Endophthalmitis in comparison to an intracapsular operation. The answer to this very important question would require a large clinical series, since the incidence of bacterial endophthalmitis is fortunately low.

There is a significant barrier effect produced by an intact posterior lens capsule, which inhibits the spread of bacterial infection from the anterior chamber to the vitreous cavity in the primate model. It is not possible from the data obtained in this study to answer the controversy over whether or not modern extracapsular surgery is superior to intracapsular surgery in the prevention of bacterial endophthalmitis. Despite the demonstration of a significant barrier effect to endophthalmitis produced by the posterior lens capsule, the two techniques probably vary in the frequency and/or size of inadvertent bacterial inocula introduced during surgery. A large, multicenter clinical study is necessary to answer this important question.

Key words: Endophthalmitis, cataract, posterior capsule

Acknowledgments. The authors gratefully acknowledge the assistance of James Stoutenburg, Ophthalmic Photographer, and Nadine Sokol, Ophthalmic Illustrator, in the preparation of this manuscript.

From the Bethesda Eye Institute and the Departments of Ophthalmology and Comparative Medicine, St. Louis University, School of Medicine, and the Bethesda General Hospital, St. Louis, Missouri. Supported in part by a research development grant from Research to Prevent Blindness, Inc., and from the Golden Fund, Bethesda Eye Institute, Department of Ophthalmology, St. Louis University School of Medicine. Submitted for publication January 28, 1983.

Characterization of Somatostatin-like Immunoreactivity in Vertebrate Retinas

David Marshak* and Tadaraka Yamada†

Large differences in retinal concentration of somatostatin-like immunoreactivity (SLI) were observed even among closely related species. Hog and chicken retinas, like those of goldfish and frog described previously, contained roughly equal amounts of SLI coeluting with somatostatin tetradecapeptide (S14) and octacosapeptide (S28) on Sephadex G 50 chromatography. In contrast, virtually all of the SLI from rat retina coeluted with S14, and nearly all of the bovine retinal SLI coeluted with S28. These species differences may reflect differences in post-translational processing of the various molecular forms of retinal SLI. Invest Ophthalmol Vis Sci 25:112–115, 1984.

Somatostatin-like immunoreactivity (SLI) in the retina has many of the properties of neurotransmitters. SLI is synthesized in the retina1 and stored in its intrinsic neurons (review in reference 2). Retinal SLI can be released by depolarizing stimuli in the presence of calcium, apparently by the same mechanism as neurotransmitters and other secretagogues.3 The retina is an excellent system to test whether somatostatin duplicates the natural transmitter's actions on postsynaptic cells. Such studies would be facilitated by elucidation of the structures of retinal SLI. This survey of retinal SLI in various vertebrates provides preliminary information about the structures of these molecules.

Materials and Methods. Eyes from cows, rabbits, chickens and carp were dissected within 5 min of death.
and frozen with liquid nitrogen or dry ice. Porcine retinas were dissected from ice-chilled eyes within 12 hours of enucleation. Frozen retinas were extracted in 3% acetic acid as previously described. Recovery of exogenously added S14 and S28 after extraction ranged from 89–100% and 84–96%, respectively. No conversion of S28 to S14 was observed following extraction. In some experiments, retinas were extracted in 3% acetic acid (3:1 volume: weight), and then, seven volumes of acetone were added, and the mixture was stirred for 4 hours at 20°C. After centrifuging the extract at 4°C for 15 min at 750 g, the supernatant was rotary-evaporated to remove the acetone. All samples were stored at −80°C until further evaluation.

**Gel filtration.** For analytical chromatography, small samples (1 ml) of extract were applied to a 1.0 × 120 cm Sephadex G 50 column and eluted with either 0.1 M ammonium acetate, pH 5.0, or 1% acetic acid at a flow rate of 15 ml/hr. For preparative chromatography larger samples of extract (up to 100 ml) were applied to 5.0 × 100 cm Sephadex G 50 column and eluted with 1% acetic acid at a flow rate of 72 ml/hr.

**Radioimmunoassay.** SLI was measured by radioimmunoassay as previously described using sheep antisomatostatin antiserum 1001. This antiserum is specific for the ring portion of S14 and cross-reacts 50% with S28. Half-maximal inhibition of binding occurred at 25 fmol/ml of S14.

