Studies on the Anti-allergic Mechanism of Glucocorticoids in Mice

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Glucocorticoids inhibit IgE antibody-mediated passive cutaneous anaphylaxis (PCA) and chemical mediator-induced cutaneous reactions elicited in the mouse ear. In the present study, we investigated the effect of actinomycin D, a protein synthesis inhibitor, on dexamethasone-caused inhibition of PCA and histamine-induced cutaneous reaction in the mouse ear. Tyrosine aminotransferase (TAT) activity in the liver, which was estimated as an index for protein synthesis, significantly increased by the administration of hydrocortisone, prednisolone and dexamethasone. Significant increase in TAT activity was observed from 2 h after glucocorticoid administration and peaked at 4 h, and declined gradually thereafter. Cycloheximide even at high doses of 100 and 300 mg/kg failed to affect the increase in TAT activity by dexamethasone. On the contrary, actinomycin D at doses of 1 and 10 mg/kg abrogated the TAT activity increase by dexamethasone almost completely. Treatment with 1 mg/kg of actinomycin D, however, failed to affect the inhibition of PCA and histamine-induced cutaneous reaction by dexamethasone. These results suggest that glucocorticoids exhibit their inhibitory action of PCA and chemical mediator-induced cutaneous reactions in mice through a mechanism resistant to actinomycin D treatment.

Keywords — glucocorticoid; dexamethasone; anti-allergic action; passive cutaneous anaphylaxis; histamine-induced cutaneous reaction; tyrosine aminotransferase; protein synthesis; actinomycin D

Introduction

Glucocorticoid is widely used in the treatment of inflammatory diseases including allergic inflammation. It has been generally accepted that glucocorticoid exerts its actions through inducing protein synthesis. Lipocortin,1–4 a glucocorticoid-inducible phospholipase A2 inhibitory protein, has been thought to play important roles in the anti-inflammatory action of glucocorticoid, because phospholipase A2 releases arachidonic acid from membrane phospholipids, which are converted to potent inflammatory mediators, prostaglandins and leukotrienes. It is also established, however, that glucocorticoid could exhibit the action in a way independent of lipocortin.5–7 On the other hand, glucocorticoid inhibits an increase, in vascular permeability8–10 and induces proteins which inhibit vascular permeability increase, vasoregulin11 and vasocortin.12 Furthermore, accumulation of inflammatory cells, production of interleukins 1 and 2, and biosynthesis of cyclooxygenase are also inhibited by glucocorticoid.13–15 However, the precise mechanisms involved in the anti-inflammatory action of glucocorticoid still remain to be elucidated.

In 1983, Nagai et al.17 suggested that a protein synthesis-independent mechanism(s) is involved in the anti-allergic action of glucocorticoid in rats. We also indicated that IgE antibody-mediated passive cutaneous anaphylaxis (PCA) and chemical mediator-induced cutaneous reactions elicited in the mouse ear are inhibited by glucocorticoid similarly, and that inhibition of vascular permeability increase plays an important part in the inhibition of PCA.18 Furthermore, we suggested a possibility that protein synthesis-independent mechanism(s) are also involved in the anti-allergic action of glucocorticoid in mice.19 On the other hand, tyrosine aminotransferase (TAT) in the liver is a well-defined protein which can be induced by glucocorticoid, and was demonstrated that its increased enzyme activity by glucocorticoid is a result of increased protein synthesis.20,21 In the present study, we investigated the effect of actinomycin D, a protein synthesis inhibitor, on the inhibition of PCA and histamine-induced cutaneous reaction in the mouse ear by...
dexamethasone under a condition in which actinomycin D inhibits TAT activity increase caused by dexamethasone.

Materials and Methods

Mice — Male ddY mice weighing about 30 g and female BALB/c mice weighing about 20 g were used. BALB/c mice were used only for preparing antiserum. All animals were purchased from Japan SLC, Inc. (Hamamatsu, Japan).

Drugs — Hydrocortisone (acetate, aqueous suspension, Nippon Merck-Banyu, Tokyo, Japan), prednisolone (acetate, aqueous suspension, Shionogi, Osaka, Japan), dexamethasone (acetate, aqueous suspension, Banyu, Tokyo, Japan), cycloheximide (Sigma, St. Louis, MO, U.S.A.) and actinomycin D (Sigma, St. Louis, MO, U.S.A.) were used. Glucocorticoids were diluted with saline containing 0.2% sodium carboxymethyl cellulose and administered intraperitoneally. Cycloheximide and actinomycin D were dissolved in saline and given subcutaneously.

Antigen and Antiserum — Dinitrophenylated Ascaris suum extract (DNP-As) and bovine serum albumin (DNP-BSA) were used as antigens. Anti-DNP-As serum was obtained from BALB/c mice immunized with DNP-As in the presence of aluminium hydroxide gel. IgE antibody titer of the antiserum preparation estimated by PCA in the mouse ear was 1:29.

