Inhibitory Effects of Glucocorticoids on Increased Vascular Permeability Caused by Passive Cutaneous Anaphylaxis and Some Chemical Mediators in Rats

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Abstract—Effects of hydrocortisone, prednisolone and dexamethasone on IgE antibody-mediated homologous passive cutaneous anaphylaxis (PCA) and mediator-induced skin reactions were investigated. PCA and skin reactions were evoked at the same time in the dorsal skin of a rat. Administrations of glucocorticoids inhibited not only the PCA but also the skin reactions caused by histamine, serotonin and leukotriene (LT) C4 significantly. It is suggested, therefore, that glucocorticoids inhibit the increase of vascular permeability non-specifically. This action of glucocorticoids might contribute at least in part to its inhibitory effect on the PCA.

It is generally accepted that glucocorticoids exert their potent anti-inflammatory effects through synthesis of a phospholipase A2 inhibitory protein, lipocortin (1-7). Lipocortin inhibits phospholipase A2 activity and the biosynthesis of inflammatory mediators from arachidonic acid such as prostaglandins and leukotrienes. In IgE antibody-mediated allergic reactions, it is reported that glucocorticoids inhibit mediator release from basophils and mast cells (8-12). However, it is also indicated that glucocorticoids exhibit an inhibitory effect on mediator-induced increase of vascular permeability in the hamster cheek pouch (13, 14). In the present study, therefore, effects of hydrocortisone, prednisolone and dexamethasone on IgE antibody-mediated PCA and mediator-induced skin reactions elicited at the same time in the same rat were compared.

Anti-dinitrophenylated Ascaris suum extract serum was prepared according to the method of Tada and Okumura (15). The IgE antibody titer of this antiserum estimated by 48-hr PCA in rats was 1:28. Male Wistar rats of about 200 g were obtained from Shizuoka Laboratory Animal Center (Hamamatsu) and used for PCA and skin reactions. Six reaction sites were settled on the shaved back of each rat, and 40-fold diluted antiserum in a volume of 0.1 ml was injected intradermally into one of the 6 sites. Forty-eight hours after the sensitization, saline and 3 kinds of mediator solutions in a volume of 0.1 ml were injected intradermally into 4 of the remaining 5 sites on the back skin. The last, non-treated site was used as a control for PCA (PCA(-)). Immediately after the mediator injections, the rats received an intravenous injection of 0.5% Evans blue saline solution containing 1 mg of dinitrophenylated bovine serum albumin. Thirty minutes after the injection of antigen and Evans blue, the rats were sacrificed, and the 6 reaction sites were removed. PCA and skin reactions were evaluated by measuring the amount of dye (16). As mediators, histamine, serotonin and LTC4 were used. Concentrations of these mediators were as follows: histamine: 10^-6 g/ml, serotonin: 3x10^-7 g/ml and LTC4: 3x10^-8 g/ml. Each experimental group consisted of 6 rats, and the reaction sites were rotated within a group. Hydrocortisone (acetate, aqueous suspension, Nippon Merck-Banyu), prednisolone (acetate, aqueous suspension, Shionogi) and dexamethasone (acetate, aqueous suspension, Banyu) were diluted with saline containing 0.2% sodium carboxymethyl cellulose.
and given to rats intraperitoneally 2 hr prior to the elicitation of PCA and skin reactions. The control group received an intraperitoneal injection of saline containing 0.2% sodium carboxymethyl cellulose.

As indicated in Fig. 1, administrations of 3 kinds of glucocorticoids inhibited the PCA dose-dependently, and the inhibition was significant at a 10 mg/kg dose of hydrocortisone or prednisolone and at 0.1 and 1 mg/kg doses of dexamethasone. These glucocorticoids also inhibited the skin reactions caused by histamine, serotonin and LTC₄, elicited at the same time, dose-dependently. The inhibition of these skin reactions was significant at the same doses that inhibited the PCA. Furthermore, the amount of dye detected in the saline-injected sites (indicated as dotted areas in skin reactions) was also reduced dose-dependently.

Rat mast cells contain histamine and serotonin (17), and these mediators are released by IgE antibody-mediated allergic reactions (18, 19). However, although the rat PCA is inhibited by antihistamines and antiserotonins (20, 21), it is not inhibited by cyclooxygenase inhibitors (22–24) and a 5-lipoxygenase inhibitor (25). These results suggest that histamine and serotonin are the major mediators, but indicate that prostaglandins and leukotrienes do not play important roles in the increase of vascular permeability caused by PCA. Furthermore, it is also suggested from these results that the phospholipase A₂ inhibitory protein does not contribute to the inhibition of PCA by glucocorticoids in rats. In the present results, histamine- and serotonin-induced skin reactions elicited at the same time with PCA were significantly inhibited by glucocorticoids. Therefore, the inhibition by glucocorticoids of the increase of vascular permeability caused by histamine and serotonin might play a role at least in part in the inhibition of PCA. On the other hand, LTC₄, LTD₄ and LTE₄ are potent bronchoconstrictors in asthmatics (26), though these leukotrienes are thought to be minor components in the rat PCA. It is reported that these leukotrienes have potent activities to increase the vascular permeability.

