Maturation of Rb⁺ and PAH Accumulation by Rabbit Anterior Uvea and Choroid Plexus

Theodore Krupin, Carol Fritz, and Bernard Becker

In vitro accumulation of radioactive para-aminobipyrinic acid (³H-PAH) and rubidium (⁸⁶Rb⁺) by the anterior uvea, ciliary processes, and the choroid plexus was evaluated in tissues from newborn and various aged rabbits. Accumulation of PAH was present in the anterior uvea at 1 day of age (tissue to media ratio, T/M, of 2.1 ± 0.2) and remained at this level for the first 14 days of life. Accumulation did not rise to adult levels until 21 days of age (T/M 5.5 ± 0.6). Rubidium accumulation in the anterior uvea, a measure of Na⁺, K⁺-pump activity, was higher than adult values 6 hr after birth (T/M 25.2 ± 0.9). Activity remained elevated through day 28 and did not fall to adult levels until day 60 (T/M 13.4 ± 0.6). Accumulation studies on isolated ciliary processes were similar to those obtained from anterior uveal tissue. Daily subcutaneous injections of penicillin (300,000 units/kg/day) for 1 week had no effect on anterior uvea PAH accumulation (penicillin T/M was 1.7 ± 0.1 and saline control T/M was 2.0 ± 0.2). Accumulation of either ³H-PAH or ⁸⁶Rb⁺ by the choroid plexus was present 1 day after birth in amounts that were similar to adult values and did not change during the 90 days of testing. Invest Ophthalmol Vis Sci 26:159-162, 1985

Active production of aqueous humor in the rabbit eye probably begins after birth. Ascorbate, which is transported from the blood into the posterior chamber, is not increased in concentration in aqueous humor until 7-9 days after birth and only reaches the increased levels found in adult animals approximately 18 days later.¹ Ciliary epithelial differentiation and maturation begins after 5-7 days and assumes the adult morphologic configuration by 30 days of age.²,³ In vitro receptor binding and active accumulation of various substances by tissues often differ between neonates and adult animals.⁴,⁵ In some tissues transport mechanisms can be stimulated by the administration of substrate.⁶ We report the accumulation of para-aminobipyrinic acid (PAH) by the anterior uvea at various ages and the failure of penicillin administration to alter the accumulation. In addition, we have studied the accumulation of radioactive rubidium (Rb⁺) as a measure of sodium transport in these tissues.

Materials and Methods

Normal pregnant albino rabbits were maintained in our animal facility at least 1 week prior to birth of the newborn animals. Animal utilization conformed to the ARVO Resolution on the Use of Animals in Research. Litters varied from seven to eleven animals. Rabbits were killed at either 6 hr, 1, 3, 7, 14, 21, 28, 60, or 90 days of age. A maximum of two animals per litter were studied at any given time. An ether chamber was used for animals 7 days or younger while older animals were killed by intracardiac air injection. The eyes were enucleated promptly, opened posteriorly, and the lens and vitreous carefully dissected free. The entire anterior uvea (the iris and ciliary body) was removed as a complete ring. In addition, individual ciliary processes from animals 1, 3, 7, 21, 28, or 60 days of age were excised using a surgical microscope. Processes of the two eyes were pooled for uptake studies on animals 7 days or younger. The water content of the anterior uvea and the ciliary processes at all test intervals was found to be approximately 85% by weighing before and after drying to constant weight. The skull cap was removed, and the lateral ventricles opened to obtain choroid plexus. The entire anterior uvea, isolated ciliary processes or choroid plexus were placed in 2 ml of Tyrode’s solution, which had been bubbled with 5%
Table 1. Effect of age on rabbit anterior uvea and choroid plexus accumulation of $^{86}\text{Rb}^+$ and $^3\text{H-PAH}$

