Cyclic nucleotides vary by area in the retina and pigmented epithelium of the human and monkey

David A. Newsome,* R. Theodore Fletcher, and Gerald J. Chader

Cyclic GMP and cyclic AMP are present in lower concentrations in the central (macular) region of the neural retina of the human and monkey than in other areas. This pattern approximates the distribution of rod photoreceptor cells. Surprisingly, an inverse gradient of cyclic GMP concentration is observed in the pigmented epithelium. Levels in the central region are over fourfold higher than in cells in the periphery, offering the first evidence of biochemical differences in this embryologically uniform cell type.

Key words: retina, pigmented epithelium, cyclic GMP, cyclic AMP, macula

The retina and pigmented epithelium (PE) form a functioning unit, translating a photic stimulus into a neural response. Cyclic nucleotides appear to have important functions in this process. Cyclic guanosine monophosphate (cyclic GMP) in particular is compartmentalized in photoreceptor outer segments, with up to a 10-fold higher concentration in dark- than in light-adapted photoreceptors. Cyclic AMP levels in photoreceptors are lower and are not responsive to light, although dark adaptation and catecholamines increase cyclic AMP in the inner layers of the retina. Nothing is known, however, concerning cyclic nucleotides in the retinal PE, the single layer of cells apposed to the retinal photoreceptor outer segments. In the present communication, we report on differences in the concentration of cyclic GMP and cyclic AMP in different areas of the light-adapted human retina and PE obtained at autopsy and in the monkey.

Materials and methods

Human eyes were obtained at autopsy and dissected within 20 hr following death. Donor ages ranged from 7 to 72 years; causes of death varied. None of the "normal" donors had a past history of ocular disease, and the eyes were anatomically normal on gross examination with the dissecting stereomicroscope. Eyes were also obtained from three patients, each with documented pathology of the retinochoroidal complex. Patient 1 was a 51-year-old black man with a 25-year history of progressive visual loss due to retinitis pigmentosa. The eye used in this study had bare light perception with no projection. Patient 2 was a 63-year-old man with moderate proliferative diabetic retinopathy in one eye which had been treated 2 years before death with panretinal photocoagulation. The other eye had very early proliferative and macular changes and was untreated. Patient 3 was a 67-year-old woman with an 8-year history of mildly reduced vision (20/40 to 20/50) presumably due to macular changes, including drusen. There had been evidence of slow progression in the year prior to death.

Retinas were dissected into three areas: (1) a 6

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Table I. Variation in cyclic nucleotide by area in neural retina and pigmented epithelium

<table>
<thead>
<tr>
<th></th>
<th>Cyclic nucleotide (Mean ± S.E.M.)</th>
<th>p value (vs. macula)</th>
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<tbody>
<tr>
<td>A. Retina cyclic GMP:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>3.2 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>Midzone</td>
<td>6.4 ± 1.1</td>
<td>0.023</td>
</tr>
<tr>
<td>Periphery</td>
<td>10.2 ± 1.8</td>
<td>0.018</td>
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<tr>
<td>B. Retina cyclic AMP:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>4.8 ± 0.6</td>
<td>—</td>
</tr>
<tr>
<td>Midzone</td>
<td>10.1 ± 1.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Periphery</td>
<td>13.2 ± 1.8</td>
<td>0.0005</td>
</tr>
<tr>
<td>C. PE cyclic GMP:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>8.5 ± 2.1</td>
<td>—</td>
</tr>
<tr>
<td>Midzone</td>
<td>3.2 ± 1.0</td>
<td>0.024</td>
</tr>
<tr>
<td>Periphery</td>
<td>2.0 ± 0.6</td>
<td>0.031</td>
</tr>
<tr>
<td>D. PE cyclic AMP:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>2.6 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Midzone</td>
<td>2.5 ± 0.4</td>
<td>Not significant</td>
</tr>
<tr>
<td>Periphery</td>
<td>3.3 ± 0.6</td>
<td>Not significant</td>
</tr>
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</table>

Values given are from 10 individual tissue samples. Duplicate radioimmunoassays at each of three different dilutions were averaged to get the final value for each of the tissue samples. Each value given therefore is based on results from up to 60 radioimmunoassays. Statistical significance (p value) was determined with a "paired t statistical" computer program. Normal eyes were obtained at autopsy (2 to 20 hr after death) from the following donors, listed by age (years), sex, and cause of death: (1) 65, F, cardiac; (2) 39, M, brain tumor; (3) 7, M, rhabdosharcoma; (4) 48, M, melanoma; (5) 52, M, oat-cell carcinoma; (6) 68, F, heart failure; (7) 47, F, melanoma; (8) 29, M, melanoma; (9) 72, F, heart failure; (10) 68, M, myocardial infarction.