PCA and Cutaneous Reaction — PCA and histamine-induced cutaneous reaction were elicited in the ear of male ddY mice as reported previously. Briefly, 10 μl of 30-fold diluted antiserum was injected into both sides of ears for sensitization. Forty-eight hours after the sensitization, PCA was elicited by injecting 0.25 ml of 0.5% Evans blue saline solution containing 0.25 mg of DNP-BSA intravenously. After 30 min, mice were sacrificed and ears were isolated for determination of extravasated dye. In the case of histamine-induced cutaneous reaction, 10 μl of 2 × 10⁻⁴ g/ml histamine was injected into both sides of ears followed by an intravenous injection of 0.25 ml of 0.5% Evans blue saline solution. After 30 min, mice were sacrificed and ears were isolated.

TAT Activity in the Liver — TAT activity in the mouse liver was measured according to the method described by Granner and Tomkins. Mice were sacrificed and livers were isolated after perfusing saline via the 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 10000 × g for 30 min at 4 °C, the enzyme activity in the supernatant was measured. The enzyme reaction was initiated by adding 0.1 ml of sample to a mixture of 0.2 ml of potassium phosphate buffer, 2.4 ml of tyrosine solution (0.007 M, pH 7.6), 0.06 ml of potassium α-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 min 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 min at 37 °C, and then the optical density was measured at 331 nm.

Statistical Analysis — Results were expressed as the mean value and the standard error. Difference between 2 experimental groups was statistically evaluated by Student's t-test or Welch's t-test.

Results

Induction of TAT Activity by Glucocorticoid

Effect of glucocorticoids on mouse liver TAT activity was examined. Hydrocortisone at a dose of 20 mg/kg, prednisolone at a dose of 5 mg/kg and dexamethasone at a dose of 1 mg/kg were given intraperitoneally 0.5—24 h prior to isolation of livers. As shown in Fig. 1, all glucocorticoids increased the TAT activity significantly, and the time courses were essentially the same. Significant increase in TAT activity was observed from 2 h after glucocorticoid administration, and peaked at 4 h, and declined gradually thereafter. In cases of prednisolone and dexamethasone, TAT activity decreased 24 h after treatment.

Effect of Cycloheximide on Dexamethasone-Induced TAT Activity Increase

Effect of cycloheximide on TAT activity in-
Fig. 1. Time Courses for the Effects of (I) Hydrocortisone, (II) Prednisolone and (III) Dexamethasone on Tyrosine Aminotransferase (TAT) Activity in the Mouse Liver

Drugs were administered intraperitoneally 0.5—24 h prior to excision of the liver. Each value expressed as a percentage of the control (C) represents the mean and S.E. of 4 mice. a) \( p<0.05 \), b) \( p<0.01 \). (I) 20 mg/kg, (II) 5 mg/kg, (III) 1 mg/kg.

TAT activity (% of control)

Control  
Dexa 1  
CH 100  
CH 100 + Dexa 1  
CH 300  
CH 300 + Dexa 1

(mg/kg)

Fig. 2. Effect of Cycloheximide (CH) on the Increase in Tyrosine Aminotransferase (TAT) Activity by Dexamethasone (Dexa) in the Mouse Liver

Dexa was administered intraperitoneally 8 h prior to excision of the liver and CH was given subcutaneously just before Dexa administration. Each value expressed as a percentage of the control represents the mean and S.E. of 4 mice. a) \( p<0.01 \).

intrapertioneally 8 h prior to isolation of livers, and actinomycin D at doses of 1 and 10 mg/kg was given subcutaneously just before glucocorticoid administration. As shown in Fig. 3, although dexamethasone significantly increased the TAT activity, the increase was abrogated almost completely by the simultaneous treatment with actinomycin D.

TAT activity (% of control)

Control  
Dexa 1  
ACD 1  
ACD 1 + Dexa 1  
ACD 10  
ACD 10 + Dexa 1

(mg/kg)

Fig. 3. Effect of Actinomycin D (ACD) on the Increase in Tyrosine Aminotransferase (TAT) Activity by Dexamethasone (Dexa) in the Mouse Liver

Dexa was administered intraperitoneally 8 h prior to excision of the liver and ACD was given subcutaneously just before Dexa administration. Each value expressed as a percentage of the control represents the mean and S.E. of 4 mice. a) \( p<0.01 \), b) not significant.
Effect of Actinomycin D on Inhibition of PCA and Histamine-Induced Cutaneous Reaction by Dexamethasone

Effect of actinomycin D on the inhibition of PCA and histamine-induced cutaneous reaction by dexamethasone was examined. Dexamethasone at a dose of 1 mg/kg was given 8 h prior to reaction, and actinomycin D at a dose of 1 mg/kg was given subcutaneously just before glucocorticoid administration. Results of PCA are shown in Fig. 4. Dexamethasone inhibited PCA by 44.0% and the inhibition was statistically significant. Treatment with actinomycin D did not affect PCA in comparison with the control. The treatment failed to affect the dexamethasone-caused inhibition of PCA at all and a significant inhibition (35.7%) was still observed. Results of cutaneous reaction are shown in Fig. 5. Dexamethasone inhibited histamine-induced cutaneous reaction by 56.8%. Although actinomycin D treatment inhibited the cutaneous reaction slightly, a significant inhibition of the reaction (55.9%) was still observed.

