INHIBITION BY HYDROXYUREA OF THE REBOUND OF GRANULOMATOUS INFLAMMATION FOLLOWING WITHDRAWAL OF GLUCOCORTICOID THERAPY

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In a previous paper (1) from this laboratory it was shown that a rebound of granulomatous inflammation of rats took place after withdrawal of glucocorticoid therapy, resulting in a rapid recovery in the wet weight of the granuloma and in the total contents of tissue DNA and non-collagen protein. In an experiment of incorporation of radioactive precursors into tissue components in vitro we also observed that the reactivation of DNA synthesis followed the reactivation of non-collagen protein synthesis and the synthetic activity of collagen overtook the control level only at the latest stage in this rebound process. The fact that the synthetic activity of DNA of the granuloma was recovered rapidly in this rebound process suggests that newly formed tissue cells play important roles in the course of the reactivation of the granuloma. The present research, therefore, was performed to investigate direct effects of DNA inhibition on the reactivation of the granulomatous tissue components. Hydroxyurea, an agent that is known to possess specific inhibitory actions on DNA synthesis with little direct action on the synthetic activities for RNA and proteins was used. (2-4).

Male rats of Donryu strain, 6–7 weeks of age, weighing 130–150 g were used. The animals were fed on laboratory chow and water ad libitum and maintained in a fixed-temperature environment. Granulomatous tissue was induced by injecting a 2% solution of carrageenin (Marine Colloid Inc., Springfield, N.J., U.S.A.) as described previously (5). Hydrocortisone acetate (Saline suspension (25 mg/ml) was obtained from Nippon Merck-Banyu Co. Ltd., Tokyo) was administered directly into the granuloma pouch in the form

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of a suspension in 0.5% carboxymethylcellulose aqueous solution. Hydroxyurea (Sigma Chemical Co.) was dissolved in pyrogen-free sterile 0.9% NaCl just before the injection into the granuloma pouch (6). Control animals were given the vehicle only. On the day of the first injection of hydrocortisone acetate, the animals were grouped at random with respect to the size of the granuloma pouch and the body weight. At the end of the experimental period, the animals were sacrificed by excision of the carotid artery. The granulomatous tissues were harvested and the wet weight and the carcass weight (designated as "net body weight") were measured. The amounts of collagen hydroxyproline and non-collagen protein were determined according to the procedures previously described (7).

Details of procedures of the extraction and determination of DNA were as described previously (1).

Hydrocortisone acetate (3 mg/kg) was daily injected into the granuloma pouch for 3 days from day 5 to day 7. Various doses of hydroxyurea (50, 150 or 250 mg/kg) were administered directly into the granuloma pouch of the hydrocortisone-treated rats at 12 hr intervals from day 8 to day 10 in order to determine the appropriate effective dose of hydroxyurea. Animals were sacrificed 12 hr after the last injection of hydroxyurea and wet weight and DNA content of the granuloma were determined. In case of the group treated with 250 mg/kg of hydroxyurea at 12 hr intervals for 6 times, strong inhibitions of the recoveries in DNA content (40% inhibition) and in wet weight of the granuloma (43% inhibition) were found, while lower doses (50 or 150 mg/kg) of hydroxyurea did not significantly affect either DNA content or wet weight of the granuloma. However, there was a marked inhibition on the body weight increase of rats treated with 250 mg/kg of hydroxyurea.

Based on the results of preliminary experiments described above, the effect of hydroxyurea on the rebound of the granuloma after withdrawal of hydrocortisone treatments was examined by using a dose of 250 mg/kg of hydroxyurea and pair-fed control animals in individual cages to synchronize their body weight changes with those of the hydroxyurea-treated animals. The results are summarized in Table 1. Wet weight and the total

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Net body wt. (g)</th>
<th>Granuloma wet wt. (g)</th>
<th>Total DNA (mg)</th>
<th>Collagen hyp. (mg)</th>
<th>Non-collagen protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone (3 mg/kg) × 3 (8-day-sacrificed)</td>
<td>8</td>
<td>152.6 ± 6.6</td>
<td>2.82 ± 0.15</td>
<td>9.8 ± 0.8</td>
<td>4.87 ± 0.15</td>
<td>151.9 ± 10.3</td>
</tr>
<tr>
<td>11-day-control (Pair-fed-control)</td>
<td>8</td>
<td>142.0 ± 6.3</td>
<td>4.97 ± 0.24</td>
<td>17.4 ± 1.1</td>
<td>8.96 ± 0.67</td>
<td>278.2 ± 10.5</td>
</tr>
<tr>
<td>Hydrocortisone (3 mg/kg) × 3 (Pair-fed-recovery)</td>
<td>8</td>
<td>143.5 ± 5.8</td>
<td>4.59 ± 0.48</td>
<td>16.2 ± 1.2</td>
<td>6.82 ± 0.59</td>
<td>245.4 ± 23.3</td>
</tr>
<tr>
<td>Hydrocortisone (3 mg/kg) × 3 + Hydroxyurea (250 mg/kg) × 6</td>
<td>8</td>
<td>143.4 ± 8.4</td>
<td>3.93 ± 0.32</td>
<td>10.5 ± 1.0</td>
<td>5.55 ± 0.41</td>
<td>206.9 ± 14.6</td>
</tr>
<tr>
<td>N.S.</td>
<td>P&lt;0.05</td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td></td>
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</tbody>
</table>

* Data are shown as means ± S.E. The percentage of the pair-fed control is shown in parenthesis. N.S. = not significant.
amounts of DNA and non-collagen protein of the granuloma in 'pair-fed-recovery group' which was pair-fed after withdrawal of the steroid treatments were recovered completely on day 11 even in the pair-fed conditions. However, total amounts of collagen hydroxyproline in the granuloma of the pair-fed recovery group did not exceed those of the pair-fed control group, although significant increase of collagen hydroxyproline was seen when compared with those of 8-day-sacrificed group. These results are virtually identical with those described in the previous paper (1) in which animals were maintained without pair-feeding.

