Iris-Ciliary Complex Inhibits Protein Synthesis by Organ Cultured Mammalian Ocular Lens Cells

M. Bagchi, A. A. Dave, A. K. Singh, and M. J. Caporale

Freshly isolated rabbit ocular lenses with or without adhering iris–ciliary complex were organ cultured. It was found that lenses with iris–ciliary complex showed decreased protein synthetic activity. Removal of the iris–ciliary complex at least partially restores the protein synthetic capability of the lens. The ocular lenses cultured with iris–ciliary complex for 48 hr showed marked equatorial opacities. Invest Ophthalmol Vis Sci 28:759–762, 1987

Rabbit ocular lenses can be maintained in organ culture for weeks if the pH, ionic, and nutritional requirements of the incubation medium can be maintained at the optimum level.1 The rabbit lenses maintained in organ culture for extended time periods retain their clarity, but the protein synthetic activity of some proteins decreases.2 It was presumed this occurred because either the incubation medium lacked some factors or the process of lens isolation caused irreversible trauma. Experiments were performed to test this hypothesis. The standard isolation technique for lens organ culture requires removal of the iris–ciliary complex (IC) from the lens3 leaving some zonule fibers for handling, and retaining the adhering vitreous humor.4 We organ-cultured freshly isolated rabbit ocular lens with attached IC for up to 2 days.5 Lens clarity and the ability of lens cells to synthesize proteins were used as markers to study the possible effect of IC on the organ cultured ocular lens. Data from these experiments revealed that the presence of attached IC on the lens resulted in diminished protein synthesis and the appearance of equatorial opacification.

Materials and Methods. Lenses were obtained from freshly killed New Zealand white rabbits of approximately 2 kg body weight and incubated in either TC-199 medium or RPMI-1640 without leucine4 for up to 2 days. In all experiments the pH of the medium was maintained at 7.4 and the molarity at 310 mosm. The pH of the medium at the end of incubation was also measured to assure maintenance of proper pH. Lenses were incubated in a closed culture system in a water bath at 33.8°C ± 1°C. Experimental lenses retained their IC, whereas, in the contralateral control lenses, the IC was removed. Six hours before the end
VIMENTIN SYNTHESIS

MP-26 SYNTHESIS

of the experiment all lenses were pulsed with $^3$H-leucine at a concentration of 50 $\mu$Ci/ml (specific activity 190 Ci/mM). After isotopic pulse lenses were isolated in cold TM buffer. Vimentin and MP-26 proteins were extracted from the superficial cortical fiber cells of the lenses. The synthetic activity of vimentin and MP-26 was measured using SDS-PAGE, densitometric gel scanning and liquid scintillation spectrometry. The specific activity of the $^3$H-leucine pool was determined by an amino acid analyzer and scintillation spectrometry.

In some experiments the epithelium of control and experimental lenses was dissolved in lysis buffer and 2-D peptide profiles were obtained. 2-D gels were photographed and dried for fluorography.

A CCRG camera system (Zeiss OPM-I microscope, Zeiss, West Germany; Pentax 35mm camera, ASHAI Pentax, New York, NY) was employed to photograph cultured ocular lenses.

Results. In the first set of experiments control and experimental (IC-attached) lenses were incubated in TC-199 medium for 6, 24, or 48 hr. The lenses were individually cultured in a closed system using 40 ml of culture medium per lens. In all experiments, lenses were pulsed with $^3$H-leucine in RPMI-1640 without leucine, 6 hr before the end of the experiment. Each lens was pulsed in 5 ml of isotopic medium. Figure 1 shows the data obtained from such experiments. It is clear from Figure 1 (top) that the rate of vimentin synthesis decreases with time in the lenses with attached IC. When compared with the control lenses at 6 hr of culture the IC attached lenses (experimental) showed a 50% decrease in the rate of vimentin synthesis, by 24 hr it decreased to 20% of control and by 48 hr it was down to 10%. In some experiments lenses were cultured with IC for 24 hr and then IC was removed and lenses cultured for another 24 hr in the same medium. The data from such experiments clearly dem-
onstrate (Fig. 1, top) that the inhibitory effect of IC is reversible. Figure 1 (bottom) shows the effect of attached IC on MP-26 synthesis by the superficial cortical fiber cells. Like vimentin, synthesis of MP-26 is drastically reduced by the presence of IC on the lens. But, unlike vimentin, inhibition of MP-26 synthesis is not time dependent. Reversal experiments showed that MP-26 synthesis can be increased by simple removal of IC from the lens and incubating it for another 24 hr. Figure 2 shows the gel pattern and fluorogram of peptides isolated from the epithelium of control and experimental lenses cultured for 48 hr. The fluorogram clearly demonstrates that the presence of IC on the lens drastically reduces the ability of the epithelial cells of the experimental lenses to incorporate 3H-leucine in their peptides. It is difficult to determine from this data whether the effect of IC on 3H-leucine incorporation is nonspecific or specific to particular proteins. The specific activity of the 3H-leucine pool in the lens superficial cortical fiber cells remained similar in the control and experimental lenses incubated for 24 hr (Table 1). However, experimental lenses cultured for 48 hr with attached IC showed a 40% decrease in the specific activity of their 3H-leucine pool. Figure 3 shows the photographs of control and experimental lenses cultured for 48 hr. Figure 3A is a control lens and it is clear. Figure 3B is an experimental lens with IC attached. Figure 3C is the same lens as 3B after IC was removed from the lens. It is apparent from this photograph that the presence of IC could cause equatorial opacities in the lens.