Fig. 1. Variation in cyclic nucleotide by area in retina and PE epithelium of rhesus monkey as a function of time after death. A, Retinal cyclic GMP. B, Retinal cyclic AMP. C, Pigmented epithelial cyclic GMP. D, Pigmented epithelial cyclic AMP. Areas of retina: □ macula; □ midzone; □ periphery. Time values refer to time after death (hours); zero time values were obtained with tissues removed from deeply anesthesized animals. Cyclic nucleotide values are averages of duplicate radioimmunoassays at each of three different dilutions.

Rhesus monkeys were maintained in the NIH animal colony and sacrificed by pentobarbital injection. To obtain a "zero" time value, the test animal was deeply anesthetized, the eye was opened in situ, and tissue samples were dissected and placed immediately into 0.5 ml of a 10% perchloric acid solution. The time lapse between opening the eye and placing the first sample (macula) into the acid solution was 30 to 60 sec. Equatorial and peripheral retinal samples then were taken as well as samples from the appropriate underlying PE areas. This process required an additional 2 to 3 min. For time periods other than the zero time point, monkeys were sacrificed, eyes were removed, and samples were later dissected and placed in perchloric acid solution at stated intervals.

Cyclic nucleotides were subsequently separated, purified, and succinylated. Radioimmunoassay was performed by the procedure of Steiner et al. with minor modification. Protein was determined by the method of Lowry et al. with bovine alubmin as standard. Results are expressed as picomoles of cyclic nucleotide per milligram of protein.

Results

Cyclic nucleotide levels varied by area in the human retina with lowest concentration observed in the central (macular) region of
Fig. 2. A, Light micrograph (hematoxylin and eosin) of a typical 18 hr postmortem specimen of neural retina demonstrating retention of outer segments (asterisk). (×420.) B, Transmission electron micrograph of PE from the same area as in (A), demonstrating the clean separation of outer segments from the PE. (×35,000.)
Regional variation of retinal cyclic nucleotides

This was found to be true for both cyclic AMP and cyclic GMP, with a twofold to threefold increase in concentration as one advanced through the midzone to the periphery. The differences in cyclic nucleotide content between macula and other retinal areas were highly statistically significant. Parameters such as age, sex, cause of death, drug treatment, etc., did not influence cyclic nucleotide levels in the cases we examined. Data from retinal samples obtained later than 20 hr after death were not included in the data analysis as discussed below. Surprisingly, the cyclic GMP level in the PE followed an inverse pattern compared to its distribution in the retina (Table I, C), i.e., twofold to fourfold higher in macula than in more peripheral regions. No such distinct pattern was observed with cyclic AMP in the different areas of the PE (Table I, D).

A similar regional distribution of cyclic nucleotides was observed in the monkey retina and PE (Fig. 1). Moreover, the influence of the time lag between death and actual retinal dissection on cyclic nucleotide levels in retina and PE was also determined in this study. For this purpose, monkeys were anesthetized, and eyes were dissected immediately (zero time) or enucleated and dissected at 1.5, 4, 6, and 24 hr after death. This study demonstrated that (1) the concentrations of cyclic nucleotides in the various retinal areas (Fig. 1, A to D) were, in fact, similar to those in the human; (2) cyclic GMP levels in retina (Fig. 1, A) were relatively stable throughout about 24 hr but declined thereafter as autolysis of the retina progressed; (3) cyclic AMP levels in retina (Fig. 1, B) were surprisingly stable in macula but suffered an initial drop (zero time to 1.5 hr) in other retinal areas; (4) in PE, concentrations of both cyclic GMP (Fig. 1, C) and cyclic AMP (Fig. 1, D) dropped threefold to fourfold within 4 hr, slowly declining thereafter; and (5) the same regional pattern of distribution was evident as in the human—in retina, both cyclic GMP and cyclic AMP lowest in macula; in PE, cyclic GMP highest in the macular region; no pattern observed with cyclic AMP. Time points at 30 and 48 hr were also studied but are not presented in Table I because they show the pattern of progression already described.

