The Influence of 12(R)-Hydroxyeicosatetraenoic Acid on Ciliary Epithelial Sodium, Potassium-Adenosine Triphosphatase Activity and Intraocular Pressure in the Rabbit

N. A. Delamere, R. R. Socci,* K. L. King, and P. Bhattacharjee

Ciliary epithelium was isolated from the rabbit eye and used as a source of plasma membrane material for sodium, potassium-adenosine triphosphatase (Na,K-ATPase) measurements. In the presence of 12(R)-hydroxyeicosatetraenoic acid [12(R)HETE], Na,K-ATPase (ouabain-sensitive ATPase) activity was reduced from 22.5 to 16.3 µM phosphate released/mg protein/hr. Ouabain-insensitive ATPase activity was not altered by 12(R)HETE. No changes in ciliary epithelium ATPase activity were observed in the presence of 12(S)HETE. In parallel studies with conscious rabbits, 12(R)HETE applied topically to the eye was found to lower intraocular pressure (IOP). It is possible that the IOP-lowering effect of 12(R)HETE may be, in part, associated with its ability to suppress the Na,K-ATPase activity of the ciliary epithelium. Invest Ophthalmol Vis Sci 32:2511–2514, 1991

12(R)hydroxy-5,8,10,14-eicosatetraenoic acid (12-(R)HETE) is a metabolic product of arachidonic acid.1,2 It has been shown that 12(R)HETE is capable of reducing sodium, potassium-adenosine triphosphatase (Na,K-ATPase) activity in membrane material obtained from the cornea and other tissues.3,4 In contrast, the stereoisomer 12(S)HETE does not alter Na,K-ATPase activity.

In a recent study, intraocular pressure (IOP) was reduced after application of 12(R)HETE to the rabbit eye.5 Because Na,K-ATPase in the ciliary epithelium is believed to be a key component of the mechanism of aqueous humor production,6,7 it was speculated that the ocular hypotensive effect of 12-(R)HETE might be related to the inhibition of Na,K-ATPase in the ciliary epithelium.5 We decided to test this notion by measuring Na,K-ATPase activity in a membrane preparation obtained exclusively from rabbit ciliary epithelium using a newly developed dissection technique.

Materials and Methods

Animals

Adult New Zealand white rabbits weighing 2–3 kg were used in these studies. The tissues were obtained from these animals immediately after they were killed with an overdose of sodium pentobarbital administered through a marginal ear vein. The procedures used in these studies conformed to the ARVO Resolution on the Use of Animals in Research.

Chemicals

The 12(R)HETE and 12(S)HETE (Cayman, Ann Arbor, MI) were obtained as an ethanol stock solution that was added to the ATPase buffer solution as necessary. Control solutions contained equal amounts of ethanol. The adenosine triphosphate (ATP), as a triethylammonium salt labeled with 32P in the terminal phosphate group, was purchased from Amersham (Arlington Heights, IL). All other chemicals were purchased from Fisher (Pittsburgh, PA) or Sigma (St. Louis, MO).

Measurement of IOP

The 12(R)HETE and 12(S)HETE were obtained as an ethanol stock solution that was added to the ATPase buffer solution as necessary. Control solutions contained equal amounts of ethanol. The adenosine triphosphate (ATP), as a triethylammonium salt labeled with 32P in the terminal phosphate group, was purchased from Amersham (Arlington Heights, IL). All other chemicals were purchased from Fisher (Pittsburgh, PA) or Sigma (St. Louis, MO).

Measurement of IOP

The IOP was measured in conscious rabbits using a Digilab applanation tonometer (Alcon, Fort Worth, TX). Before measuring the IOP, the animals were re-
strained gently, and their corneas were anesthetized with two drops of 0.5% proparacaine (Alcaine; Alcon). Measurements of IOP were made at several intervals for 1 day before the experiment to ensure that the rabbits were accustomed to the procedure and that the IOP was stable. The IOP of each rabbit was measured before and after topical administration of the 12(R)HETE or 12(S)HETE. One eye of each animal received a single 25-μl drop of the HETE at a specified concentration in saline. The contralateral (control) eye received a single drop of the vehicle. The ethanol concentration of both experimental and control solutions was <1%. The control solutions caused no detectable changes in IOP.

Ciliary Epithelium Membrane Preparation

Immediately after death, the cornea was removed and discarded. The iris, ciliary body, and lens were removed in one piece and transferred to a dish of ice-cold Kreb's solution containing 110 mM NaCl, 6 mM KCl, 1.1 mM KH2PO4, 2.5 mM CaCl2, 1 mM MgCl2, 5 mM glucose, and 25 mM NaHCO3 at pH 7.4. The solution was equilibrated with 95% O2/5% CO2. A radial cut was made through the iris from the pupil to the periphery. The iris then was peeled gently away, leaving the ciliary epithelium attached to the lens zonules. Morphologic examination of the tissue revealed that the epithelium was free from contamination by ciliary process stroma or capillaries. The two layers of epithelium remained intact and joined together but in some regions, the basolateral membrane of the pigmented cells appeared damaged. The lens zonules were cut to free the ciliary epithelium. This was transferred immediately to an ice-cold douncer and homogenized in 0.5 ml of a solution containing 300 mM KC1, 3 mM MgCl2, 100 mM NaCl, 11 μg/ml amiloride in pH 7.4, and a calcium-EGTA buffer to give a free calcium concentration of 10-8 M. Immediately before the assay, 12(R)HETE or 12(S)HETE were added to the buffer as a concentrated stock solution in ethanol. Controls received ethanol alone. In each experiment, assays were done in the presence and absence of 1 mM ouabain.

