Insulin concentrations in aqueous humor after paracentesis and feeding of rabbits.

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Although the lens has been shown to have the capacity to respond to insulin in vitro, little is known concerning the biochemical relationships of insulin to the lens in vivo. Therefore we have measured insulin in the aqueous humor of rabbits by a sensitive radioimmunoassay after paracentesis and feeding. The insulin concentration in aqueous humor was 3% of that in plasma. One hour after paracentesis the aqueous humor insulin concentration was increased sixfold, apparently due to breakdown of the blood-aqueous barrier, but 1 week after paracentesis it had returned to its original level, apparently because of restoration of the blood-aqueous barrier within that time. After feeding, the aqueous humor insulin concentration was increased by 30% compared to a 175% increase in plasma. Factors influencing the aqueous humor insulin concentration and the possibility of insulin influence on lens metabolism are discussed.

The hormone insulin exerts a broad spectrum of metabolic effects on many mammalian tissues and is capable of influencing the metabolism of carbohydrates, lipids, proteins, and nucleic acids as well as sodium-potassium transport. However, very little is known about the influence of insulin on the ocular lens. Supraphysiologic concentrations of insulin have been shown to stimulate glucose utilization, mitosis, fiber cell differentiation, microtubule assembly, and $\beta$-crystallin synthesis in lenses or lens epithelium preparations cultured in vitro. $^{1-4}$ The effect of insulin on cell growth also implies stimulated protein and lipid biosynthesis. Furthermore, the presence of insulin receptor sites in intact rabbit lens epithelial cells propagated in culture has been demonstrated. $^{5}$ Therefore it is clear that the lens has the capacity to respond to insulin, but it is not known whether insulin influences the lens metabolism under physiologic conditions. The metabolic relationships between insulin and the lens are of special interest because altered availability of insulin to the lens could conceivably play a role in the development of diabetic cataract by altering the enzyme activities of the lens in such a way as to favor the accumulation of sorbitol, which is known to be a key causal factor in this type of cataract. $^{6,7}$ We recently demonstrated that insulin is present in the aqueous humor of rabbits at concentrations of approximately 2% to 3% of the plasma concentration. $^{8}$ This report represents an effort to further characterize the physiologic relationships between insulin and the lens and describes investigations on the effects of paracentesis and feeding on the insulin concentration in the aqueous humor of rabbits.

Materials and methods. New Zealand white rabbits, weighing 3 to 4 kg and maintained on Purina Rabbit Chow were used in this investigation. Anesthetization, paracentesis, and bleeding
Table I. Insulin and protein concentrations in aqueous humor after paracentesis of rabbits

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of rabbits</th>
<th>Paracentesis</th>
<th>Insulin (pg/ml)</th>
<th>Protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>First</td>
<td>54 ± 3*</td>
<td>41 ± 2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second—1 hr later</td>
<td>329 ± 42</td>
<td>1818 ± 105</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>First</td>
<td>51 ± 4</td>
<td>37 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second—1 wk later</td>
<td>52 ± 4†</td>
<td>37 ± 2†</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M. (n = 12 for insulin; n = 24 for protein).†Not significantly different from first paracentesis by paired t test.

of rabbits and assay procedures for insulin and protein have been described previously.8

Two experiments were performed to study the effects of paracentesis on insulin and protein concentrations in aqueous humor. In the first experiment, rabbits were fasted overnight and anesthetized, and primary aqueous humor was withdrawn; secondary aqueous humor was withdrawn 60 min later. In the second experiment, rabbits never before subjected to paracentesis were fasted overnight and anesthetized, and aqueous humor was withdrawn; this procedure was repeated with the same rabbits 1 week later.

In order to study the insulin concentrations in aqueous humor after feeding, four groups of six rabbits each were fasted overnight, and rabbits within each group were randomly assigned for blood collection, anesthetization, and paracentesis at 0 (fasting), 1, 2, 3, 4, and 5 hr after feeding. Food was removed after 1 hr to prevent continued feeding. The procedure was repeated at weekly intervals for 12 weeks with the same groups of rabbits. This schedule was followed so that each rabbit underwent paracentesis only once a week in order to allow restoration of the blood-aqueous barrier and normal aqueous humor composition before paracentesis was repeated. Data from the four groups of rabbits were combined.

Results. The mean insulin and protein concentrations in aqueous humor of fasted rabbits withdrawn 1 hr after initial paracentesis were increased sixfold and 43-fold, respectively, but when a week was allowed to elapse before paracentesis was repeated, the insulin and protein concentrations were not significantly different from those of primary aqueous humor (Table I).

