fluid would tend to ensure adequate supplies of amino acid to the cells under even marginal conditions of supply.

While there is no evidence to support impermeability of cells to α-AIB from the stroma the resistances to permeability from the surface of the epithelium is significant. By comparing the relative uptake of α-AIB when the molecule was present in the tear solution rather than the stromal water, as in Table I, E/S = 1.56, E/T = 0.52, the concentration of α-AIB by epithelial cells from the tear surface is substantially less than the concentration seen when the uptake occurs from the stromal water.

As in the case of glucose, the primary source of amino acids to the corneal epithelium is aqueous humor. By blocking the access of aqueous humor to the cornea with a gas bubble, the flux of α-AIB across the anterior stromal surface falls to 30 per cent of normal. This amount presumably diffuses into the cornea from the limbal region.

The quantity of amino acid required by the normal turnover of epithelial cells in the rabbit has been estimated by Maurice and Riley on the basis of a seven day replacement time. Depending upon the specific amino acid, permeability coefficients of 0.005 cm. per hour (alanine and proline) to 0.06 cm. per hour (aspartate) were calculated. The permeability coeffi-
cient for α-AIB in these experiments is 0.23 cm. per hour, determined in the same way by dividing the rate of loss of α-AIB from the anterior stromal surface, in micromoles per square centimeter per hour, by the aqueous humor concentration, in micromoles per cubic centimeter. This permeability represents eight times that estimated by Maurice and Riley18 for amino acids through the endothelium. One possible source of error in our determination of permeability is the contribution of the limbal supply to the flux. In addition, the aqueous humor concentration of α-AIB is 1.3 μM per milliliter, several times the concentration of all the amino acids except glycine, which is equal to the α-AIB level and had a permeability coefficient of 0.006 cm. per hour. Whether or not the same permeability would be found at lower levels of α-AIB has not been determined. The estimates of Maurice and Riley18 represent minimal permeabilities required for amino acid supply, whereas our figure obtained for α-AIB is a maximal permeability, representing an upper limit on amino acid availability to the corneal epithelium. It would appear that the availability of amino acid to the anterior stromal surface is at least 10 times greater than the amount required for normal turnover of epithelial cells.

REFERENCES