INHIBITION OF PROSTAGLANDIN SYNTHESIZING ENZYMES BY HAPTOGLOBIN AND PLASMA OF RATS WITH INFLAMMATION

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Accepted June 30, 1980

Abstract—Administration of carrageenin or adjuvant to rats to induce inflammation produced increases in the plasma haptoglobin level. Simultaneously, there was an increase in the activity of the plasma inhibitor of prostaglandin E\textsubscript{2} synthesis. Partially purified haptoglobin inhibited the microsomal over-all conversion of arachidonic acid to prostaglandin E\textsubscript{2}. The addition of the haptoglobin inhibited two heme-dependent reactions catalyzed by a purified enzyme of seminal vesicle microsome, i.e., the prostaglandin G\textsubscript{2} synthesis from arachidonic acid and the conversion of prostaglandin G\textsubscript{2} to H\textsubscript{2}. However, the plasma inhibitory activity was accounted for only partially by the haptoglobin contained in the plasma from treated rats.

Prostaglandins (PGs) have been implicated as modulators in various aspects of inflammation (1). Recently, an endogenous inhibitor of PG synthetase with anti-inflammatory activity was found in mammalian plasma (2). The inhibitory activity was explained at least partially by haptoglobin (Hp) contained in the plasma (3). Albumin was also found to have this inhibitory activity (4), and it has also been reported that the serum of carrageenin-treated rat inhibited the PG release by leukocytes phagocytosing killed bacteria (5).

PG biosynthesis proceeds in a series of enzymatic reactions involving fatty acid cyclooxygenase (arachidonic acid→PGG\textsubscript{2}) and PG hydroperoxidase (PGG\textsubscript{2}→PGH\textsubscript{2}). These two reactions require hematin or hemoglobin as an activator (6, 7). In view of the haptoglobin-hemoglobin interaction (8, 9), we investigated the inhibitory effect of Hp and the plasma from rats with inflammation on these heme-dependent syntheses of PG endoperoxides, and our findings are reported herein.

MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats weighing 150–200 g, were used in all experiments. A suspension of 1% carrageenin in 0.1 ml of saline or 0.6 mg of killed Mycobacterium butyricum (Difco) in 0.1 ml of liquid paraffin was injected into the right hind paw to induce inflammation. Blood was collected from the eye ground after the rats had been anesthetized with ether.

Purification and determination of haptoglobin: Rat Hp was partially purified from
plasma of carrageenin-treated rats, according to the method of Javid and Liang (10). Rat plasma was collected 24 hr after carrageenin injection and was passed through a rat hemoglobin affinity column. The purity of the preparation was about 87%, as examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The level of plasma Hp was measured on the basis of the peroxidase activity of Hp-hemoglobin complexes and was expressed as the hemoglobin binding capacity in mg per 100 ml of plasma (11).

Assay of prostaglandin biosynthesis: The microsomal over-all synthesis of PGE$_2$ from arachidonic acid was assayed in the following reaction mixture: 1 mg protein/ml bovine seminal vesicle microsome (Miles Laboratories), 0.25 nM hemoglobin, 5 mM tryptophan, 0.3 mM glutathione and 33 nM arachidonic acid (Sigma) and 50 mM Tris-HCl buffer (pH 7.5) in a total volume of 0.2 ml. Reactions were carried out at 37°C for 8 min, and the reaction mixture was subjected directly to the PGE$_2$ bioassay on rat stomach fundus, with authentic PGE$_2$ as a standard.

The fatty acid cyclooxygenase activity was determined using 100 nM [1-$^{14}$C] arachidonic acid (Radiochemical Centre) and the PG hydroperoxidase activity with 50 nM [1-$^{14}$C] PGG$_2$, as described previously (7). The enzyme used was a purified preparation of PG endoperoxide synthetase of bovine seminal vesicle microsome (6). The enzyme unit was defined as described elsewhere (6).