The mean insulin concentration in plasma was 1354 pg/ml in fasting animals and rose to a peak 175% higher at 1 hr after feeding (Fig. 1, A); by the second hour, the insulin concentration had fallen to a level not significantly different from the fasting concentration. The mean insulin concentration in aqueous humor was 45 pg/ml in fasting animals and rose to a peak 30% higher at one hour (p < 0.001) (Fig. 1, B). The aqueous humor insulin concentration remained higher than the fasting concentration from 1 to 3 hr after feeding and fell to a level not significantly different from the fasting concentration by the fourth hour. Calculation of the aqueous/plasma concentration ratios for insulin and total protein in the fasting animals gave values of 3.30% ± 0.37 (mean ± S.E.M.) for insulin and 0.67% ± 0.02 for total protein; the ratio for insulin is five times greater than that of total protein (p < 10^-6).

Discussion. These investigations clearly show that insulin was present in the aqueous humor of rabbits at a concentration of approximately 3% of that in plasma. Its concentration rose dramatically 1 hr after paracentesis, apparently due to a breakdown of the blood-aqueous barrier, but after 1 week, its concentration had returned to its original level, apparently because of restoration of the blood-aqueous barrier within that time. Secondary aqueous humor has been shown to be capable of inducing mitosis in cultured rabbit lenses,9 and since insulin has also been shown to be capable of the same effect,6 it is conceivable that the increased insulin concentration in secondary aqueous humor is responsible for its mitogenic effect.

The increase in the aqueous humor insulin concentration after feeding confirmed our previous investigation.8 The increase was only 30% compared to a 175% increase in the plasma insulin concentration, but the increase above the fasting concentration was sustained for a longer period of time in aqueous humor than in plasma. The smaller but more sustained insulin increase in aqueous humor was appropriate for the relatively slow aqueous humor turnover rate of approximately 0.01 min^-1 (ref. 10). The finding that the aqueous/plasma concentration ratio is five times greater than that for total protein suggests that insulin crosses the blood-aqueous barrier more readily than do most plasma proteins; this is not surprising, since insulin is a relatively small protein with a molecular weight of only 6000.

These studies suggest that the major factors
influencing the concentration of insulin in aqueous humor are the plasma insulin concentration, the permeability of the blood-aqueous barrier, and the aqueous humor turnover rate; it is conceivable that the blood-aqueous barrier may have the capability of degrading insulin and that this also could influence the insulin concentration in aqueous humor.

It is not yet known whether or not insulin influences the lens metabolism under physiologic conditions. The presence of insulin receptor sites in the lens suggests that such an influence might exist. Appropriate studies of various metabolic effects of insulin on the lens in vitro at insulin concentrations known to occur physiologically would be expected to help in assessing the effects of insulin on lens metabolism.

From the Division of Research, Scott and White Memorial Hospital, Scott, Sherwood and Brindley Foundation, Temple, Texas. Supported by NIH Research Grant EY 00404, awarded by the National Eye Institute. Submitted for publication May 1, 1980. Reprint requests: Dr. J. B. Coulter, III, Scott and White Memorial Hospital, 2401 South Thirty-First Street, Temple, Texas 76508.

Key words: insulin concentration, aqueous humor, paracentesis, blood-aqueous barrier, lens, diabetic cataract

REFERENCES


The efficacy of ascorbate treatment after severe experimental alkali burns depends upon the route of administration. Roswell R. Pfister, Christopher A. Paterson,* John W. Spiers,*, and Sonia Anderson Hates.

Rabbit eyes were subjected to severe alkali burns (35 sec, 12 mm, 1N sodium hydroxide). In one experiment, rabbits in the treated group received a daily subcutaneous injection of neutralized ascorbic acid solution (0.5 gm/kg body weight), while control animals received no treatment. At the termination of the experiment (30 days), 11 of 16 eyes (68.8%) in the control group had ulcerated or formed descemetoceles, and in the experimental (treated) group, 15 of 20 eyes (75%) had ulcerated, formed descemetoceles, or perforated. In a second experiment, burned rabbits received topical 10% ascorbic acid while control eyes were given the vehicle only. At the termination of the experiment (34 days), 16 of 20 eyes (80%) in the control group had ulcerated or perforated, compared to five of 18 eyes (27.8%) in the ascorbate treated groups.

The failure of systemic administration of ascorbic acid to prevent corneal ulceration could be explained on the basis of inadequate penetration of ascorbic acid into the anterior segment of severely burned rabbit eyes. On the other hand, immediate topical treatment of identically burned rabbit eyes achieved greatly elevated aqueous humor ascorbate levels and provided substantial protection from corneal ulceration and perforation.

After 20 sec, 12 mm, 1N sodium hydroxide burns of the rabbit eye, the level of ascorbic acid in the aqueous remains persistently depressed. It has been established that the incidence of corneal ulceration and perforation induced by such burns can be significantly reduced by elevating the aqueous humor ascorbate levels by subcutaneous injection or by topical administration of ascorbic acid. This beneficial effect was considered to be the result of restoring corneal levels of ascorbic acid, which is essential for collagen synthesis.

The purpose of this study was to determine whether parenteral and topical administration of ascorbic acid would each be effective in reducing...