One possible explanation for our results would be that the retinal outer segments (rods and/or cones) were broken off during dissection and were retained by the underlying PE cells. At the macula, this could lead to artifactually low cyclic nucleotide levels and concomitantly high levels in the underlying PE. However, careful examination by both light and electron microscopy (Fig. 2) revealed that the bulk of the outer segments in the macular as well as peripheral areas of the retina was retained by the retina and was not artifactually associated with the underlying PE. The sample shown is a typical 18 hr postmortem human specimen and demonstrates that even though progressive autolytic changes were evident, the bulk of the outer segments remained with the retina (Fig. 2, A). Apical pigmented epithelial processes were infrequently seen by this time (Fig. 2, B).

In preliminary studies, we have found that cyclic nucleotide concentrations actually do appear to be altered in the human retina in some diseased states. For example, in the single case of advanced retinitis pigmentosa we have been able to examine (n = 1), cyclic GMP values were decreased fivefold to 10-fold (0.5, 0.6, and 2.0 pmol/mg in macula, midzone, and periphery, respectively), whereas cyclic AMP values were virtually normal (6.7, 13.8, and 11.2 pmol/mg in the three areas). In diabetes mellitus (one retinal sample from each eye of patient, n = 2), retinal cyclic AMP levels were greatly elevated (13.0, 39.3, and 33.2 pmol/mg in the three areas), whereas cyclic GMP values were virtually normal (3.4, 6.7, and 8.8 pmol/mg in the three areas). In an eye exhibiting many drusen, levels of both cyclic nucleotides were increased, and a reversed pattern of distribution observed for cyclic AMP, i.e., highest in macula (cyclic AMP 34.2, 23.9, and 9.7 pmol/mg and cyclic GMP 12.6, 17.6, and 9.4 in the three areas).
Discussion

Only a few animals, including man, possess a specialized retinal area, the macula, which subserves high-resolution visual acuity. Although morphological differences between macular and nonmacular areas of the retina are well known (e.g., rod/cone distribution), this report is the first to demonstrate biochemical differences between these areas. Such differences might be basic in understanding clinical conditions which demonstrate specific regional patterns. In contrast to the cellular heterogeneity of the retina, the PE has been thought to be a relatively uniform tissue because it is only a single cell layer thick and all cells are thought to have similar functions. The finding of a high cyclic GMP content in PE cells in the macular region was quite surprising, especially in light of the relatively low cyclic GMP content of the overlying neural retina. For the first time, therefore, significant differences in cell metabolism in different areas of the PE are indicated. We may ultimately be able to correlate these biochemical changes with differences in PE cell morphology. Of some importance, besides the actual empirical values for the two nucleotides, may be the cyclic GMP/cyclic AMP ratio. This is relatively uniform in the various areas of the retina (macula 0.7; midzone 0.6; periphery 0.8), but in the PE, the ratio drops from 3.3 in the macular zone to 1.5 in the midzone and only 0.6 in the periphery. The uniform ratio in retina is also interesting because it indicates that there may be no preferential concentration of either nucleotide in the cone-rich macula vs. the more peripheral rod-rich areas.

From our present and previous results and more detailed studies of Orr et al., and Ferrendelli and Cohen, and Farber and Lolley, it appears that two distinct pools of cyclic nucleotides can be discerned in retina. First, a relatively labile pool (visual cycle pool) appears to be present primarily in the photoreceptor region and probably is involved in the rapid reactions of the visual cycle and ensuing neurotransmitter events. The present human and primate study was obviously not designed to investigate this pool, since studies on small rodent eyes have shown that there are very rapid changes in retinal cyclic nucleotides after death. The second pool (basal pool) is one that appears to be relatively stable and may be involved in the more general functions of the retina as in other parts of the central nervous system. Perturbations of this second pool in particular could be associated with retinal pathology. Our preliminary results on several human retinal conditions indicate that study of cyclic nucleotide perturbations may yield valuable information about the etiology of some of these diseases and possibly form a rational basis for disease treatment.

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