Cholic acid accumulation by the ciliary body and by the iris of the primate eye

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Cholic acid accumulates in both the ciliary body and the iris of the primate eye during in vitro incubations at 37°C for 1 hr. Incubation at 0°C depresses uptake in both tissues. The washout of preaccumulated cholic acid occurs some 3.4 times faster from the iris than from the ciliary body. The mechanism of cholic acid accumulation in both tissues is less sensitive to inhibition by high iodipamide concentrations and also is less sensitive to inhibition by high hippurate concentrations than the mechanism of p-aminohippurate (PAH) accumulation. Therefore, although overlap may exist, the cholic acid–uptake mechanism differs from the PAH-uptake mechanism in both the primate ciliary body and the primate iris.

Key words: cholic acid, bile acids, p-aminohippuric acid, transport, washout, blood-aqueous barrier, aqueous humor, ciliary body, iris, inhibition, rhesus monkey

The existence of a transport system in the primate iris that accumulates p-aminohippuric acid (PAH) is described in the accompanying paper, and this system parallels that present in the primate ciliary body.1 The present study explores the question of whether or not other transport systems, present in the ciliary body, also exist in the primate iris.

Bárány has described a liverlike transport mechanism in the anterior uvea, using iodipamide as the transport substrate.2 A wide variety of anions,3,4 including the bile acids,5 can inhibit this liverlike mechanism. Most of these inhibitors preferentially affect the hippurate-uptake system,6 analogous to the PAH system mentioned above, but the bile acids are one class of inhibitors with greater affinity for the iodipamide-uptake system.5

Indeed, the rabbit anterior uvea also accumulates bile acids.6 Iodipamide only partly inhibits the uptake of bile acids by anterior uvea, but it can completely inhibit o-iodohippurate accumulation. However, the iodipamide-sensitive component of bile acid accumulation is more sensitive to iodipamide inhibition than is o-iodohippurate accumulation. On the basis of a mathematical analysis of inhibition studies of the accumulation of iodipamide, o-iodohippurate, and two bile acids, Bárány has postulated four overlapping iodipamide-sensitive transport systems in the rabbit: the hippurate system, a liverlike system moderately resistant to hippurate inhibition (L1), a liverlike system very resistant to hippurate inhibition (L2), and a system more equally inhibited by hippurate and iodipamide than the others. The L2 system transports iodipamide but little of the bile acids.

Most of this work has been performed in rabbit uvea, although a few preliminary experiments have confirmed that iodipamide also accumulates in primate ciliary bodies.5

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WASHOUT TIME (min)

Fig. 1. Washout of cholic acid from ciliary body and from iris. Each point represents the mean ± S.E. from four half-ciliary body and four half-iris specimens studied at 37° C with a washout reservoir of plain Tyrode's solution. Inset, Semilog plots of the same data, as described in the text. Error bars not shown are hidden by the symbol. The values for kw represent the mean ± S.E. from a total of 12 such specimens studied under the same conditions except for differences in the time of reservoir sampling.

Because the rabbit ciliary processes extend onto the posterior iris surface, independent study of the iris is not possible in this species. In the primate eye, the iris is anatomically distinct and easily separable from the ciliary body. 3H-cholic acid was employed in the present study to examine further the nature of liverlike transport in the primate ciliary body and to define further the role of the iris in anion transport.

Methods

The source of rhesus monkey eyes and the dissection techniques were identical to those previously described.1 In general, the ciliary body and iris were halved or quartered, with one segment serving as the control for other segments from the same eye.

After dissection, the tissue was incubated in 4 ml of Tyrode's solution buffered to pH 7.4 with 95% O2 and 5% CO2. The incubation medium contained 3H-cholic acid (14 Ci/mmol; New England Nuclear, Boston, Mass.) and enough unlabeled sodium cholate (Sigma Chemical Co., St. Louis, Mo.) so that the two radiolabeled compounds had similar specific activity. The tissue was incubated with gentle shaking in a water bath at 37° C for 1 hr.