Tubes containing 350 μl of Buffer B and 1 μg of membrane protein were warmed to 37°C for 5 min before the ATPase reaction was initiated by adding 32P-labeled ATP to a final concentration of 1 mM. After 20 min, the reaction was stopped with ice-cold 10% trichloroacetic acid. In separate experiments, we determined that ATP hydrolysis is linear over this period. The amount of ATP hydrolyzed during the 20-min incubation was determined by counting the radiolabeled inorganic phosphate released. The method used to measure phosphate release was described in detail by others.9 The Na,K-ATPase activity was determined as the difference between ATPase activity in the presence and absence of ouabain; ATPase activities are expressed as micromoles of phosphate released per milligram of protein per hour.

Results

The Influence of 12(R)HETE on IOP

The 12(R)HETE was applied topically to one eye of each rabbit. After doses of 1 and 2 μg/eye, a modest but significant reduction of IOP was observed in the treated eye (Table 1). The largest changes of IOP (3–4 mm Hg) were seen at 30 and 60 min after treatment; the IOP returned to the control value by 4 hr post-treatment (Fig. 1). The contralateral eye, which received saline vehicle, did not show a change in IOP.

In separate experiments, the stereoisomer, 12-(S)HETE, was applied topically to the eye. No signifi-

<table>
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<tr>
<th>12(R)HETE (μg/eye)</th>
<th>Initial</th>
<th>60 min after treatment</th>
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<tbody>
<tr>
<td>0</td>
<td>23.0 ± 0.45(19)</td>
<td>23.1 ± 0.53(19)</td>
</tr>
<tr>
<td>0.5</td>
<td>23.0 ± 0.53(5)</td>
<td>20.8 ± 2.03(5)</td>
</tr>
<tr>
<td>1.0</td>
<td>24.4 ± 0.90(5)</td>
<td>19.7 ± 1.13(7)*</td>
</tr>
<tr>
<td>2.0</td>
<td>22.4 ± 0.37(7)</td>
<td>19.9 ± 0.77(7)*</td>
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Data are mean ± SE. Values in parentheses are the number of rabbits tested.

* Significantly different from intraocular pressure measured at the same time in the contralateral eye (P < 0.05)

Table 1. The influence of 12(R)HETE on intraocular pressure
Effect of 12(R)HETE on intraocular pressure.

Fig. 1. The influence of 12(R)HETE on intraocular pressure (IOP) in the rabbit. The decrease of IOP (control eye — treated eye) is given in units of mm Hg. The data are presented as the mean of measurements from at least five rabbits. The standard error is indicated by a vertical bar. The dosages of 12(R)HETE received by the rabbits were 0.5 μg/eye (open bar), 1.0 μg/eye (hatched bar), or 2.0 μg/eye (filled bar).

Significant changes of IOP were observed in response to 12(S)HETE at doses up to 2 μg/eye.

**Na,K-ATPase Measurements**

The ATPase activity was measured in membrane preparations obtained from ciliary epithelium dissected from control, untreated rabbits. This activity was measured under four conditions: (1) control, (2) in the presence of 10⁻³ M ouabain, (3) in the presence of 10⁻⁶ M 12(R)HETE, and (4) in the presence of 10⁻⁶ M 12(R)HETE and 10⁻³ M ouabain. The data obtained are shown in Table 2. In the absence of ouabain, 12(R)HETE significantly reduced ATPase activity. The activity measured in the presence of ouabain was unaltered by 12(R)HETE. The 12(R)HETE reduced the ouabain-inhibitable component of ATPase activity, Na,K-ATPase activity, by approximately 30%. At concentrations less than 10⁻⁶ M, statistically significant changes of ATPase activity could not be detected with 12(R)HETE. However, Na,K-ATPase inhibition of 15% or less would be undetectable due to the inherent variability of this assay.

The influence of 12(S)HETE on Na,K-ATPase activity also was examined; the compound was tested at concentrations of 10⁻⁸ to 10⁻⁶ M. The 12(S)HETE did not change ATPase activity. In the presence of 10⁻⁶ 12(S)HETE, Na,K-ATPase activity was 101.4 ± 22.0%, and ouabain-insensitive ATPase activity was 100.7 ± 17.1% of the control values, respectively (mean ± standard error).

**Discussion**

To test the hypothesis that 12(R)HETE might inhibit ciliary epithelial Na,K-ATPase, we developed a new tissue preparation. Pure samples of ciliary epithelium were dissected from the rabbit eye, and this mate-

<table>
<thead>
<tr>
<th>Table 2. The influence of 12(R)HETE on Na, K-ATPase activity*</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Total ATPase activity</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>10⁻⁴ M 12(R)HETE</td>
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</tbody>
</table>

*ATPase activities are presented as nmoles phosphate released/μg membrane protein/hr. The data are given as mean ± SE with the number of measurements in parentheses.

†Significantly different from control (P = 0.01).

††Significantly different from control (P = 0.001).
was much shorter in duration. In our studies, IOP is only one point on the pathway of aqueous humor flow through the eye; 12(R)HETE also might cause changes in outflow mechanisms or blood flow to the ciliary process. These changes could either antagonize or enhance any effect on IOP due to altered ciliary epithelium Na,K-ATPase activity.

Key words: Na,K-ATPase, intraocular pressure, 12(R)-HETE, ciliary epithelum

References