In the group treated with 6 times injections of hydroxyurea after withdrawal of the steroid treatments, a strong inhibition in the recovery of DNA contents occurred. This is in agreement with the results of the preliminary experiments described above. Accompanying the DNA inhibition, significant inhibitions of the recovery in the wet weight of the granuloma and in the amounts of collagen and non-collagen protein were also found in the hydroxyurea-treated group, while those values, except for collagen contents, of the pair-fed recovery group recovered to the levels of the pair-fed control. These results, therefore, suggest that the inhibition of DNA synthesis gives a strong influence on the reactivation of the other components of the granulomatous tissue after cessation of the steroid treatments.

A second series of experiments were done in order to investigate whether hydroxyurea has an anti-inflammatory activity which may cause inhibition of the rebound of granuloma. Hydroxyurea (250 mg/kg) was injected into the pouch of normal granuloma 6 times at 12 hr intervals from day 5 to day 7. The granulomatous tissues were harvested 12 hr later after the last injection of hydroxyurea. Wet weight and total contents of DNA, non-collagen protein and collagen hydroxyproline of the granuloma were determined. As shown in Table 2, a marked reduction in DNA contents by the consecutive treatments of hydroxyurea was demonstrated without any significant changes in the wet weight and other components of the granuloma, suggesting that hydroxyurea did not display any anti-inflammatory or anti-exudative actions during the experimental period of the present study. From all of the results described above, it can be postulated that rebound phenomenon.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Net body wt. (g)</th>
<th>Granuloma wet wt. (g)</th>
<th>Total DNA (mg)</th>
<th>Collagen hyp. (mg)</th>
<th>Non-collagen** protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-day-control</td>
<td>7</td>
<td>145.0±7.3</td>
<td>4.18±0.15</td>
<td>10.3±0.2</td>
<td>4.16±0.44</td>
<td>51.4±3.0</td>
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<tr>
<td>8-day-control (Pair-fed)</td>
<td>7</td>
<td>138.8±5.6</td>
<td>4.50±0.29</td>
<td>16.2±0.7</td>
<td>8.51±0.96</td>
<td>78.3±4.7</td>
</tr>
<tr>
<td>Hydroxyurea (250 mg/kg)×6</td>
<td>7</td>
<td>136.4±9.0</td>
<td>4.45±0.39</td>
<td>10.7±0.6</td>
<td>9.10±0.85</td>
<td>71.4±3.9</td>
</tr>
</tbody>
</table>

N.S. = not significant.

* Data are shown as means±S.E. The percentage of 8-day-control is shown in parenthesis. N.S. = not significant.

** The collagen-free residue was obtained by autoclaving the tissue at 120°C for 1 hr.
is eventually prevented due to an interruption of cellular proliferations, which is caused by a strong reduction in DNA contents of granulomatous tissue in rats treated with hydroxyurea.

REFERENCES

INHIBITIONS BY COLCHICINE, VINBLASTINE AND CYTOCHALASIN-B OF THE STIMULATORY EFFECT OF THE CYTOPLASMIC FRACTION ON CATECHOLAMINE RELEASE FROM ADRENOCLIVAL GRANULES

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Microtubules and microfilaments have been regarded as being involved in release of hormones and amines, since agents, such as colchicine and vinblastine, and cytochalasin-B, which disrupt microtubules and microfilaments, respectively, inhibit the release of hormones or amines from cells (1-11). Stimulation of the release of catecholamine from perfused adrenal gland by acetylcholine or nicotine is also inhibited by colchicine, vinblastine or cytochalasin-B (12-15).

In our previous studies on the intracellular mechanism of catecholamine release, using isolated adrenal medullary granules, it was demonstrated that the release of catecholamine is stimulated by ATP-Mg$^{2+}$ in the presence of a low concentration of Ca$^{2+}$ (<10$^{-5}$ M) (16, 17) and that a cytoplasmic fraction of adrenal medulla (supernatant obtained by centrifugation at 105,000 g for 2 hr) accelerates ATP-Mg$^{2+}$ stimulated catecholamine release from the granules (18).

In the present work, the effects of colchicine, vinblastine and cytochalasin-B on the stimulatory effect of the cytoplasmic fraction on catecholamine release were examined, to determine whether microtubules and microfilaments-like components are involved in the stimulatory effect of the cytoplasmic fraction. Catecholamine storage granules and the cytoplasmic fraction from bovine adrenal medulla were prepared as described previously (18). Incubations were carried out as described in the footnote of Fig. 1.

Results are summarized in Fig. 1. During the incubation period, 18.2 ± 3.6% (mean ±