![Fig. 1. Effects of hydrocortisone, prednisolone and dexamethasone on 48-hr PCA and mediator-induced skin reactions in rats. Skin reactions were caused by 10⁻⁵ g/ml of histamine, 3×10⁻⁷ g/ml of serotonin and 3×10⁻⁶ g/ml of LTC₄. Glucocorticoids were given to rats intraperitoneally 2 hr prior to the elicitation of PCA and skin reactions. Each value represents the mean and the standard error of 6 rats. Shaded areas indicate the values of control sites for PCA (PCA(−) site) or skin reactions (saline-injected site). Statistical analysis was performed by Student’s t-test using the values subtracted by the value of each control site. *P<0.05, **P<0.01, ***P<0.001.](image-url)
(27–31). Therefore, in bronchial asthma, histamine and leukotrienes are thought to be important mediators for causing not only bronchoconstriction but also edema formation. Since the LTC4-induced skin reaction was also inhibited by glucocorticoids, it may possible that the edema formation by histamine and leukotrienes in bronchial asthma is inhibited by glucocorticoids.

It is suggested that glucocorticoids inhibit the edema formation caused by serotonin and dextran (4, 32) through the synthesis of vascular permeability inhibitory protein (33, 34). This glucocorticoid-induced protein is distinct from lipocortin and inhibits the increase of vascular permeability non-specifically. Therefore, it is suggested that the biosynthesis of this protein may be involved in the inhibition of PCA in rats. However, Nagai et al. (11) indicated that the inhibition of PCA requires a latent period, but demonstrated that the protein synthesis inhibitor cycloheximide failed to recover the inhibition. Therefore, a protein biosynthesis-independent mechanism can also be considered. The precise inhibitory mechanism of glucocorticoids on PCA is not yet clear.

Glucocorticoids inhibit the mediator release from basophils and mast cells (8–12). Here, we indicated that glucocorticoids inhibit the increase of vascular permeability non-specifically. These actions of glucocorticoids might contribute simultaneously to their anti-allergic effects.

References

1 Thompson, E.B. and Lippman, M.E.: Mechanism of action of glucocorticoids. Metabolism 23, 159–202 (1974)
2 Flower, R.J.: Steroidal anti-inflammatory drugs as inhibitors of phospholipase A2. Adv. Prostaglandin Thromboxane Res. 3, 105–112 (1978)
3 Danon, A. and Assouline, G.: Inhibition of prostaglandin biosynthesis by corticosteroids requires RNA and protein synthesis. Nature 273, 552–554 (1978)
4 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)
5 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)
6 Hirata, F., Schiffermann, 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)
7 Russo-Marie, F. and Duval, D.: Dexamethasone-induced inhibition of prostaglandin production does not result from a direct action on phospholipase activity but is mediated through a steroid-inducible factor. Biochim. Biophys. Acta 712, 177–185 (1982)
8 Schleimer, R.P., Lichtenstein, L.M. and Gillespie, E.: Inhibition of basophil histamine release by anti-inflammatory steroids. Nature 292, 454–455 (1981)
9 Andersson, P., Brattsand, R., Brange, C., Källström, L. and Stahre, G.: Protective effects of budesonide on lung anaphylaxis in actively sensitized guinea pigs. Inhibition of "IgE"-mediated anaphylaxis. Eur. J. Respir. Dis. 63, 260–262 (1982)
10 Daéron, M., Sterk, 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)
11 Nagai, H., Takizawa, T., Nakatomi, I., Matsuura, N. and Koda, A.: Anti-allergic action of glucocorticoids in rats. Japan. J. Pharmacol. 33, 349–355 (1983)
12 Grasman, N. and Jensen, S.M.: Influence of glucocorticoids on histamine release and 45Ca2+ uptake by isolated rat mast cells. Agents Actions 14, 21–30 (1984)
13 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)
14 Svensjö, E. and Roempke, K.: Time-dependent inhibition of bradykinin and histamine-induced increase in microvascular permeability by local glucocorticoid treatment. In Glucocorticoids, Inflammation and Bronchial Hyperreactivity, Edited by Hogg, J.C., Ellul-Micallef, R. and Brattsand, R., p. 136–144, Excerpta Medica, Amsterdam (1985)
15 Tada, T. and Okumura, K.: Regulation of homocytotropic antibody formation in the rat. I. Feedback regulation by passively administered antibody. J. Immunol. 106, 1002–1011 (1971)
16 Katayama, S., Shionoya, H. and Ohtake, S.: A new method for extraction of extravasated dye
in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. Microbiol. Immunol. 22, 89–101 (1978)