At the conclusion of the incubation, tissue-medium (T/M) ratios were determined as previously described.1 The washed pellet from the centrifugation contained less than 5% of the 3H counts for both the ciliary body and the iris specimens. All specimens were counted in Aquasol scintillation counting solution (New England Nuclear) on a Beckman LS-355 scintillation counter. All specimens were tested with an external-standard channels ratio, and a quenching correction proved to be unnecessary.

In the zero degree incubations, the vials were placed in an insulated ice bucket attached to the moving platform in the water bath. The incubation vials were allowed to equilibrate to the temperature of the ice bath prior to insertion of the tissues. Both the vials at zero degrees and the control vials at 5°C contained 3.5 x 10^-6M 3H-cholic acid.

For the washout studies, half-ciliary body and disinserted half-iris specimens were preincubated at 37°C with 10^-4M 3H-cholic acid. At 10^-4M, bile acids are not toxic to the uptake mechanisms under study.5 At the conclusion of the preincubation, each tissue segment was quickly washed in plain Tyrode's solution and then placed in its own 10 cc reservoir of plain Tyrode's solution, pH 7.4, in a constant temperature shaking water bath, generally at 37°C. Small aliquots from the washout reservoir were sampled and counted at regular time intervals. At the conclusion of each washout study, the tissue was blotted, weighed, ground, spun, and counted as outlined above. From the residual tissue counts and from the washout data, the total uptake at the conclusion of the washout was calculated. The percent of preaccumulated cholic acid remaining in the tissue as a function of washout time was calculated. Separate washout experiments were conducted at 0° C, 10^-4M sodium cholate and with 10^-4M 2,4-dinitrophenol (Fisher Scientific Co., Fairlawn, N. J.) in the washout reservoir. In all cases, one half of the ciliary body or iris was incubated under the experimental conditions, and the other half of the tissue from the same eye was incubated as a control in a washout reservoir of Tyrode's solution at 37°C with no added chemicals.

In studies testing the simultaneous uptake of 3H-cholic acid and 14C-PAH, the concentration of both transport substrates was 5 x 10^-6M. One quarter of the ciliary body and iris was incubated as an uninhibited control; each of the other three quarters was incubated in the presence of a different concentration of an inhibitor, either sodium hippurate (Sigma) or sodium iodipamide (prepared with iodipamide acid from Mr. S. J. Lucania, The
Squibb Institute, Princeton, N. J.). After 1 hr incubations, T/M ratios were determined for each radiolabeled anion and for each tissue. According to the method of Bárany,6 "active uptake" was defined here as T/M ratio at 37° C minus T/M ratio at 0° C. Neither hippurate nor iodipamide affect the binding of bile acids at 0° C in rabbit anterior uvea,6 and it was assumed that a similar situation applies in the monkey eye. Therefore the nonactive uptake for each tissue was estimated as the T/M ratio for 3H-cholic acid or for 14C-PAH after 1 hr incubation at 0° C in the absence of inhibitor, as determined in separate experiments. Thus the expression $100 \times (T/M$ inhibited $- T/M$ zero degree$)/(T/M$ uninhibited $- T/M$ zero degree) with the appropriate T/M values for each anion and for each tissue was used to give the percent active uptake in the presence of inhibitor.$^6$ In the present experiments, each inhibited value was compared to the control from the same eye, and then the data were averaged.

**Results**

**Accumulation.** The ciliary body and iris specimens were divided in half. One half was incubated at 37° C, and other half at 0° C. After a 1 hr incubation at 37° C, the ciliary body halves achieved a T/M ratio of 7.03 ± 0.54, and the iris halves achieved a T/M ratio of 1.77 ± 0.16. After a 1 hr incubation at 0° C, the ciliary body halves accumulated cholic acid only to a T/M ratio of 0.64 ± 0.04, and the iris halves accumulated cholic acid only to a T/M ratio of 0.91 ± 0.06 (mean ± S.E. from four eyes).

