Mechanisms for Glucocorticoid Inhibition of Immediate Hypersensitivity Reactions in Rats

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ABSTRACT—The inhibitory mechanisms of immediate hypersensitivity reactions by glucocorticoid (GC) were studied in rats. Homologous passive cutaneous anaphylaxis (PCA) mediated by IgE antibodies and cutaneous reactions caused by histamine, serotonin and leukotriene C₄ were elicited at the same time in the same rats. Three kinds of GC, hydrocortisone, prednisolone and dexamethasone, inhibited all these reactions significantly. Although mediator-induced cutaneous reactions were inhibited transiently around 2 hours after GC administration, inhibition of PCA was more potent and lasted longer. A time lag seemed to be essential for both inhibitions. IgE antibody-mediated histamine release in vivo in the rat peritoneal cavity was also inhibited by GC administration significantly, and the inhibition was long lasting when compared to those of the mediator-induced cutaneous reactions. Tyrosine aminotransferase (TAT) activity in the rat liver increased significantly by GC administration, and the increased TAT activity was completely abrogated by simultaneous administration of 5 mg/kg of cycloheximide (CH). In the same experimental condition, although inhibition of histamine-induced cutaneous reaction by GC was completely abrogated, the inhibition of PCA elicited at the same time in the same rats was only partially attenuated. Furthermore, the same dose of CH little affected the dexamethasone inhibition of histamine release in the rat peritoneal cavity, although the increase of TAT activity in the liver of the same rats was completely abrogated. These results demonstrate that PCA is inhibited by GC through at least 2 mechanisms, inhibition of mediator release from mast cells and non-specific inhibition of vascular permeability increase caused by released mediators. Although the latter action of GC is dependent upon protein synthesis, the former seems to be mediated by a unique mechanism independent of protein synthesis.

Keywords: Glucocorticoid, Anti-allergic action, Passive cutaneous anaphylaxis, Cycloheximide, Protein synthesis

Glucocorticoid (GC) is widely used in the treatment of inflammatory diseases including allergic inflammation. It is generally accepted that GC exerts its actions through the induction of protein synthesis. Lipocortin (1–4), a GC-inducible phospholipase A₂ inhibitory protein, has been believed to play important roles in the anti-inflammatory action of GC, because phospholipase A₂ releases arachidonic acid from membrane phospholipids, which is converted to potent inflammatory mediators, prostaglandins and leukotrienes. It is also established, however, that GC could exhibit the action in a way independent of lipocortin (5–7). On the other hand, GC inhibits vascular permeability increase (8–10), and induces vascular permeability increase inhibitory proteins, vasoregulin (11) and vasocortin (12). Furthermore, accumulation of inflammatory cells, interleukin 1 production and biosynthesis of cyclooxygenase are also inhibited by GC (13–15). These reports suggest the multiple actions of GC. However, the precise mechanisms involved in the anti-inflammatory action of GC still remain to be elucidated.

In 1983, Nagai et al. (16) suggested the possibility that a protein synthesis-independent mechanism is involved in the anti-allergic action of GC in rats. We also indicated that IgE antibody-mediated passive cutaneous anaphylaxis (PCA) in rats, an important immediate hypersensitivity reaction model, is inhibited by GC, and that inhibition of vascular permeability increase plays an important part in the inhibition of PCA (17). On the other hand, tyrosine aminotransferase (TAT) in the liv-
er is a well-defined protein which can be induced by GC, and it has been demonstrated that its increased enzyme activity by GC is a result of increased protein synthesis (18, 19). In the present study, therefore, further attempts were made to elucidate the inhibitory mechanisms of PCA by GC in rats by examining the TAT activity.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing about 200 g and female Wistar rats weighing about 150 g were used. Female rats were used only for preparing antiserum. All animals were purchased from Japan SLC, Inc. (Hammatsu, Japan).

Drugs

Hydrocortisone (Hydro; sodium phosphate injection, Yamanouchi, Tokyo, Japan), prednisolone (Pred; sodium phosphate injection, Banyu, Tokyo, Japan), dexamethasone (Dexa; sodium phosphate injection, Takeda, Osaka, Japan) and cycloheximide (CH; Sigma, St. Louis, MO, USA) were used. GCs were diluted with saline and administered intravenously. CH was dissolved in saline and given subcutaneously.

Antigen and antiserum

Dinitrophenylated Ascaris suum extract (DNP-As) (20) and bovine serum albumin (DNP-BSA) were used as antigens. Anti-DNP-As serum was prepared according to the method described by Tada and Okumura (21) with a slight modification. The IgE antibody titer of the antiserum preparation estimated by homologous PCA was 1:29.