Discussion. It is evident from these data that the presence of IC on the lens causes decreased protein synthesis by both superficial cortical fiber cells and epithelial cells. This is rather unexpected because IC is always present in the in-vivo lens and it functions properly. Why in organ culture do we see this anomalous behavior? One of the basic differences between the in-vivo and organ culture conditions of the lens is that in the in-vivo situation the iris is in contact with the lens only in the pupillary region, and the rest of the anterior surface of the lens is separated from the iris as the aqueous humor flows from the posterior to the anterior chamber. Whereas, in-vitro the IC complex lays on the lens and, thus, it could limit nutrient supply. Lack of essential nutrients could cause decreased metabolic activities manifested by decreased protein synthesis and appearance of equatorial opacities. Or could it be a pH effect? The experimental lenses have extra tissue (IC), and it could cause a decrease in the pH of the culture medium. The authors used 40 ml of medium per lens, and the pH of the culture medium at the end of the culture period showed no change. But, this does not rule out a pH change in the microenvironment of the lens, as the IC lays on top of the lens.

Table 1. Specific activity of the 3H-leucine pool

<table>
<thead>
<tr>
<th></th>
<th>Micromole of leucine in 1 ml supernatant</th>
<th>Counts per minute (cpm) in 50 µl of supernatant</th>
<th>Specific activity of the 3H-leucine pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (24 hr)</td>
<td>0.041</td>
<td>45,814</td>
<td>2.2 × 10^6</td>
</tr>
<tr>
<td>Experimental (24 hr)</td>
<td>0.040</td>
<td>44,368</td>
<td>2.2 × 10^6</td>
</tr>
<tr>
<td>Control (48 hr)</td>
<td>0.068</td>
<td>95,967</td>
<td>2.8 × 10^6</td>
</tr>
<tr>
<td>Experimental (48 hr)</td>
<td>0.066</td>
<td>57,455</td>
<td>1.7 × 10^6</td>
</tr>
</tbody>
</table>
Fig. 3. (A) Anterior view of ocular lens cultured for 48 hr. (B) Anterior view of lens with IC cultured for 48 hr. (C) Anterior view of 3B lens after removal of IC. z = zonule fibers; IR = iris; and $\uparrow$ = open arrow indicates equatorial opacity.

lens protein synthesis did not change. Thus, it seems that the effect of IC is not because of some physical changes during the removal of the IC from the lens but because of physiologic activity of the IC. It could be that metabolic breakdown products of the IC are not being properly washed out from the lens surface, thus causing damage to the lens protein synthesis or IC produces some protein synthesis inhibitory factors, which then migrate to the lens causing diminished protein synthesis. But then the question can be raised, why does this not happen in vivo? It could occur because there are so many growth-stimulating factors in the eye that protein synthetic inhibitory factors from the IC maintain a low, stable rate of protein synthesis in the in-vivo lens. Thus, in organ culture, where there are no growth factors, only the protein synthetic inhibitory effect of the IC is apparent. The decreased protein synthetic activity of the experimental lenses incubated for 48 hr can result partly because the decrease in the specific activity of the $^3$H-leucine pool, but it still does not explain the appreciable decrease in the rate of protein synthesis after 24 hr of incubation of the lenses with adhering IC.

The equatorial opacity produced by the IC in the lenses cultured for 48 hr is puzzling. So far, in studies with protein synthesis, the authors have seen no correlation between decreased protein synthesis and the production of lenticular opacity in organ cultured lenses. This equatorial opacity develops only after 48 hr of culture with IC. The presence of IC on the lens may cause changes in the permeability properties of the lens cells and the equatorial opacity may be due to hydration. Owers and Duncan reported that when bovine lenses were cultured with iris for 24 hr the Na$^+$ levels doubled. It is possible that the increased sodium levels in the lenses cultured with adhering IC causes diminished protein synthetic activity.

Key words: iris–ciliary complex, protein synthesis, organ culture, mammalian lens, cataract.

Acknowledgment. The authors thank B. Chakrapani of Dr. V. Reddy’s laboratory for performing total leucine estimation.

From the Department of Anatomy and Cell Biology, School of Medicine, Wayne State University, Detroit, Michigan. Supported by EY-01848 and EY-04068 (core grant) from the National Institutes of Health, Bethesda, Maryland. Submitted for publication: June 10, 1986. Reprint requests: M. Bagchi, Department of Anatomy and Cell Biology, School of Medicine, Wayne State University, Detroit MI 48201.

References