**Washout.** Fig. 1 shows a typical washout study. As in the washout of PAH,$^1$ these curves were exponential in form. These studies were designed so that the cholic acid concentration in the washout reservoir was small compared to the tissue concentration in the initial portions of the washout and generally throughout the entire washout study. With PAH washout, it thus appears that following first-order rate equation applies to the washout kinetics:

$$\frac{T'}{T^0} = e^{-k_w t}$$

where $T'$ = tissue concentration of cholic acid at time $t$, $T^0$ = initial tissue cholic acid concentration, and $k_w$ = a washout constant. The initial portions of the semilog plots, In

**Table I. Effects on $k_w$ of altering washout conditions**

<table>
<thead>
<tr>
<th>Washout conditions</th>
<th>Ciliary body</th>
<th>Iris</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-4M cholate</td>
<td>104 ± 10</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>0°C</td>
<td>41 ± 3</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>15^-4M DNP</td>
<td>134 ± 17</td>
<td>100 ± 3</td>
</tr>
</tbody>
</table>

DNP = dinitrophenol.

*Each value represents the mean ± S.E. from four eyes.

$(T'/T^0)$ vs. $t$, are linear, but both plots deviate from linearity as washout progresses. As in the case of PAH washout,$^1$ tissue binding, reaccumulation, and postmortem tissue changes may be contributing to these findings, but the present study does not allow characterization of these effects during the latter part of the washout studies. As discussed below, reuptake of cholic acid during the initial washout period seems to occur in the ciliary body only. The slopes of the initial linear portion of the semilog plots, where postmortem changes are least, give values for $-k_w$. It is assumed that these values reasonably approximate the true washout rate for both tissues. Fig. 1 shows the mean values for $k_w$. Cholic acid washout from the iris thus occurred some 3.4 times faster than cholic acid washout from the ciliary body.

To further explore the washout phenomenon, washout was studied in the presence of 10^-4M cholic acid, at 0° C, and in the presence of 10^-5M dinitrophenol. This dinitrophenol concentration is the concentration that inhibits PAH uptake approximately 50% in both ciliary body and iris.$^1$ Table I shows the effects of these washout conditions on $k_w$.

**Iodipamide inhibition.** Fig. 2 illustrates the effects of increasing iodipamide concentration on the simultaneous uptake of 3H-cholic acid and 14C-PAH in both the ciliary body and the iris. As in the rabbit uvea,$^6$ a fraction of cholic acid uptake was insensitive to inhibition by high iodipamide concentrations. This iodipamide-insensitive uptake was about 20% for the primate ciliary body and about 32% for the primate iris. In the rabbit uvea, iodipamide fully depresses o-iodohippurate uptake.$^6$ Unlike the rabbit, however, in the primate tissue a small frac-
Fig. 2. Effect of iodipamide on the simultaneous uptake of $^3$H-cholic acid and $^{14}$C-PAH in the ciliary body and in the iris. The data are corrected to show active uptake, as defined in the text. Each point represents the mean ± S.E. for tissues from four eyes incubated at 37° C for 1 hr.

Fig. 3. Inhibition by iodipamide of iodipamide-sensitive uptake in the ciliary body and in the iris. See text for explanation.

tion of PAH uptake persisted in the presence of high iodipamide concentration, 8% for the ciliary body and 17% for the iris.

Data on iodipamide-sensitive uptake was derived from the experiments shown in Fig. 2 in a manner similar to Bárany’s technique, except that a correction for the small iodipamide-insensitive component of PAH uptake was also included. The percent uptake for each anion and for each tissue that occurred at 3000 $\mu$M iodipamide was taken as an estimate of the iodipamide-insensitive uptake for each anion and each tissue. For those eyes in which one quarter of the tissue was incubated with 3000 $\mu$M iodipamide, the data from the other three quarters were corrected with the iodipamide-insensitive uptake from the same eye. For those eyes in which no incubation vial contained 3000 $\mu$M iodipamide, the appropriate mean value of the percent uptake at 3000 $\mu$M iodipamide was used. For all values, the data were corrected first and then averaged. Fig. 3 shows the results of these corrections and indicates the inhibition by iodipamide of the iodipamide-sensitive uptake of both anions in both tissues.