PCA and cutaneous reactions

PCA and cutaneous reactions caused by allergic chemical mediators were examined at the same time in the same rats (17, 22). As allergic chemical mediators, histamine (dihydrochloride, Nacalai Tesque, Kyoto, Japan), serotonin (creatinine sulfate, Merck, NJ, USA) and leukotriene C4 (LTC4; Wako, Osaka, Japan) were used. Histamine and serotonin were dissolved in saline, and 5 × 10^-5 g/ml of LTC4 in 50% methanol was diluted with saline. On the shaved dorsal skin of rats, 6 reaction sites were marked, and 0.1 ml of 40-fold diluted antiserum was injected intradermally into 1 of the 6 reaction sites for passive sensitization. At the same time, 0.1 ml of saline was injected into another site as a control for PCA. Forty-eight hours after the sensitization, 0.1 ml of 10^-5 g/ml of histamine, 3 × 10^-7 g/ml of serotonin and 3 × 10^-8 g/ml of LTC4 were injected into 3 of the 4 remaining sites intradermally. The last site was used as a control for cutaneous reactions, and received an injection of an equivalent volume of saline. Immediately after the injection of mediator solutions and saline, 1 mg of DNP-BSA and 5 mg of Evans blue dye dissolved in saline were given intravenously. Thirty minutes later, rats were sacrificed and reaction sites were excised for determination of extravasated dye. Each experimental group consisted of 6 rats and the reaction sites were rotated within the group. When PCA and the histamine-induced cutaneous reaction were elicited at the same time, 2 of the 6 reaction sites on the dorsal skin of rats were used for PCA, 2 for the histamine-induced cutaneous reaction, and the 2 remaining sites were controls for PCA and the cutaneous reaction. Extravasated dye in each reaction site was extracted and determined colorimetrically according to the method described by Katayama et al. (23).

Passive peritoneal anaphylaxis

IgE antibody-mediated in vivo histamine release in the rat peritoneal cavity was performed according to the method of Orange and Austen (24). In brief, rats were passively sensitized by an intraperitoneal injection of 2 ml of 30-fold diluted antiserum. Forty-eight hours after the sensitization, 1 mg of DNP-As dissolved in saline was injected intravenously. Exactly 1 minute later, rats were sacrificed and the peritoneal cavity was washed with 10 ml of Tyrode solution containing 5 units/ml of heparin. The peritoneal washings were centrifuged at 600 rpm for 10 minutes at 4°C, and the concentration of histamine in the supernatant was determined by a fluorescence method described by May et al. (25).

TAT activity in the liver

TAT activity in the rat liver was measured according to the method described by Granner and Tomkins (26). Rats were sacrificed and livers were isolated after perfusing saline via portal vein. A twenty percent liver homogenate was prepared in 0.125 M potassium phosphate buffer, pH 7.6, containing 5 mg/ml bovine serum albumin, 10^-3 M ethylenediaminetetraacetic acid and 10^-3 M dithiothreitol. After centrifuging the homogenate at 12,000 rpm for 30 minutes at 4°C, the enzyme activity in the supernatant was measured. The enzyme reaction was initiated by adding 0.1 ml of 40-fold diluted sample to a mixture of 0.2 ml of potassium phosphate buffer, pH 7.6, 0.06 ml of potassium a-ketoglutarate solution (0.5 M, pH 7.0) and 0.03 ml of pyridoxal 5'-phosphate solution (0.005 M, pH 6.5). After incubating for 20 minutes at 37°C, the reaction was terminated by adding
0.21 ml of 10 N potassium hydroxide solution. The reaction mixture was further incubated for 30 minutes at 37°C, and then the optical density was measured at 331 nm.

Statistical analyses

Results were expressed as the mean value and the standard error. Multiple comparisons were made to examine the statistical significance. When uniform variance of data was identified by Bartlett's analysis (P < 0.05), one-way analysis of variance was used to test for statistical differences. Significant differences (P < 0.05) were identified, after which the data were further analyzed by Duncan's multiple range test for significant differences between individual pairs of means. If uniform variance of data was not demonstrated, nonparametric multiple comparisons were made. After confirming significant differences (P < 0.05) by Kruskal-Wallis' analysis, the differences between individual pairs of means were examined by Williams-Wilcoxon analysis.

