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

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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 Ophthal 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.1 Chlorpromazine has also been found to have a high affinity of melanin granules in ocular tissues.2 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.3-5 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.6 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 4,500 g 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-
suspended in 250 mM sucrose and centrifuged at the same force. The final pellet was resuspended in 250 mM sucrose, and the final suspension was used as the lysosomal fraction.

The lysosomal extract was prepared as described previously. Following seven-time freeze-thawing, the lysosomal fraction was centrifuged at 25,000 g for 20 min. The clear supernatant obtained was used as a lysosomal extract.

The effect of the drug on enzyme activity in the lysosomal extract was measured as follows: the lysosomal extract (1 mg) was incubated in 250 mM sucrose-40 mM Tris-acetate buffer (pH 7.4) at 37°C for 60 min, with or without the drug or Triton X-100, in a total volume of 1 ml. After incubation, each tube was cooled to 4°C and centrifuged at 25,000 g for 20 min. Enzyme activity after incubation was compared to that before incubation. Enzyme assays were described in the Materials and Methods.

The effects of chlorpromazine on the release of enzyme from lysosomes were studied in the presence of air and light as previously reported. The lysosomal fraction (5 mg protein) was incubated with continuous shaking in 250 mM sucrose-40 mM Tris-acetate buffer (pH 7.4) at 37°C for 60 min, with or without the drug chlorpromazine or Triton X-100, in a total volume of 1 ml. After incubation, each tube was cooled to 4°C, and centrifuged at 25,000 g for 20 min to pellet the intact lysosomes. Enzyme activity in the supernatant was determined. The enzyme activity after incubation was compared to that treated with Triton X-100 (0.1%).

Cathespin D [EC 3.4.23.5.] activity was spectrophotometrically measured by the release of tyrosine as described previously: the mixtures were incubated in a total volume of 0.5 ml for 20 min at 37°C in 0.1 M-citrate-sodium citrate buffer at pH 4.0 with bovine serum albumin (10 mg/0.5 ml) as substrate. The released tyrosine was measured spectrophotometrically.

### Table 1. Effect of chlorpromazine on the enzyme activities in lysosomal extract†

<table>
<thead>
<tr>
<th>Chlorpromazine</th>
<th>Incubation time (min)</th>
<th>Cathespin D</th>
<th>Arylsulfatase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>None</td>
<td>60</td>
<td>97.5 ± 1.6</td>
<td>99.8 ± 3.4</td>
<td>88.6 ± 3.8</td>
</tr>
<tr>
<td>2 × 10⁻³ M</td>
<td>60</td>
<td>96.5 ± 2.7</td>
<td>103.3 ± 3.9</td>
<td>72.4 ± 2.3</td>
</tr>
<tr>
<td>1.5 × 10⁻³ M</td>
<td>60</td>
<td>100.2 ± 2.9</td>
<td>103.3 ± 3.1</td>
<td>74.6 ± 2.7</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>60</td>
<td>104.3 ± 3.1</td>
<td>103.4 ± 2.8</td>
<td>76.1 ± 3.1</td>
</tr>
<tr>
<td>7.5 × 10⁻⁴ M</td>
<td>60</td>
<td>103.9 ± 4.0</td>
<td>103.3 ± 3.1</td>
<td>78.6 ± 2.3</td>
</tr>
<tr>
<td>5 × 10⁻⁴ M</td>
<td>60</td>
<td>104.5 ± 2.1</td>
<td>103.3 ± 1.2</td>
<td>81.6 ± 5.7</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>60</td>
<td>100.7 ± 4.0</td>
<td>96.6 ± 3.6</td>
<td>86.6 ± 3.8</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>60</td>
<td>100.2 ± 2.9</td>
<td>97.5 ± 3.5</td>
<td>89.3 ± 3.1</td>
</tr>
</tbody>
</table>

* Values are the Mean ± SEM of three separate experiments.
† The lysosomal extract (one mg protein) was incubated in 250 mM sucrose-40 mM Tris-acetate buffer (pH 7.4) at 37°C for 60 min in a total volume of one ml. After incubation, each tube was cooled and centrifuged at 25,000 g for 20 min. Enzyme activity in the supernatant was measured and was compared to that before incubation. Enzyme assays were described in the Materials and Methods.