RESULTS

Plasma inhibitor of prostaglandin E$_2$ biosynthesis: Rat plasma collected 24 hr after carrageenin injection inhibited the PGE$_2$ synthesis catalyzed by bovine seminal vesicle microsome. The extent of inhibition was dependent on the concentration of the plasma (Fig. 1A). This plasma at 1% concentration inhibited the PGE$_2$ biosynthesis by about 80% whereas the plasma of untreated rats at the same concentration showed only 20% inhibition. On the other hand, the Hp content 24 hr after carrageenin injection was 158.6±16.6 mg/100 ml while that of untreated animals was 53.8±7.0 mg/100 ml. Thus, the plasma from carrageenin-treated rat showed a higher inhibitor activity of the PGE$_2$

![Fig. 1. Effect of plasma from treated rats and that of Hp on over-all PGE$_2$ synthesis. The PGE$_2$ synthesis by bovine seminal vesicle microsome was determined, as described in Materials and Methods, in the presence of plasma collected from rats 24 hr after carrageenin injection (A) or partially purified Hp (B).](image-url)
synthesis and an elevated Hp level, as compared with the plasma of untreated rats.

As shown in Table 1, the PGE₂ synthesis was also inhibited by the plasma collected 7, 14 and 21 days after adjuvant injection. The plasma Hp level in arthritis-induced rats was about 3–4 times higher than the normal level during a period of 1 to 3 weeks after adjuvant injection. The plasma collected from animals with severe arthritis inhibited the PGE₂ biosynthesis to a greater extent and showed a higher Hp level (Table 2).

**Effect of haptoglobin on prostaglandin E₂ biosynthesis:** Either the partially purified Hp (63 to 500 µg/ml) or the plasma from treated rats (Hp content, 5 to 40 µg/ml) inhibited PGE₂ synthesis in a dose dependent manner (Fig. 1). When Fig. 1A and B were compared,

### Table 1. Changes in the inhibitory effect of plasma on PGE₂ biosynthesis and the plasma Hp level after adjuvant injection

| Experiments                  | Time after adjuvant injection | PGE₂ biosynthesis | Plasma Hp level |
|------------------------------|-------------------------------|-------------------|-----------------|
|                              | 7 days                        | 14 days           | 21 days         |
| No addition                  |                               |                   |                 |
| Normal plasma                | 0.18±0.06                      | 0.15±0.01         | 0.27±0.02       |
| Plasma from treated rats     | 0.15±0.01                      | 0.14±0.02         | 0.21±0.03       |
|                              | (16.7)                         | (6.7)             | (22.2)          |
| 1%                           | 0.04±0.01**                    | 0.05±0.01*        | 0.03±0.01**     |
|                              | (77.8)                         | (66.7)            | (88.9)          |
| 2%                           | NT                            | 0.03±0.00*        | NT              |
|                              | (80.0)                         |                   |                 |
| Normal plasma                | 83.8±8.7                       | 67.7±6.9          | 61.5±1.8        |
| Plasma from treated rats     | 220.8±6.7**                    | 276.1±5.2**       | 237.1±10.1**    |

Rats were given the adjuvant, and blood was collected at the indicated time. The PGE₂ biosynthesis by bovine seminal vesicle microsome and the plasma Hp level were determined, as described in Materials and Methods. a) Mean±s.e.m. of 4 to 6 assays with pooled plasma. b) Mean±s.e.m. of 6 to 16 rats. Significant difference from normal rat plasma (*, p<0.01, **, p<0.001)

### Table 2. Inhibitory effect of plasma on PGE₂ biosynthesis and the plasma Hp level in rats with mild and severe adjuvant arthritis

| Inflammation | Score | PGE₂ biosynthesis | Hp |
|--------------|-------|--------------------|----|
| Mild         | 2.3±0.4| 27.3±3.2           | 166.3±15.1 |
| Severe       | 13.0±0.0*| 61.9±2.2*          | 266.7±3.8** |