RESULTS

Effects of GC on PCA and mediator-induced cutaneous reactions elicited at the same time in the same rats

Table 1 shows the results of dose-response studies of Hydro, Pred and Dexa on PCA and mediator-induced cutaneous reactions elicited at the same time in the same rats. GCs were administered intravenously 2 hours prior to induction of reactions. Hydro at doses of 5–20 mg/kg, Pred at doses of 0.2–2 mg/kg and Dexa at doses of 0.01–0.1 mg/kg significantly inhibited PCA. At the same time, these GCs also inhibited histamine-, serotonin- and LTC₄-induced cutaneous reactions dose-dependently. PCA was inhibited by any GC more potently than the mediator-induced cutaneous reactions. Next, we performed time course studies using 20 mg/kg of Hydro, 2 mg/kg of Pred and 0.1 mg/kg of Dexa. As shown in Table 2, GCs administered simultaneously with the challenge or 30 minutes prior to it did not inhibit PCA and the cutaneous reactions at all. The LTC₄-induced cutaneous reaction was significantly potentiated by Dexa given 30 minutes before. Figure 1 shows the results of GCs administered 1–12 hours prior to challenge. Hydro inhibited PCA significantly when given 1–4 and 12 hours before the challenge. The maximum inhibition observed at 2 hours was 69.9 ± 7.6%. Cutaneous reactions elicited at the same time were also inhibited significantly by Hydro administered 1 and 2 hours before. Maximum inhibitions of histamine-, serotonin- and LTC₄-induced cutaneous reactions observed at 2 hours were 58.0 ± 4.9%, 64.5 ± 3.7% and 60.1 ± 4.9%, respectively. The LTC₄-induced cutaneous reaction at 8 hours was significantly potentiated. In the case of Pred, PCA was also inhibited biphasically at 2–4 and 8–12 hours. The maximum inhibition, 64.4 ± 5.0%, was observed at 2 hours. Histamine-, serotonin- and LTC₄-induced cutaneous reactions were inhibited at 2 hours; and their inhibitions were 62.5 ± 3.9%, 53.4 ± 4.3% and 54.2 ± 4.7%, respectively. Dexa inhibited PCA persistently between 2–12 hours and maximally at 4 hours. The inhibition at 4 hours was 89.8 ± 4.6%. The histamine-induced cutaneous reaction was significantly inhibited by Dexa administered 2–4 hours before the challenge, and the

Table 1. Dose-response effects of hydrocortisone, prednisolone and dexamethasone on PCA and cutaneous reactions induced by histamine, serotonin and LTC₄ elicited at the same time in the same rats

| Dose mg/kg | Control PCA | 14.65 ± 0.84 | 12.71 ± 1.98 | 9.86 ± 0.76 |
|-----------|-------------|--------|--------|--------|
| Hydrocortisone 5 | 2.47 ± 0.52 (87.1)** | 10.69 ± 1.55 (23.0)* | 7.67 ± 0.61 (39.7)* | 6.07 ± 0.60 (37.4)* |
| 10 | 3.74 ± 1.10 (80.5)* | 9.97 ± 1.56 (31.9)* | 7.94 ± 0.99 (37.5)* | 5.62 ± 1.09 (43.0)** |
| 20 | 1.49 ± 0.44 (92.2)** | 7.06 ± 0.99 (51.8)** | 5.85 ± 0.73 (54.0)** | 4.75 ± 1.36 (51.8)** |
| Control | 13.93 ± 3.05 | 11.66 ± 1.53 | 11.12 ± 0.84 | 7.63 ± 1.04 |
| Prednisolone 0.2 | 7.24 ± 2.02 (48.0)* | 10.37 ± 1.06 (11.1) | 8.86 ± 0.53 (20.3)* | 7.11 ± 0.83 (6.8) |
| 0.5 | 6.26 ± 1.84 (53.1)* | 9.18 ± 1.14 (21.3) | 7.45 ± 0.70 (33.0)** | 6.61 ± 0.97 (13.4) |
| 2 | 3.78 ± 1.08 (72.9)** | 7.88 ± 0.78 (32.4)** | 6.37 ± 0.70 (42.7)** | 5.14 ± 0.40 (32.6) |
| Control | 13.39 ± 2.83 | 10.53 ± 1.24 | 9.41 ± 1.17 | 7.63 ± 1.04 |
| Dexamethasone 0.01 | 4.97 ± 1.56 (62.9)** | 7.00 ± 0.46 (33.5)* | 6.75 ± 0.57 (28.3)* | 6.79 ± 1.05 (11.0) |
| 0.05 | 3.24 ± 1.78 (75.8)** | 6.39 ± 1.07 (39.3)* | 5.74 ± 0.85 (39.0)** | 5.20 ± 0.99 (31.8) |
| 0.1 | 4.54 ± 1.81 (66.1)** | 5.67 ± 0.79 (46.2)** | 5.04 ± 0.80 (46.4)** | 4.36 ± 0.72 (42.9)* |

