Metabolic Influences on the Absorption of Serous Subretinal Fluid

Shin-ichiro Kawano* and Michael F. Marmor

Experimental detachments were made in Dutch rabbit eyes by injecting autologous serum or Hanks’ solution into the subretinal space through a glass micropipette. The serum was resorbed at a rate of 0.064 ± 0.023 μl/mm²/hr, which is approximately half as fast as Hanks’ solution. Both cyclic GMP and dibutyryl cyclic GMP accelerated serum resorption by 23%, whereas cyclic AMP and dibutyryl cyclic AMP (both used with IBMX) decreased the absorption rate by 46%. The absorption rate for Hanks’ solution did not differ significantly between light and dark. Intravenous administration of acetazolamide (50 mg/Kg) and mannitol (2.5 g/kg) failed to alter the serum resorption rate significantly. Thus, cyclic nucleotides (and presumably metabolic transport) are important to absorption of serous fluid, but acetazolamide and hyperosmotic agents have less effect than on nonproteinaceous subretinal fluid. Invest Ophthalmol Vis Sci 29:1255–1257, 1988

Our studies of experimental nonrhegmatogenous retinal detachments have shown that saline fluids injected into the subretinal space are resorbed rapidly, mainly by metabolic activity of the retinal pigment epithelium (RPE).1-3 The resorption of balanced salt solution is greatly reduced by general metabolic inhibition with dinitrophenol4 and can also be modified by more specific metabolic agents such as cyclic nucleotides and acetazolamide,4 and by physical agents such as hyperosmotic solutions.5 However, clinical detachments contain proteinaceous fluid which is absorbed more slowly from experimental detachments.6 It is important to know whether the same balance of metabolic and osmotic forces that remove saline subretinal fluid is responsible for removing serous subretinal fluid, and whether agents that enhance saline fluid absorption are similarly effective for serous fluid (and might be considered for clinical application). Thus, we have studied the effects of metabolic and hyperosmotic agents upon the absorption of subretinal serum.

Materials and Methods

These investigations adhered to the ARVO Resolution on the Use of Animal in Research. Dutch rabbits weighing 1–1.5 kg were sedated with acepromazine maleate (1.0 mg/kg, IM) and anesthetized with urethane (1.0 g/kg, IP), supplemented occasionally with ketamine hydrochloride (20 mg/kg, IM). The pupils were dilated with 1% atropine sulphate and 10% phenylephrine drops.

Small retinal detachments (blebs) were made in the avascular posterior pole, as described previously.1 In brief, a micropipette was passed through a limbal incision, and across the vitreous, to penetrate the subretinal space into which an experimental fluid was injected. Two different measurement methods for evaluation of the resorption of this fluid were employed: resorption time and resorption rate. Resorption times were measured by observing the blebs every 10–15 min until they decreased in size sufficiently (50% of the bleb diameter had flattened). Blebs were made as close to a constant size (2.5–3.0 mm diameter) as possible, since larger blebs absorb more slowly.6 Resorption rates were calculated from measurements of bleb height, using a YAG laser system. The dual He-Ne laser beams were focussed through a Goldmann fundus lens upon three points along the horizontal diameter of the bleb, and the relative heights of these points were measured with a micrometer attached to the slit lamp apparatus. Details of this method have already been published.3

Autologous serum for injection into the subretinal space was prepared by collecting autologous blood from an artery in the ear and centrifuging it at 1500 RPM for 15 min. Experimental agents used were sodium acetazolamide (Lederle Laboratories, Wayne, NJ); cyclic nucleotides (cGMP, dibutyryl cGMP, cAMP, dibutyryl cAMP—all from Sigma Chemical...
Fig. 1. Effect of cyclic nucleotides on the rate of serum resorption from the subretinal space. Data from cyclic and dibutyryl cyclic nucleotides have been combined; cAMP was always combined with IBMX. Error bars indicate 1 SEM; parentheses indicate the number of experiments.

Co., St. Louis, MO); isobutylmethylxanthine (IBMX, from Sigma Chemical Co.); and mannitol (20% solution, Abbott Laboratories, N. Chicago, IL).

Results

Cyclic nucleotides and facilitatory substances were injected both into the mid-vitreous and into the blebs themselves, to yield a 1 mM concentration. Thus, blebs were formed with 1 mM concentrations of drugs added to the autologous serum, and drugs dissolved in Hanks’ solution were injected into the vitreous in amounts sufficient to produce a 1 mM concentration after diffusion into the full vitreous volume. Figure 1 shows the effects of cyclic nucleotides on autologous serum resorption. Autologous serum was resorbed at a rate of 0.064 ± 0.023 (1 SD), which is close to our previous measurement.3 Cyclic GMP or dibutyryl cGMP accelerated resorption by 23% (both forms of cGMP gave similar results, so the data have been combined to give greater statistical significance). Cyclic AMP and dibutyryl cAMP (both used with IBMX) decreased the absorption rate by 46%. Statistically, the cGMP and dibutyryl cGMP effect was barely significant (P < 0.05) by student t-test, whereas the cAMP and dibutyryl cAMP effect was highly significant (P < 0.001).