<table>
<thead>
<tr>
<th>Time after birth</th>
<th>Anterior uvea wet weight (mg ± SEM)</th>
<th>$^{86}\text{Rb}^+$ T/M* anterior uvea</th>
<th>$^{86}\text{Rb}^+$ T/M choroid plexus</th>
<th>$^3\text{H-PAH}$ T/M* anterior uvea</th>
<th>$^3\text{H-PAH}$ T/M choroid plexus</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hr</td>
<td>1.7 ± 0.1</td>
<td>25.2 ± 0.9</td>
<td>12.5 ± 2.5</td>
<td>2.1 ± 0.2</td>
<td>4.8 ± 1.2</td>
</tr>
<tr>
<td>1 day</td>
<td>1.6 ± 0.2</td>
<td>30.9 ± 2.3</td>
<td>13.5 ± 4.5</td>
<td>2.0 ± 0.1</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>3 days</td>
<td>2.4 ± 0.2†</td>
<td>29.2 ± 1.4</td>
<td>13.8 ± 2.1</td>
<td>2.1 ± 0.2</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>7 days</td>
<td>4.1 ± 0.4†</td>
<td>25.6 ± 1.8</td>
<td>14.8 ± 3.6</td>
<td>2.3 ± 0.1</td>
<td>5.9 ± 1.0</td>
</tr>
<tr>
<td>14 days</td>
<td>8.6 ± 0.7†</td>
<td>26.7 ± 2.8</td>
<td>14.4 ± 1.9</td>
<td>6.0 ± 0.6</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>21 days</td>
<td>16.3 ± 0.5</td>
<td>27.0 ± 1.5</td>
<td>13.2 ± 0.9</td>
<td>7.1 ± 0.8</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td>28 days</td>
<td>20.1 ± 0.9†</td>
<td>23.5 ± 2.0</td>
<td>14.4 ± 1.9</td>
<td>6.9 ± 0.7</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>60 days</td>
<td>43.4 ± 1.7†</td>
<td>13.4 ± 0.6‡</td>
<td>23.5 ± 2.0</td>
<td>6.0 ± 0.6</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>90 days</td>
<td>43.4 ± 1.4</td>
<td>14.0 ± 0.7</td>
<td>13.2 ± 0.9</td>
<td>7.1 ± 0.8</td>
<td>4.6 ± 1.2</td>
</tr>
</tbody>
</table>

* Each test time represents the mean ± SEM of five to eleven animals for $^{86}\text{Rb}^+$ and five animals for $^3\text{H-PAH}$. Significantly different from previous time interval. Student's t-test. † P < 0.01, ‡ P < 0.005.

CO$_2$–95% O$_2$ to adjust the pH to 7.4. The Tyrode's solution had the following composition (milliequivalent per liter): Na$^+$ 149, K$^+$ 3.0, Ca$^{++}$ 3.5, Mg$^{++}$ 1.0, Cl$^-$ 144, PO$_4$$^{3-}$ 0.4, HCO$_3$ 12, glucose 5.5; $^3$H-PAH (New England Nuclear, specific activity 2.1 Ci/mmol) or $^{86}\text{Rb}^+$ (Amersham Searle, 5–8 mCi/mg) was added in the Tyrode's media to 1 μCi/10 ml.

Incubations were carried out with gentle shaking in a water bath at 37°C for 60 min. Tissues were blotted on filter paper, weighed and solubilized in 0.5 ml 1 N sodium hydroxide for 30 min in a 60°C water bath. Following this the mixture was neutralized with 0.5 ml 1 N hydrochloric acid. Tissues were killed on the eighth day for $^3\text{H-PAH}$ anterior uvea accumulation studies. Animals were studied 24 hr after the last injection of penicillin to eliminate residual penicillin in the plasma or tissue. Residual penicillin would inhibit competitively PAH accumulation and mask substate-induced stimulation.

Results are presented as the mean ± SEM. Statistical analysis was performed using the unpaired, two-tailed Student's t-test.

Table 2. Effect of age on rabbit ciliary process accumulation of $^{86}\text{Rb}^+$ and $^3\text{H-PAH}$

<table>
<thead>
<tr>
<th>Time after birth (days)</th>
<th>Ciliary process wet weight (mg ± SEM)</th>
<th>$^{86}\text{Rb}^+$ T/M</th>
<th>$^3\text{H-PAH}$ T/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4 ± 0.6</td>
<td>36.1 ± 3.2</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>1.6 ± 0.5</td>
<td>31.2 ± 2.4</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>1.7 ± 0.6</td>
<td>27.5 ± 2.1</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>21</td>
<td>1.8 ± 0.5</td>
<td>28.1 ± 1.1</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>28</td>
<td>2.3 ± 0.4</td>
<td>24.0 ± 2.1</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>60</td>
<td>4.2 ± 0.4</td>
<td>15.3 ± 2.1</td>
<td>7.7 ± 0.5</td>
</tr>
</tbody>
</table>