Drugs were administered intravenously 2 hours prior to challenge. Each value represents the mean and S.E. of 6 rats. *: P < 0.05, **: P < 0.01
Table 2. Effects of hydrocortisone, prednisolone and dexamethasone on PCA and cutaneous reactions induced by histamine, serotonin and LTC₄ elicited at the same time in the same rats

| Administration time (prior to challenge) | PCA | Amount of dye, μg (% inhibition) | Histamine | Serotonin | LTC₄ |
|-----------------------------------------|-----|---------------------------------|-----------|-----------|------|
| Hydrocortisone, 20 mg/kg                |     |                                 |           |           |      |
| Control                                 | 16.74 ± 1.53 |                                 | 18.05 ± 0.94 | 14.72 ± 1.18 | 11.97 ± 1.07 |
| 0 hr                                    | 18.45 ± 2.18 (-10.2) |                                 | 19.39 ± 1.83 (-7.4) | 17.73 ± 1.36 (-20.4) | 14.17 ± 1.23 (-18.4) |
| 0.5 hr                                  | 16.29 ± 3.89 (2.7) |                                 | 17.78 ± 3.01 (1.5) | 18.94 ± 4.01 (-28.7) | 10.53 ± 1.74 (12.0) |
| Prednisolone, 2 mg/kg                   |     |                                 |           |           |      |
| Control                                 | 20.20 ± 2.20 |                                 | 25.13 ± 1.72 | 18.13 ± 0.93 | 16.43 ± 1.03 |
| 0 hr                                    | 20.83 ± 4.09 (-3.1) |                                 | 22.27 ± 2.96 (11.4) | 17.42 ± 2.84 (3.9) | 16.54 ± 2.41 (-0.6) |
| 0.5 hr                                  | 14.06 ± 3.65 (30.4) |                                 | 19.87 ± 1.38 (20.9) | 17.05 ± 1.92 (6.0) | 16.16 ± 1.56 (1.6) |
| Dexamethasone, 0.1 mg/kg                |     |                                 |           |           |      |
| Control                                 | 15.23 ± 2.36 |                                 | 15.62 ± 1.74 | 11.84 ± 2.23 | 10.01 ± 1.28 |
| 0 hr                                    | 17.62 ± 4.88 (-15.7) |                                 | 19.40 ± 1.86 (-24.2) | 15.37 ± 2.60 (-29.8) | 13.90 ± 1.18 (-38.9)* |
| 0.5 hr                                  | 14.87 ± 1.89 (2.4) |                                 | 17.46 ± 2.35 (-11.8) | 15.50 ± 2.39 (-30.9) | 11.57 ± 0.50 (-15.6) |

Drugs were administered intravenously just before or 0.5 hour prior to the challenge. Each value represents the mean and S.E. of 6 rats. *: P < 0.05

Fig. 1. Time courses for the effects of hydrocortisone, prednisolone and dexamethasone on PCA and cutaneous reactions induced by histamine, serotonin and LTC₄ elicited at the same time in the same rats. Drugs were administered intravenously 1–12 hours prior to challenge. Each value expressed as a percentage of the control represents the mean and S.E. of 6 rats. Control values of PCA, cutaneous reactions caused by histamine, serotonin and LTC₄ were: hydrocortisone, 31.75 μg, 17.98 μg, 20.74 μg and 10.60 μg; prednisolone, 24.91 μg, 16.20 μg, 13.45 μg and 10.64 μg; dexamethasone, 19.10 μg, 12.56 μg, 10.98 μg and 8.06 μg, respectively. ○: PCA, ●: histamine, ▲: serotonin, ■: LTC₄, *: P < 0.05, †: P < 0.01.
maximum inhibition observed at 2 hours was 50.0 ± 6.4%. The serotonin-induced cutaneous reaction was also inhibited at 2–4 hours, but the maximum inhibition, 53.1 ± 3.1%, was observed at 4 hours. The LTC4-induced cutaneous reaction was significantly inhibited at 2 hours, and the inhibition was 48.4 ± 8.7%.