**Results.** The concentrations of SLI in various vertebrate retinas are listed and compared in Table 1 to the findings in other published reports. Bovine retinas not only had the highest concentration of SLI per gram but also had the greatest mass of any species examined. When the same batch of frozen bovine retinal tissue was used, the yield with the simpler acetic acid extract, 39 pmol/g, was similar to the yield with the more elaborate acetic acid/acetone procedure, 44 pmol/g.

Two peaks of immunoreactivity were eluted on Sephadex G 50 chromatography of retinal extracts. Retinas of all species studied contained a form of SLI, which coeluted with S14. Essentially all of the rat retinal SLI was comprised of this molecular form (Fig. 1A). In contrast, only half of the SLI found in porcine and chicken retina coeluted with S14 (Figs. 1B and C). On chromatography of crude bovine retinal extracts, a molecular form of SLI eluted as a peak near the void volume as though it were a much larger molecule; however, treatment with 8 M urea apparently converted this peak to a form coeluting with S14 (Fig. 2), suggesting that it consisted of S14 noncovalently bound to a larger molecule. The vast majority of bovine retinal SLI, however, coeluted with synthetic S28.

**Discussion.** We detected SLI in all species examined in this study. The concentrations of SLI that we observed were comparable to those obtained by others using different antisera in their radioimmunoassays. As in our study, the highest contents of retinal SLI were previously observed in bovine tissues. It is interesting to note that closely related species may have markedly different concentrations; for example, retinal SLI concentration in goldfish was 8.5 times higher than in carp. Similar findings have been reported by Eskay, who noted that retinal SLI concentrations were 3.5 times higher in *Rana catesbiana* than in *Rana pipiens*. Some of the observed species differences, as well as differences in retinal SLI concentrations reported by different laboratories, may reflect differences in cross-reactivity of the two forms of retinal SLI with the various antisera used for assay. In addition, differences in SLI concentration expressed as a fraction of retinal weight may reflect varying amounts of vitreous humor adherent to the retina on dissection.

Most of the retinal extracts we examined contained two peaks of SLI on column chromatography, one that coeluted with S14 and the other that coeluted with S28. In other studies, we have purified the bovine retinal SLI, which coeluted with S28 and demonstrated that its amino acid sequence is indeed identical to that of S28. While we have not yet purified the small molecular form of retinal SLI, all previous purifications of the smaller SLI from other tissues have produced sequences identical to that of S14.

Although others have observed a molecular form of SLI larger than S28 in neural tissues, we were unable to detect such a peak in the retina. In the case of bovine retina, a small peak of SLI was observed in the void volume on Sephadex G 50 gel filtration, but chro-

### Table 1. Concentrations of somatostatin-like immunoreactivity in extracts of retinas from a variety of vertebrate species. Data obtained in our laboratory are listed on the left. The hog and rabbit retinas were extracted in acid/acetone; the rest were extracted in 3% acetic acid. Results from other laboratories are listed on the right. BU = Buckerfield et al.; E = Eskay et al.; L = Lake and Patel; R = Rorstad et al.; S = Shapiro et al.

<table>
<thead>
<tr>
<th>Species</th>
<th>Per gram</th>
<th>Per retina</th>
<th>Per gram</th>
<th>Per retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish</td>
<td>13.6</td>
<td>4.0</td>
<td>3.5</td>
<td>(R)</td>
</tr>
<tr>
<td>Carp</td>
<td>1.6</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>25.3</td>
<td>3.5</td>
<td>62.2</td>
<td>(E)</td>
</tr>
<tr>
<td>Rat</td>
<td>7.5</td>
<td>0.20</td>
<td>31.7</td>
<td>(E)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
<td>(E)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
<td>(R)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>4.3</td>
<td>0.16</td>
<td>4.3</td>
<td>(E)</td>
</tr>
<tr>
<td>Hog</td>
<td>9.8</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>39.4</td>
<td>15.1</td>
<td>29.9</td>
<td>(E)</td>
</tr>
</tbody>
</table>
Fig. 1. Sephadex G 50 sf chromatography of retinal extracts from rat (a), hog (b), and chicken (c). The elution volume (Ve) had been expressed as Kav (equal to Ve-Vo/Vs-Vo) to normalize for differences in column size. The columns were calibrated by noting the elution volume of the NaCl (Vs), bovine serum albumin (Vo), somatostatin-14 (S14) and somatostatin-28 (S28).

matography under dissociative conditions indicated that this peak probably represents a noncovalent complex of S14 with a larger molecule. In our studies with frog retina, we observed two peaks of biosynthetic SLI near the void volume, but on radioimmunoassay of Sephadex G 50 eluates of whole retinal extracts, these peaks were not detected. The relatively slow turnover rate of somatostatin in the retina may account for the presence of only minute or undetectable amounts of molecular forms larger than S28, presumably precursor forms. It is possible that our antibody did not cross-react with molecular forms larger than S28.