**Hippurate inhibition.** Fig. 4 illustrates the effects of increasing hippurate concentration on the simultaneous uptake of $^3$H-cholic acid and $^{14}$C-PAH in both primate tissues. As in
Fig. 4. Effect of hippurate on the simultaneous uptake of $^3$H-cholic acid and $^{14}$C-PAH in the ciliary body and in the iris. The data were corrected to show active uptake, as defined in the text. Each point represents the mean for tissues from four eyes incubated at 37° C for 1 hr. Error bars show ±S.E. unless hidden by the symbol.

Fig. 5. Inhibition by hippurate of hippurate-sensitive uptake in the ciliary body and in the iris. See text for explanation.

the rabbit, a fraction of cholic acid uptake was also insensitive to inhibition by high hippurate concentrations. The hippurate-insensitive cholic acid uptake was about 31% of control uptake for the ciliary body and about 50% of control uptake for the iris. At 10,000 µM hippurate, one of the values for cholic acid accumulation in the iris was excessively high. This value, however, was included in the analysis, since no obvious explanation for it was present. A significantly smaller fraction of PAH uptake occurred at high hippurate concentrations, 6% for the ciliary body and 21% for the iris.

A correction was also made in the hippurate data to evaluate hippurate-sensitive uptake. The percent uptake at 10,000 µM hippurate was used as the insensitive uptake for cholic acid uptake in the ciliary body and for PAH uptake in both tissues. Because of the extraneous value for cholic acid uptake in the iris at 10,000 µM hippurate, the uptake at 3000 µM hippurate was used to estimate hippurate-insensitive uptake for cholic acid.
accumulation in the iris only. The uptake data from the experiments shown in Fig. 4 were then corrected by subtracting the appropriate hippurate-insensitive uptake from the percent uptake values for each anion and each tissue. Wherever possible, each data point was corrected with the value for insensitive uptake from the same eye. If the experiments on the quartered uveal tissue of a particular eye did not happen to include an incubation at the hippurate concentration used to estimate insensitive uptake, the data were corrected with mean values for insensitive uptake from the eyes studied at these concentrations. In all cases, the data were corrected first and then averaged. Fig. 5 contains the results of these corrections. It shows the inhibition by hippurate of the hippurate-sensitive uptake of both anions in both tissues.

Discussion
The present study demonstrates that cholic acid accumulates at 37°C in both the ciliary body and the iris of the primate eye and that incubation at 0°C significantly depresses uptake in both tissues. The T/M ratios of the ciliary body for cholic acid are higher than those reported for iodipamide in the rhesus monkey. The higher ratios for cholic acid may in part relate to the presence of an iodipamide-insensitive uptake mechanism for bile acids that presumably carries no iodipamide and may in part relate to the use of fresher tissue in the present study. No previous studies of liverlike transport in the iris exist.

The washout of cholic acid is slightly slower from both tissues than the washout of PAH. As in PAH washout, however, iris washout of cholic acid occurs more rapidly than ciliary body washout. In fact, the ratio of iris $k_w$ to ciliary body $k_w$ for cholic acid is 3.4, greater than the value of 2.5 found for PAH washout. Since the studies reported here represent relatively long 1 hr incubations and since the T/M ratios indicate net accumulation, the existence of such rapid iris washout for cholic acid implies that the activity of the iris uptake mechanism in relation to the ciliary body uptake mechanism is greater than indicated by the relatively low iris T/M ratios.

The difference in the washout rates probably arises from anatomical differences between the two tissues—the compact ciliary body stroma and its highly convoluted epithelium in contrast to the loose iris stroma and the smoother iris epithelium. Such differences between the two tissues make meaningful kinetic comparisons difficult. The experimental and theoretical difficulties in the use of these primate tissues for kinetic studies have been discussed elsewhere.