**Effects of GC on passive peritoneal anaphylaxis**

Effects of GCs on IgE antibody-mediated in vivo histamine release in the rat peritoneal cavity were examined. Hydro at a dose of 20 mg/kg, Pred at a dose of 2 mg/kg and Dexa at a dose of 0.1 mg/kg were given intravenously 1–12 hours prior to challenge. The results are shown in Fig. 2. Hydro significantly inhibited the histamine release when given 1–4 and 8–12 hours before the challenge. The maximum inhibition, 48.2 ± 8.6%, was observed at 1 hour. Pred and Dexa inhibited the histamine release persistently from 1 to 12 hours, and the maximum inhibitions were 53.8 ± 6.0% at 1 hour and 81.2 ± 4.5% at 6 hours, respectively. It was confirmed, however, that these GCs when administered simultaneously with challenge did not inhibit the histamine release at all (data not shown).

**Increase in liver TAT activity by GC**

Effects of GCs on TAT activity in the rat liver were
examined. Hydro at a dose of 20 mg/kg, Pred at a dose of 2 mg/kg and Dexa at a dose of 0.1 mg/kg were given intravenously 1–12 hours prior to excision of the livers. The results are shown in Fig. 3. Hydro and Pred significantly increased the TAT activity administered at 1–4 hours, and maximum TAT activities, 207.5 ± 21.4% and 248.4 ± 16.5% (against control), respectively, were observed at 2 hours. These GCs also increased the activity at 12 hours; and in the case of Pred, it was significant. Dexa administered 1–12 hours before increased the TAT activity significantly, and the maximum increase observed at 8 hours was 474.7 ± 35.2%.

*Effects of CH treatment on the inhibition of immediate hypersensitivity reaction and the increase in TAT activity by GC*

![Graph showing the effects of hydrocortisone, prednisolone, and dexamethasone on TAT activity.](image)

**Fig. 3.** Time courses for the effects of hydrocortisone, prednisolone, and dexamethasone on tyrosine aminotransferase (TAT) activity in the rat liver. Drugs were administered intravenously 1–12 hours prior to excision of livers. Each value expressed as a percentage of the control represents the mean and S.E. of 4 rats. *: P < 0.05, †: P < 0.01.

Effect of CH treatment on increased liver TAT activity by GCs was studied. Hydro at a dose of 20 mg/kg, Pred at a dose of 2 mg/kg and Dexa at a dose of 0.1 mg/kg were administered intravenously 2 hours prior to excision of livers. CH at a dose of 5 mg/kg was given subcutaneously just before GC administration. As shown in Fig. 4, the TAT activity was significantly increased by Hydro, Pred and Dexa, and the activities were 2.08, 2.00 and 1.64 times of the control, respectively. CH treatment decreased spontaneous TAT activity to about one half. The CH treatment completely abrogated the increase in TAT activity observed by GC administration. The TAT activities in CH-treated groups were lower than that in the control. Under the same experimental condition, effects of CH on the inhibition of PCA and the histamine-induced cutaneous
reaction elicited at the same time in the same rats were studied. The results are shown in Fig. 5. Three kinds of GC significantly inhibited both PCA and the histamine-induced cutaneous reaction, and the inhibition of PCA was more potent than that of the histamine-induced cutaneous reaction in each GC as mentioned above. CH treatment did not significantly affect either reaction. Although the inhibition of the histamine-induced cutaneous reaction caused by GCs was completely abrogated, the inhibition of PCA examined in the same rats was only partially reduced, and inhibitions of about 40% were still observed.

Next, we investigated the effect of CH treatment on the inhibition of histamine release and the increase in TAT activity by Dexa in the same rats. Dexa at a dose of 0.1 mg/kg was administered 6 hours before the challenge, a better experimental condition for detecting Dexa effects. CH at a dose of 5 mg/kg was given subcutaneously just before the administration of glucocorticoid. Immediated after recovering the peritoneal washings for the histamine assay, the livers were excised for determination of TAT activity. The results of TAT activity are shown in Fig. 6. Dexa treatment increased the TAT activity to 4.45 times the control level. CH treatment did not affect the control TAT activity significantly. Treatment with CH abrogated the increase in TAT activity by Dexa almost completely. Results of histamine release examined at the same time are shown in Fig. 7. Dexa treatment significantly inhibited the histamine release. CH treatment did not affect the histamine release significantly. The CH treatment failed to affect the inhibition of histamine release by Dexa.