Species differences in the relative distribution of molecular forms of SLI corresponding to S14 and S28 are of some interest. While virtually all of the SLI in rat coeluted with S14, bovine retinal SLI mostly coeluted with S28. In other species, the relative distributions of the two forms of retinal SLI were more equal. The stability of exogeneously added S28 and S14 indicates that these differences do not reflect artifacts of extraction. The differences may be a reflection of differences in post-translational processing of S28 and S14. Alternatively, it is possible that differences in processing of the somatostatin gene generates different products, namely S14 and S28.

Fig. 2. Sephadex G 50 chromatography of bovine retinal extracts. Extracts were chromatographed before and after one hour of incubation in 8 M urea. Both samples were applied to the same column (1 X 120 cm) of Sephadex G 50 sf equilibrated in 0.1 M ammonium acetate, pH 5.0. Each 2 ml fraction that was eluted was assayed for SLI. The column was calibrated as noted in Figure 1.
No. 1 Reports

Key words: retina, neuropeptides, somatostatin, radioimmunoassay, prosomatostatin

Acknowledgments. We wish to thank Bruce Osadchey and Mimi Takami for their technical assistance and Anna Holland for the preparation of this manuscript.

From the Medical and Research Divisions, VA Wadsworth Medical Center, and The Jules Stein Institute, UCLA School of Medicine, Los Angeles, California. Supported by USPHS grants AM 17328, EY 01190 and EY 04172 and VA research funds. *Present address: The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138. §Present address: Gastroenterology Division, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109. Submitted for publication February 18, 1983. Reprint requests: Tadataka Yamada, M.D., Gastroenterology Division, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109.

References


Effect of Chlorpromazine In Vitro on Release of Enzymes from Lysosomes of the Bovine Retinal Pigment Epithelium

Takashi Shiono, Seiji Hayasaka, and Katsuyoshi Mizuno

The effects of chlorpromazine on lysosomal enzymes and the release of enzymes from lysosomes of bovine retinal pigment epithelial cells were studied in vitro, using cathepsin D, arylsulfatase, and acid phosphatase as lysosomal marker enzymes. Chlorpromazine had little effect on the enzyme activity of cathepsin D and arylsulfatase and slightly decreased that of acid phosphatase. Chlorpromazine accelerated considerably the release of cathepsin D and arylsulfatase, but only minimally affected the release of acid phosphatase. The release of these enzymes from lysosomes depended on the dose of chlorpromazine. Invest Ophthalmol Vis Sci 25:115–117, 1984

Chlorpromazine has been widely used for the treatment of schizophrenia. Changes in the retinal pigment epithelium of patients receiving high doses of the psychotropic drug have been demonstrated. Chlorpromazine has also been found to have a high affinity of melanin granules in ocular tissues. However, the pathogenetic mechanisms responsible for chlorpromazine-induced retinopathy remain unclear.

The retinal pigment epithelium contains both a large amount of pigment granules and high activity of lysosomal enzymes. Because of their hydrolytic capacity, lysosomal enzymes in the retinal pigment epithelium take an important role in pathologic tissue injuries as well as in physiologic processes.

The purpose of the present study was, therefore, to investigate the in vitro effect of chlorpromazine on lysosomal enzyme activities and release of the enzymes from lysosomes of the bovine retinal pigment epithelium, using cathepsin D, arylsulfatase, and acid phosphatase as marker enzymes.

Materials and Methods. Lysosomal fractions of the bovine retinal pigment epithelial cells were prepared as described previously. Sixty bovine eyes maintained at 4°C from the time of slaughter were used in one experiment. The bovine retinal pigment epithelial cells were gently brushed out of the eye-cup in 250 mM sucrose. The isolated cells were homogenized using a Potter-Elvehjem homogenizer, and the homogenate was centrifuged at 4°C for 10 min to obtain the supernatant. The pellet was resuspended in 250 mM sucrose, and centrifuged at the same force. The supernatants were combined and centrifuged at 25,000 g for 20 min. After centrifugation, the pellet was re-

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933111/ on 10/02/2017