Table 1. Effects of illumination of the resorption time of Hanks’ solution

<table>
<thead>
<tr>
<th></th>
<th>Average resorption time</th>
<th>Average bleb diameter</th>
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<tbody>
<tr>
<td></td>
<td>(min ± 1 SEM)</td>
<td>(min ± 1 SEM)</td>
</tr>
<tr>
<td>Light</td>
<td>175 ± 7</td>
<td>2.77 ± 0.03</td>
</tr>
<tr>
<td>Dark</td>
<td>167 ± 9</td>
<td>2.76 ± 0.05</td>
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Since local cyclic nucleotide levels might be expected to vary with light and dark (see Discussion) we looked for differences in the resorption of subretinal fluid under light and dark conditions. The “light” condition was our usual protocol for measuring resorption time: the experiments were performed in room lighting with high intensity microscope illumination used to form blebs and observe them. For the “dark” condition, all procedures to make blebs were performed under dim red microscope light after which the animals were kept in darkness except for brief periods of microscopic observation under the same dim red light every 10–15 min. We used Hanks’ solution rather than serum for the initial experiments with light and dark, because the less viscous fluid is easier to inject and follow. Since we failed to observe any significant difference between the resorption times of Hanks’-filled blebs under light and dark (Table 1), serum experiments were never done.

To study the effects of acetazolamide and mannitol on serum resorption, blebs were formed and the resorption rate measured between 30 and 75 min after bleb formation to establish a baseline. Acetazolamide (50 mg/kg) or mannitol (2.5 g/kg) was injected intravenously at this point and the measurements of bleb volume (resorption rate) were continued to determine whether the rate changed. We failed to observe a significant effect on serum resorption from either of those drugs (Table 2).

Table 2. Effects of acetazolamide and mannitol on the resorption rate of serum

<table>
<thead>
<tr>
<th>Drug</th>
<th>Before drug injection</th>
<th>After drug injection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(µl/mm²/hr ± 1 SEM)</td>
<td>(µl/mm²/hr ± 1 SEM)</td>
</tr>
<tr>
<td>Acetazolamide (50 mg/kg)</td>
<td>0.068 ± 0.008</td>
<td>0.067 ± 0.010</td>
</tr>
<tr>
<td>Mannitol (2.5 g/kg)</td>
<td>0.067 ± 0.011</td>
<td>0.064 ± 0.007</td>
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</table>

Discussion

Our previous work indicated that saline subretinal fluid is removed across the RPE into the choroidal space primarily by RPE metabolic activity. We found that cGMP, acetazolamide and hyperosmotic agents facilitated the resorption of Hanks’-filled blebs and speculated that they might have beneficial effect on the treatment of clinical detachments. However, clinical retinal detachments invariably contain protein which slows the absorption of fluid and we had no evidence on whether those drugs were also effective on serum resorption.

The average rate of fluid resorption from serum-filled blebs was 0.064 µl/mm²/hr, which is approxi-
approximately half of the rate for Hanks' solution. Serum resorption was accelerated 23% by cGMP and decreased 46% by cAMP, compared to changes in Hanks' solution resorption of 33% by cGMP and 25% by cAMP. These results suggest that cyclic nucleotides and presumably active ion transport are involved in the clearance of proteinaceous as well as saline fluid from the subretinal space.

Cyclic nucleotide levels are different between light and dark in retina and in the rod outer segments. cGMP is directly involved in the control of sodium channel conductivity in response to light and dark. In lower vertebrates, retinomotor movement and pigment movement in RPE vary with light and dark, and cAMP has been suggested as a mediator. However, we found that the resorption times of Hanks'-filled blebs were no different in light than dark, indicating that illumination-induced changes of local cyclic nucleotide concentrations, if any, were not sufficient to cause major changes in rate of fluid resorption.

In our previous study, acetazolamide had little effect on Hanks' solution resorption at conventional clinical doses (15 mg/kg), but increased resorption significantly at higher doses (50 mg/kg). In the present study, we failed to enhance the resorption rate of serum even with the higher dose. We do not know whether the serum interferes pharmacologically with acetazolamide (which seems unlikely) or the beneficial effects of acetazolamide are simply too weak in the rabbit to overcome the added osmotic burden of protein in the subretinal space. We also failed to modify the resorption of serum-filled blebs with intravenous mannitol, despite well documented effects on Hanks'-filled blebs. The descrepancy with respect to mannitol is easier to explain, since subretinal protein removes much of the osmotic gradient between choroid and subretinal space by which mannitol is presumed to work. The fact that both drugs have less effect on proteinaceous than saline subretinal fluid is disappointing from the clinical point of view. It is also possible that the limitations of our method prevented us from recognizing small effects and that small effects could have been masked by the drug-induced fall of intraocular pressure (which would have a small effect on serum resorption).

Key words: subretinal fluid, nonrhegmatogenous detachment, retinal pigment epithelium, epithelial water transport, light/dark, cyclic nucleotide, acetazolamide

References