**DISCUSSION**

In the present study, time course experiments of GC actions on IgE antibody-mediated PCA, chemical mediator-induced cutaneous reactions, IgE antibody-dependent in vivo histamine release in the peritoneal cavity and TAT activity in the liver were performed in rats. PCA and mediator-induced cutaneous reactions elicited at the same time in the same rats were inhibited by GC administration. Since cutaneous reactions caused by 3 different chemical mediators were inhibited similarly, a common mechanism is suggested to be involved in their inhibition. PCA was always inhibited more potently than mediator-induced cutaneous reactions, and it was also inhibited when no inhibition of mediator-induced cutaneous reactions was observed. PCA is a reaction characterized by increased vascular permeability which is caused by chemical mediators released from antigen-stimulated mast cells. GC is reported to inhibit histamine release from mast cells and basophils (27, 28). Actually, IgE antibody-dependent histamine release in the peritoneal cavity was inhibited by GC, and the inhibition was long lasting. Although
the inhibitory time courses for PCA, the mediator-induced cutaneous reaction and histamine release were different from each other, that for PCA seems to be constructed by combining those of the mediator-induced cutaneous reaction and histamine release. All these results collectively indicate that PCA is inhibited by GC through at least 2 mechanisms, as suggested previously (17). One is non-specific inhibition of vascular permeability increase and the other is inhibition of chemical mediator release from mast cells. Furthermore, although the mediator-induced cutaneous reaction was inhibited only transiently around 2 hours after GC administration, prolonged inhibition was observed in the cases of PCA and histamine release, indicating that the inhibition of both vascular permeability increase and histamine release contributes to that of PCA in the early phase after GC administration, but that in later phase, only the inhibition of histamine release is responsible. We also confirmed that a time lag is essential for these actions of GCs. On the other hand, it is interesting that the inhibition of PCA by Hydro and Pred was biphasic as reported previously (16). Furthermore, biphasic actions of Hydro and Pred were also observed in TAT activity. Since histamine release was inhibited potently 12 hours after Hydro or Pred administration, this may be responsible for the second phase inhibition of PCA. These biphasic actions of Hydro and Pred may have some relation to the plasma corticosterone levels.

Fig. 5. Effect of cycloheximide (CH) on the inhibition by hydrocortisone (Hydro), prednisolone (Pred) and dexamethasone (Dexa) of PCA and histamine-induced cutaneous reaction elicited at the same time in the same rats. Glucocorticoids were administered intravenously 2 hours prior to the challenge. CH was given subcutaneously just before the administration of glucocorticoid. Each value represents the mean and S.E. of 6 rats. Figures in parentheses indicate the percent inhibition. *: P < 0.05, †: P < 0.01, N.S.: not significant.
On the contrary, biphasic action was not observed in the case of Dexa, which exhibits potent and long lasting actions.

It is well-accepted that GC exhibits its actions through synthesizing functional proteins and that some actions of GC are clearly prevented by protein synthesis inhibitors (5-7, 14). In our experience, however, it was difficult to demonstrate whether protein synthesis is really inhibited by these agents or not (29). Tryptophan oxygenase and TAT activities increase in the liver after GC treatment, and increased TAT activity is caused by increased enzyme protein (18, 19, 30, 31). In the present study, therefore, TAT activity was used to examine the effectiveness of both GC and the protein synthesis inhibitor CH. According to the results of preliminary experiments, we decided to use CH at the dose of 5 mg/kg. GC administration clearly increased TAT activity in the rat liver, and simultaneous treatment with CH completely abrogated the increase. In the present experimental conditions, therefore, it is obvious that CH treatment effectively inhibits protein synthesis induced by GC. Under the same experimental conditions, in-

Fig. 6. Effect of cycloheximide (CH) on the increase in tyrosine aminotransferase (TAT) activity in the rat liver by dexamethasone (Dexa). Dexa was administered intravenously 6 hours prior to excision of the liver. CH was given subcutaneously just before the administration of Dexa. Each value expressed as a percentage of the control represents the mean and S.E. of 4 or 6 rats.  †: P < 0.01, N.S.: not significant.

Fig. 7. Effect of cycloheximide (CH) on the inhibition by dexamethasone (Dexa) of IgE antibody-mediated histamine release in the rat peritoneal cavity. Dexa was administered intravenously 6 hours prior to challenge. CH was given subcutaneously just before the administration of Dexa. Each value represents the mean and S.E. of 4 or 6 rats. Figures in parentheses indicate the percent inhibition. †: P < 0.01.
hbinition of the histaminic-induced cutaneous reaction by GC was completely abrogated, suggesting that protein synthesis is involved in the inhibition. However, inhibition of PCA elicited at the same time was attenuated only partially. It is suggested, therefore, that the inhibition of PCA by GC involves a mechanism resistant to CH treatment, and that the attenuated part of the inhibition indicates the participation of vascular permeability increase inhibition by GC. It should be stressed that the complete recovery of vascular permeability increase inhibition and partial attenuation of PCA inhibition were demonstrated in the same rats at the same time. Furthermore, CH treatment little affected the inhibition of histamine release in the rat peritoneal cavity by Dexa, although in the same rats, the increase in liver TAT activity was abrogated almost completely. These results suggest that the CH treatment resistant process involved in PCA inhibition is the inhibition of histamine release.