Discussion

In the present study, we showed that actinomycin D could not recover the dexamethasone-caused inhibition of both PCA and histamine-induced cutaneous reaction in mice even in an experimental condition where the agent inhibited the dexamethasone-induced increase in TAT activity almost completely. It has been well-accepted that glucocorticoid exhibits its actions through synthesizing functional proteins and that some actions of glucocorticoid are clearly prevented by portein synthesis inhibitors. In 1980, Tsurufuji et al. indicated that dexamethasone inhibited the bradykinin-induced edema in mice, and that the inhibition was completely abrogated by the simultaneous treatment with protein synthesis inhibitors. According to their reports, we have made various attempts, but we could not demonstrate the involvement of protein synthesis in the inhibition of PCA and chemical mediator-induced cutaneous reactions by glucocorticoid in mice. In these experiments, it was difficult to demonstrate whether protein synthesis is really inhibited by protein synthesis inhibitors or not. TAT is a well-defined protein and it is demonstrated that its increased enzyme activity by glucocorticoid is a result of increased protein synthesis. In the present study, therefore, we selected the TAT activity as an indicator for protein synthesis, and indicated that the dexamethasone inhibition of both PCA and histamine-induced cutaneous reaction in mice does not involve a process of protein synthesis.

Previously, we indicated that hydrocortisone,
 prednisolone and dexamethasone significantly inhibited PCA and chemical mediator-induced cutaneous reactions elicited in the mouse ear. These glucocorticoids inhibited PCA with similar inhibitory time courses, and exhibited the maximum inhibition when administered 8 h prior to the reaction. Furthermore, dexamethasone inhibited cutaneous reactions caused by chemical mediators, such as histamine, serotonin, platelet activating factor, leukotriene C\(_4\) and leukotriene D\(_4\), with similar inhibitory time courses to that of PCA. The inhibitions of mediator-induced cutaneous reactions were comparable to that of PCA. Furthermore, we confirmed that histamine and serotonin play important roles to cause vascular permeability increase in the PCA. These results suggest that a common mechanism is involved in the inhibition of both PCA and mediator-induced cutaneous reaction in mice, and that the inhibition of vascular permeability increase is important for the PCA inhibition by glucocorticoid. In the present results, actinomycin D did not affect the dexamethasone-caused inhibition of both PCA and histamine-induced cutaneous reaction, indicating that the common mechanism, the inhibition of vascular permeability increase, by glucocorticoid is resistant to actinomycin D treatment.

In rats, similarly to mice, glucocorticoid inhibits both PCA and chemical mediator-induced cutaneous reaction significantly. However, the inhibition of PCA is always more potent than that of mediator-induced cutaneous reactions, suggesting that PCA is inhibited by at least 2 mechanisms, inhibition of vascular permeability increase and inhibition of chemical mediator release from mast cells. Actually, antigen-induced histamine release in the rat peritoneal cavity in vivo is inhibited by glucocorticoid significantly. Furthermore, although the inhibition of vascular permeability increase is prevented by the simultaneous treatment with cycloheximide, the inhibition of histamine release is not affected by the treatment. It is suggested, therefore, that the mechanism of glucocorticoid inhibition of vascular permeability increase in mice is quite different from that in rats, although different protein synthesis inhibitors were employed. In mice, cycloheximide failed to affect the glucocorticoid-induced increase in TAT activity even in high doses. Cycloheximide may not be effective in the present experimental condition, as mice are reported not to be so sensitive to the inhibitor as rats.

In the present results, hydrocortisone, prednisolone and dexamethasone significantly increased the TAT activity in the mouse liver, and 20 mg/kg of hydrocortisone, 5 mg/kg of prednisolone and 1 mg/kg of dexamethasone showed equivalent potencies. It may be interesting to note, however, that the durations of their effect on TAT activity were similar. As mentioned above, these glucocorticoids inhibit PCA with similar time courses. On the contrary, in rats, the durations of effects of these glucocorticoids are quite different from each other, especially in PCA inhibition and increase in TAT activity. These results also suggest the species differences between mice and rats on the action of glucocorticoid.

Lately, the glucocorticoid receptor was reported to interact with other transciption factors and to inhibit the expression of functional proteins. This mechanism may explain not only the glucocorticoid inhibition of de novo synthesis of interleukins 1 and 2, and cyclooxygenase but also the immunosuppressive property of glucocorticoid. It is well known that a latent period is essential for glucocorticoid to exhibit the action, and the latent period has been discussed in relation to the induction of functional proteins. In the case of PCA and chemical mediator-induced cutaneous reactions, a process of protein synthesis is considered not to play important roles for the inhibition by glucocorticoid, although a latent period was observed. 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. Therefore it may be interesting to consider this mechanism for the anti-allergic actions of glucocorticoid in mice. However, the precise mechanisms involved in the PCA inhibition are not clear at present.

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References