Rats were given the adjuvant, and blood was collected after 21 days. The PGE₂ biosynthesis by bovine seminal vesicle microsome and the plasma Hp level were determined, as described in Materials and Methods. The values are presented as mean ±s.e.m. of 3 rats. The severity of inflammation was evaluated by observations of the peripheral joints, tail and ears and expressed by the score with a maximum of 16 (12). Significant difference from rats with mild inflammation (*, p<0.01 and **, p<0.001)
the extent of inhibition by the plasma from treated rats was accounted for only in part by the inhibitory effect of the Hp contained in the plasma. ID50 of the plasma from treated rats with respect to Hp concentration was 11 μg/ml whereas ID50 of Hp as such was 175 μg/ml. As the concentration was raised, almost complete inhibition was observed with the plasma from treated rats but not with Hp itself.

**Effects of haptoglobin on fatty acid cyclooxygenase and prostaglandin hydroperoxidase reactions:** Using the purified enzyme of bovine seminal vesicle microsome (6) the two enzyme reactions (arachidonic acid → PGG₂ and PGG₂ → PGH₂) were investigated, with respect to the inhibitory effect of Hp. The cyclooxygenase activity was demonstrated with arachidonic acid as substrate in the presence of either hematin (6, 7) or manganese protoporphyrin (13). The hydroperoxidase activity was assayed with PGG₂ as substrate in the presence of both hematin and tryptophan (7). As shown in Fig. 2A, both the hematin-activated cyclooxygenase and hydroperoxidase reactions were inhibited by Hp in a dose dependent manner. In contrast, the manganese protoporphyrin-activated cyclooxygenase was affected to a much lesser extent. Plasma from treated rats gave essentially identical results, and was much more effective in terms of Hp concentration (Fig. 2B). Neither of the two enzyme activities was significantly affected by the normal plasma, as shown in Fig. 2C.

**DISCUSSION**

As reported by other investigators (5) and also described herein, the activity of PGE₂ synthesis inhibitor increased in the plasma of carrageenin-treated rats. We noted similar findings in the case of adjuvant arthritis. Concomitantly, there was an increase in the plasma Hp level. On the other hand, the addition of partially purified Hp inhibited the PGE₂ synthesis from arachidonic acid catalyzed by bovine seminal vesicle microsome. These findings suggested that the endogenous inhibitor activity is partly due to the plasma Hp. However, the actual concentration of Hp in the plasma from treated rats was too low, indicating a
possible presence of another inhibitor such as albumin, as reported by Kendall et al. (4). Furthermore, the possibility cannot be ruled out that such an inhibitor still contaminating the purified Hp preparation is actually working to inhibit the enzymes.

It is well known that Hp binds to hemoglobin by the so-called protein-protein interaction (8, 9). The earlier steps of PG biosynthesis (cyclooxygenase and hydroperoxidase reactions) are heme-dependent reactions (6, 7, 13). On the basis of these observations, the effect of Hp on these two enzymatic steps was investigated. We found that both the cyclooxygenase and hydroperoxidase activities demonstrated in the presence of hematin were markedly affected by Hp as such and also by the Hp-containing plasma of rats with inflammation. The lack of inhibition of the manganese protoporphyrin-activated cyclooxygenase with the addition of Hp suggested an interaction of Hp with hematin, although the actual binding of these two components was not experimentally demonstrated. Our experiments using the purified enzyme clarified to some extent, the mechanism of PG synthesis inhibition by Hp, one of the endogenous inhibitors in the plasma.

Acknowledgments: The work of K. M. and S. Y. was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture of Japan, by a research grant from the Intractable Diseases Division, Public Health Bureau, Ministry of Health and Welfare of Japan, and by grants from the Japanese Foundation on Metabolism and Diseases, the Naito Foundation, the Asahi Scholarship Promotion Fund and the Japan Research Foundation for Clinical Pharmacology.

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