The mechanism of cholic acid washout is similar to that of PAH washout. The failure of 100 μM cholic acid in the washout medium to affect the ciliary body washout rate suggests that ciliary body washout is occurring by simple passive diffusion rather than by carrier-mediated mechanisms. Since the present study includes only one cholic acid concentration, however, the existence of a more complex mechanism as seen in the kidney system cannot be totally excluded. The small decrease in the iris washout rate with 100 μM cholic acid most probably reflects experimental variation. The depression of washout at 0°C in both tissues can result from a decrease in passive diffusion across cell membranes occurring at low temperatures and does not necessarily imply the depression of an active component of washout. Dinitrophenol depresses the cholic acid washout from the ciliary body, a finding consistent with the suppression of a reaccumulation mechanism. No dinitrophenol effect is observed in the iris, perhaps because the cholic acid molecules are removed from the uptake site before reaccumulation can occur.

As with PAH washout, then, the washout of cholic acid probably occurs by a passive diffusional mechanism in both tissues. A small reuptake process also appears to occur in the ciliary body. However, a more complex process cannot be excluded on the basis of present data, and further studies to characterize the washout process are necessary.

In other tissues, the kidneylike and liverlike transport systems have overlapping spe-
cificities.\textsuperscript{2} Does cholic acid accumulate by the same mechanism by which PAH accumulates in the ciliary body and iris, or are there differences in the mechanisms by which the two anions accumulate? The studies of the simultaneous uptake of \textsuperscript{3}H-cholic acid and \textsuperscript{14}C-PAH represent an approach to answer this question.\textsuperscript{10}

Fig. 2 shows that high concentrations of iodipamide inhibit the uptake of both cholic acid and PAH in both tissues but that PAH uptake is more effectively inhibited. These findings are similar to Bárány's findings in the rabbit uvea,\textsuperscript{6} in which iodipamide can fully depress iodohippurate uptake but leaves some 30\% of cholate uptake. Thus there exists an iodipamide-insensitive mechanism that accumulates cholic acid but handles little PAH in both the ciliary body and the iris of the primate eye.

Similarly, Fig. 4 shows that high hippurate concentrations effectively depress the uptake of PAH in both primate tissues but that some 30\% of control cholic acid uptake remains in the ciliary body and some 50\% of control cholic acid uptake remains in the iris under these conditions. Therefore there also exists a hippurate-insensitive mechanism that accumulates cholic acid in both tissues but carries little PAH. These findings are also similar to Bárány's findings in rabbit uvea,\textsuperscript{6} in which high hippurate concentrations fail to completely suppress cholate uptake.

Thus the primate ciliary body the the primate iris both contain a mechanism for the accumulation of cholic acid that, relative to the PAH uptake mechanism, is insensitive to inhibition by both iodipamide and hippurate. Therefore, although overlap may exist, the mechanisms by which cholic acid and PAH accumulate are different in both tissues. That is, both the ciliary body and the iris contain a liverlike mechanism for the accumulation of cholic acid. The relationship between the iodipamide-insensitive and the hippurate-insensitive cholic acid uptake cannot be determined from the present studies.

Figs. 3 and 5 represent attempts to use the present data to characterize further the liverlike mechanism in both tissues. Because of the small sample sizes and the data scatter at the low inhibitor concentrations, no detailed attempt to define the individual components of the liverlike system is made, and only tentative conclusions can be drawn about the characteristics of liverlike transport in primate uvea.

Fig. 3 shows that, for the ciliary body, the iodipamide-sensitive uptake of cholic acid is more sensitive to iodipamide inhibition than the iodipamide-sensitive uptake of PAH. These findings are similar to Bárány's findings concerning bile acid uptake in rabbit uvea\textsuperscript{6} and indicate the existence of an iodipamide-sensitive liverlike mechanism different from the PAH mechanism in the primate ciliary body. For the iris, however, iodipamide inhibition of sensitive cholic acid uptake parallels iodipamide inhibition of sensitive PAH uptake. Thus cholic acid appears to accumulate in the ciliary body by a mechanism somewhat different from the mechanism in the iris.

Fig. 5 shows that hippurate more effectively inhibits sensitive cholic acid uptake than sensitive PAH uptake, in both the ciliary body and the iris. This finding is opposite to the rabbit uvea in which hippurate more effectively inhibits iodohippurate uptake than cholic acid uptake.\textsuperscript{6} Cholic acid accumulation in the primate eye apparently has a different specificity from that in the rabbit eye.

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REFERENCES


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