In 1987, Carnuccio et al. (32) reported that vasocortin recovered from the peritoneal cavity of rats treated with Dexa inhibits dextran edema in rats. Vasocortin is also reported to inhibit histamine release from rat peritoneal exudate cells caused by IgE antibody-dependent stimuli, such as dextran and concanavalin A (33). These reports strongly suggest that the inhibition of histamine release by GC is mediated through protein synthesis and that the protein vasocortin plays an important role in the inhibition of PCA by GC. Furthermore, Ialenti et al. (34) reported that the anti-inflammatory effect of GC depends on the induction of both lipocortin and vasocortin. Vasocortin inhibits the early phase of carrageenin paw edema, and antiflammin, a nonapeptide fragment of lipocortin with inhibitory activity for phospholipase A2 in vitro, inhibits the late phase in rats. In spite of these reports, however, roles of these proteins in the anti-inflammatory or anti-allergic action of GC have not yet been established, and lipocortin is considered not to play important roles in the inhibition of PCA by GC in mice and rats (35, 36).

It is well-known that GC needs a latent period to exhibit its actions. The time lag has been discussed in relation to the induction of protein synthesis. In the present study, although we indicated that the inhibition of histamine release by GC was not recovered by CH, it was also confirmed that GC did not inhibit the histamine release when given simultaneously with the challenge. Lately, the GC receptor was reported to interact with other transcription factors and to inhibit the expression of functional proteins (37–39). This mechanism may explain the inhibition of de novo synthesis of cyclooxygenase (15). A latent period could be present in the process from inhibition of de novo synthesis of some protein to expression of the decreased function of the protein. It may be interesting to consider this mechanism for the inhibition of histamine release by GC. However the precise mechanisms are not clear at present.

In summary, IgE antibody-mediated PCA was inhibited by GC through at least 2 mechanisms. One is the inhibition of mediator release from mast cells and the other is the inhibition of vascular permeability increase caused by released chemical mediators. Although the latter action of GC is dependent upon protein synthesis, the former seems to be mediated by a unique mechanism independent of protein synthesis.