### Table 2. Effect of chlorpromazine on release of enzymes from lysosomes* |

<table>
<thead>
<tr>
<th>Additions</th>
<th>Cathespin D†</th>
<th>Arylsulfatase‡</th>
<th>Acid phosphatase§</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>51.2 ± 6.4 (18.5)</td>
<td>28.8 ± 2.5 (9.9)</td>
<td>50.9 ± 3.7 (8.5)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>160.3 ± 8.6 (58.0)</td>
<td>234.9 ± 12.5 (80.9)</td>
<td>121.6 ± 5.3 (20.3)</td>
</tr>
<tr>
<td>2 × 10⁻³ M</td>
<td>131.0 ± 3.6 (47.4)</td>
<td>159.5 ± 5.2 (51.1)</td>
<td>86.9 ± 5.0 (14.5)</td>
</tr>
<tr>
<td>1.5 × 10⁻³ M</td>
<td>102.4 ± 8.2 (37.0)</td>
<td>95.7 ± 5.4 (32.9)</td>
<td>77.1 ± 4.2 (13.0)</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>81.7 ± 8.0 (29.5)</td>
<td>51.1 ± 5.2 (17.6)</td>
<td>59.7 ± 7.2 (10.0)</td>
</tr>
<tr>
<td>7.5 × 10⁻⁴ M</td>
<td>58.7 ± 7.1 (21.2)</td>
<td>32.2 ± 3.2 (11.1)</td>
<td>58.1 ± 5.0 (9.7)</td>
</tr>
<tr>
<td>5 × 10⁻⁴ M</td>
<td>48.9 ± 7.3 (17.7)</td>
<td>28.3 ± 2.1 (9.9)</td>
<td>51.3 ± 3.0 (8.6)</td>
</tr>
<tr>
<td>10⁻⁴ M</td>
<td>49.3 ± 6.7 (17.8)</td>
<td>28.8 ± 3.3 (9.9)</td>
<td>52.8 ± 3.0 (8.8)</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>20.1 ± 10.2 (100)</td>
<td>290.0 ± 8.5 (100)</td>
<td>598.3 ± 14.7 (100)</td>
</tr>
</tbody>
</table>

* Values are the Mean ± SEM of three separate experiments. Numbers in parentheses represent percentages of total activity released by 0.1% Triton X-100. The lysosomal fraction (5 mg protein) was incubated in 250 mM sucrose-40 mM Tris-acetate buffer (pH 7.4) at 37°C for 60 min with or without additions in a total volume of one ml with continuous shaking. After incubation, each tube was cooled and centrifuged at 25,000 g for 20 min. Enzyme activity in the supernatant was measured and compared to that treated with Triton X-100 (0.1%).
† μg tyrosine released per 20 min per ml.
‡ μg p-nitrocatechol released per 20 min per ml.
§ μg p-nitrophenol released per 20 min per ml.
in the same way as described previously: the mixtures were incubated in a total volume of 0.5 ml for 20 min at 37 °C with 3 μ moles of p-nitrocatechol sulfate as substrate in 0.1 M acetate-sodium acetate buffer (pH 5.3). The released p-nitrocatechol was measured spectrophotometrically.

Acid phosphatase [EC. 3.1.3.2.] activity was spectrophotometrically measured by the release of p-nitrocatechol described previously: the mixture was incubated in a total volume of 0.5 ml for 20 min at 37 °C with 0.5 μ mol of p-nitrophenyl phosphate as substrate in 0.1 M acetate-sodium acetate buffer at pH 4.5.

Protein content was determined by the method of Lowry and co-workers with bovine-sodium albumin as the standard.

Chlorpromazine, p-nitrophenol, and p-nitrophenyl phosphate were obtained from Sigma Chemical Co. (St. Louis, MO). All common chemicals used in the present experiment were reagent grade.

Results. Chlorpromazine showed little effect on the activities of either cathepsin D and arylsulfatase (Table 1); however, at concentrations greater than 5 × 10^-4 M the drug slightly decreased the activity of acid phosphatase.

Chlorpromazine also appeared to cause the apparent release of cathepsin D and arylsulfatase from lysosomes (Table 2). The enzyme activities released in the supernatant after incubation were dose dependent.

Discussion. In this experiment, it has been observed that chlorpromazine appears to increase the activity of lysosomal enzymes in the supernatant after incubation. This finding strongly suggests that chlorpromazine plays a role in the release of these enzymes from lysosomes, since the drug showed little effect on the activities of either cathepsin D and arylsulfatase and slightly decreased acid phosphatase activity. The increased activity of lysosomal enzymes in the supernatant after incubation was not due to changes in pH or osmolarity in the incubation mixture by chlorpromazine, because the concentration of drug used in the present experiment had little effect on these factors.

Interestingly, the release of cathepsin D and arylsulfatase appeared to be influenced more than that of acid phosphatase. A similar differential release of enzymes more lysosomes has also been reported in several tissues. The observed difference in the enzyme release by chlorpromazine may suggest a specific change of membrane permeability of lysosomes rather than disruption. This effect of chlorpromazine may be the result of lipid peroxidation since this experiment was done in the presence of air and a pH-dependent effect of chlorpromazine on the release of enzyme from rat liver lysosomes has also been described.

Under acidic conditions, at a concentration of 5 × 10^-4 M, chlorpromazine decreased the release of acid phosphatase from lysosomes. At neutral pH, on the other hand, the drug increased the release.

Retinal pigmentary changes have also been reported in patients receiving high doses of chlorpromazine. Although high doses of chlorpromazine were required to produce the effect, it is possible that chlorpromazine may accumulate in the retinal pigment epithelium to such high concentrations, because of its high affinity for melanine granules. This could result in the release of hydrolytic enzymes from lysosomes. The hydrolytic enzymes released by chlorpromazine may damage neighboring tissues, resulting in fundus changes seen in patients receiving high doses of the drug.

Key words: chlorpromazine, lysosome, retinal pigment epithelium

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