* Pooled processes from the two eyes for animals age 1, 3, and 7 days. Individual eye weights for older animals each test time represents six to eight animals.

neously. Injections were started on the first day and continued through the seventh day of life. Animals were killed on the eighth day for $^3\text{H-PAH}$ anterior uvea accumulation studies. Animals were studied 24 hr after the last injection of penicillin to eliminate residual penicillin in the plasma or tissue. Residual penicillin would inhibit competitively PAH accumulation and mask substate-induced stimulation.

Results

The anterior uvea weight 6 hr after birth was 1.7 ± 0.1 mg. The weight showed a progressive and significant increase from day 3–60 (Table 1). The 60- and 90-day weights were similar ($P > 0.3$). Eyelids were closed during the first 7 days of life and opened either on day 8 or 9.

The T/M for $^{86}\text{Rb}^+$ accumulation by the anterior uvea tissue was 25.2 ± 0.9 6 hr after birth. The ratio showed any significant ($P > 0.3$) age-related change through 7 days of life. There was a significant ($P < 0.01$) reduction in the ratio between day 3 ($29.2 ± 1.4$) and day 28 ($23.5 ± 2.0$). Between days 28 and 60, $^{86}\text{Rb}^+$ accumulation continued to decrease, after which time it remained unchanged. The T/M for $^{86}\text{Rb}^+$ accumulation by pieces of ciliary processes showed similar results as those obtained on the entire anterior uvea (Table 2). Accumulation 1, 3, 7, 21, and 28 days of age were significantly ($P > 0.01$) greater than the adult 60-day old ratio. The T/M for $^{86}\text{Rb}^+$ accumulation by the choroid plexus did not show any significant ($P > 0.3$) age-related change (Table 1).

The anterior uvea accumulation ratios for $^3\text{H-PAH}$ were approximately 2.0 for animals killed on days 1,
Anterior uveal weights on day 8 of life were similar (P > 0.2) in the rabbits receiving penicillin injections (4.9 ± 0.2 mg) and those receiving control saline injections (5.1 ± 0.2 mg). The T/M for 3H-PAH accumulation by the anterior uvea was also similar (P > 0.7) in the penicillin- (1.7 ± 0.1) and saline- (2.0 ± 0.2) treated animals.

Discussion

The inner, nonpigmented, ciliary epithelium differs in the newborn and adult rabbit. The epithelial cell membrane in the newborn has few infoldings and lacks the complicated intercellular interdigitations characteristic of the adult rabbit. These membrane characteristics do not appear until after day 7, and it is not until day 30 when they assume the adult appearance. The cell nucleus is apical in location in the newborn and does not reach the adult basal cellular location until day 30. These morphologic maturational changes that are characteristic of actively transporting epithelia correlate with the onset of aqueous humor production between days 7–9 after birth.

Ascorbic acid is accumulated actively by the ciliary body in vitro and actively transported from the blood into the posterior chamber aqueous humor. Newborn aqueous humor ascorbic acid concentration is similar to blood levels, the aqueous humor concentration begins to increase about day 8 and reaches adult levels around day 30. Aqueous humor that is not present in the anterior chamber during the first week of life. Stimulation at later times may again relate to the more mature blood–brain barrier present at birth.

The excised process that contains the secretory ciliary epithelial elements would be expected to have higher accumulation than the entire anterior uvea, which contains nonesecretory elements. However, results are similar for these two tissues. Reasons for this discrepancy may include nonsecretory tissue in the excised processes as well as possible tissue damage during process excision and incubation.

Age-dependent PAH or Rb accumulation by the anterior uvea and by isolated pieces of ciliary processes...
show similar maturation changes. This suggests a true maturation of the ciliary epithelial secretory cells and not just an age-dependent change in the relationship of the secretory to the nonsecretory tissue.

Key words: anterior uvea, choroid plexus, rubidium, para-aminohippuric acid, rabbit

References