REFERENCES

1. Blackwell, G.J., Carnuccio, R., Di Rosa, M., Flower, R.J., Parente, L. and Persico, P.: Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. Nature 287, 147–149 (1980)
2. Hirata, F., Schiffmann, E., Venkatasubramanian, K., Salomon, D. and Axelrod, J.: A phospholipase A2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. Proc. Natl. Acad. Sci. U.S.A. 77, 2533–2536 (1980)
3. Russo-Marie, F. and Duval, D.: Dexamethasone-induced inhibitions of prostaglandin production does not result from a direct action on phospholipase activities but is mediated through a steroid-inducible factor. Biochim. Biophys. Acta 712, 177–185 (1982)
4. Flower, R.J.: Background and discovery of lipocortins. Agents Actions 17, 255–262 (1985)
5. Tsurufuji, S. and Sugio, K.: Molecular mechanism in the manifestation of antiinflammatory activity of glucocorticoids. Eur. J. Rheumatol. Inflamm. 1, 226–231 (1978)
6. Tsurufuji, S., Sugio, K. and Takemasa, F.: The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone. Nature 280, 408–410 (1979)
7. Tsurufuji, S., Sugio, K., Takemasa, F. and Yoshizawa, S.: Blockade by antiguocorticoids, actinomycin D and cycloheximide of anti-inflammatory action of dexamethasone against bradykinin. J. Pharmacol. Exp. Ther. 212, 225–231 (1980)
8. Sugio, K., Ohuchi, K., Sugata, M. and Tsurufuji, S.: Suppression by dexamethasone of vascular permeability responses induced with leukotrienes C4 and D in the rat skin. Prostaglandins 21, 649–653 (1981)
9. Svensjö, E. and Roempke, K.: Time-dependent inhibition of bradykinin- and histamine-induced microvascular permeability increase by local glucocorticoid treatment. Prog. Resp. Res. 19, 173–180 (1985)
10. Björk, J., Goldschmidt, T., Smedegård, G. and Arfors, K.-E.: Methylprednisolone acts at the endothelial cell level reducing inflammatory responses. Acta Physiol. Scand. 123, 221–223 (1985)
11. Oyanagui, Y. and Suzuki, S.: Vasoregulin, a glucocorticoid-inducible vascular permeability inhibitory protein. Agents Actions 17, 270–277 (1985)
12. Di Rosa, M., Calignano, A., Carnuccio, R., Lalenti, A. and Sautebin, L.: Multiple control of inflammation by glucocorti-
oids. Agents Actions 17, 284–289 (1985)
13 Snyder, D.S. and Unanue, E.R.: Corticosteroids inhibit murine macrophage la expression and interleukin 1 production. J. Immunol. 129, 1803–1805 (1982)
14 Tsurufuji, S., Kurihara, A. and Ojima, F.: Mechanisms of anti-inflammatory action of dexamethasone: blockade by hydrocortisone mesylate and actinomycin D of the inhibitory effect of dexamethasone on leukocyte infiltration in inflammatory sites. J. Pharmacol. Exp. Ther. 229, 237–243 (1984)
15 Fu, J.-Y., Masterrer, J.L., Seibert, K., Raz, A. and Needleman, P.: The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes. J. Biol. Chem. 265, 16737–16740 (1990)
16 Nagai, H., Takizawa, T., Nakatomi, J., Matsuura, N. and Koda, A.: Anti-allergic action of glucocorticoids in rats. Japan. J. Pharmacol. 33, 349–355 (1983)
17 Inagaki, N., Miura, T., Nagai, H. and Koda, A.: Inhibitory effects of glucocorticoids on increased vascular permeability caused by passive cutaneous anaphylaxis and some chemical mediators in rats. J. Pharmacol. 46, 189–192 (1988)
18 Lin, E.C.C. and Knox, W.E.: Adaptation of the rat liver tyrosine-a-ketoglutarate transaminase. Biochim. Biophys. Acta 26, 85–88 (1957)
19 Kenney, F.T. and Flora, R.M.: Induction of tyrosine-a-ketoglutarate transaminase in rat liver. 1. Hormonal nature. J. Biol. Chem. 236, 2699–2702 (1961)
20 Strejan, G. and Campbell, D.H.: Hypersensitivity to ascaris antigen. IV. Production of homocytotropic antibodies in the rat. J. Immunol. 101, 628–638 (1968)
21 Tada, T. and Okumura, K.: Regulation of homocytotropic antibody formation in the rat. I. Feed-back regulation by passively administered antibody. J. Immunol. 106, 1002–1011 (1971)
22 Koda, A., Miura, T., Inagaki, N., Sakamoto, O., Arimura, A., Nagai, H. and Mori, H.: A method for evaluating anti-allergic drugs by simultaneously induced passive cutaneous anaphylaxis and mediator cutaneous reactions. Int. Arch. Allergy Appl. Immunol. 92, 209–216 (1990)
23 Katayama, S., Shionoya, H. and Ohtake, S.: A new method for extraction of extravasated dye in the skin and influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. Microbiol. Immunol. 22, 89–101 (1978)
24 Ornage, R.P. and Austen, K.F.: Pharmacologic dissociation of immunologic release of histamine and slow reacting substance of anaphylaxis in rats. Proc. Soc. Exp. Biol. Med. 129, 836–841 (1968)
25 May, C.D., Lyman, M., Alberto, R. and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. J. Allergy 46, 12–20 (1970)
26 Granner, D.K. and Tomkins, G.M.: Metabolism of amino acids and amines: tyrosine aminotransferase (rat liver). In Methods in Enzymology, Edited by Tabor, H. and Tabor, C.W., Vol. 17A, p. 633–637, Academic Press, New York (1970)
27 Schleimer, R.P., Lichtenstein, L.M. and Gillespie, E.: Inhibition of basophil histamine release by anti-inflammatory steroids. Nature 292, 454–455 (1981)
28 Deacon, M., Steak, A.R., Hirata, F. and Ishizaka, T.: Biochemical analysis of glucocorticoid-induced inhibition of IgE-mediated histamine release from mouse mast cells. J. Immunol. 129, 1212–1218 (1982)
29 Figelson, P. and Greengard, O.: Immunohistological evidence for increased titers of liver tryptophan pyrrolase during substrate and hormonal enzyme induction. J. Biol. Chem. 237, 3714–3717 (1962)
30 Taira, M., Kohno, S.W., Yamamura, H. and Ohlata, K.: Lack of involvement of leukotriene and platelet activating factor in passive cutaneous anaphylaxis in rats. Agents Actions 24, 189–195 (1988)
31 Jonat, C., Rahmsdorf, H.J., Park, K.-K., Cato, A.C.B., Gebel, S., Ponta, H. and Herrlich, P.: Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell 62, 1189–1204 (1990)