17 Douglas, W.W.: Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. In The Pharmacological Basis of Therapeutics, Edited by Gilman, A.G., Goodman L.S., Rall, T.W. and Murad, F., seventh edition, p. 605–638, Macmillan, New York (1985)

18 Schwartz, L.B. and Austen, K.F.: Mast cells and mediators. In Clinical Aspects of Immunology, Edited by Lachman, P.J. and Peters, D.K., p. 130–157. Blackwell Scientific Publications, Oxford (1982)

19 Kaliner, M.: Mast cell mediators and asthma. Prog. Resp. Res. 19, 17–29 (1985)

20 Katayama, S., Akimoto, N., Shionoya, H., Morimoto, T. and Katoh, Y.: Anti-allergic effect of azelastine hydrochloride on immediate type hypersensitivity reactions in vivo and in vitro. Arzneimittelforschung 31, 1196–1203 (1981)

21 Chand, N., Harrison, J.E., Rooney, S.M., Sofia, R.D. and Diamantis, W.: Inhibition of passive cutaneous anaphylaxis (PCA) by azelastine: dissociation of its antiallergic activities from antihistiminc and antiserotonin properties. Int. J. Immunopharmacol. 7, 833–838 (1985)

22 Azuma, H., Banno, K. and Yoshimura, T.: Pharmacological properties of N-(3',4'-dimethoxy-cinnamoyl)anthranilic acid (N-5'), a new antistopic agent. Br. J. Pharmacol. 58, 483–488 (1978)

23 Takashima, T., Ono, T., Ohtsuka, M., Mori, J. and Kumada, S.: The mode of action of antianaphylactic effect of tiaramide hydrochloride. Arzneimittelforschung 29, 903–910 (1979)

24 Yokoya, F., Nakayama, T., Sakamoto, K., Ohhira, K., Ohshika, Y., Mori, Y., Toyoshi, K., Nagai, H. and Koda, A.: Effect of (±)-2-(p-(2-thenoyl)-phenyl)propionic acid (suprofen) on experimental allergic reactions. Japan. J. Pharmacol. 34, 23–31 (1984)

25 Ashida, Y., Saijo, T., Kuriki, H., Makino, H., Terao, S. and Maki, Y.: Pharmacological profile of AA-861, a 5-lipoxygenase inhibitor. Prostaglandins 26, 955–972 (1983)

26 Barnes, N.C. and Costello, J.F.: Leukotrienes and asthma. In Asthma. Clinical Pharmacology and Therapeutic Progress. Edited by Kay, A.B., p. 194–204. Blackwell Scientific Publications, Oxford (1986)

27 Welton, A.F., Crowley, H.J., Miller, D.A. and Yacemko, B.: Biological activities of chemically synthesized form of leukotriene E_4. Prostaglandins 21, 287–296 (1981)

28 Peck, M.J. and Williams, T.J.: The effect of leukotrienes C_4 and D_4 on the microvasculature of guinea pig skin. Prostaglandins 21, 315–321 (1981)

29 Ueno, A., Tanaka, K., Katori, M., Hayashi, H. and Ariy, A.: Species difference in increased vascular permeability by synthetic leukotriene C_4 and D_4. Prostaglandins 21, 637–648 (1981)

30 Dahlén, S.-E., Björk, J., Hedqvist, P., Arfors, K.-E., Hammarström, S., Lindgren, J.-A. and Samuelsson, B.: Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. Proc. Natl. Acad. Sci. U.S.A. 78, 3887–3891 (1981)

31 Inagaki, N., Goto, S., Yamasaki, M., Nagai, H. and Koda, A.: Studies on vascular permeability increasing factors involved in 48-hour homologous PCA in the mouse ear. Int. Arch. Allergy Immunol. 80, 285–290 (1986)

32 Calignano, A., Carnuccio, R., Di Rosa, M., Ilentl, A. and Moncada, S.: The anti-inflammatory effect of glucocorticoid-induced phospholipase inhibitory proteins, Agents Actions 16, 60–62 (1985)

33 Öyanagui, Y. and Suzuki, S.: Vasoregulin, a glucocorticoid-inducible vascular permeability inhibitory protein. Agents Actions 17, 270–277 (1985)

34 Di Rosa, M., Calignano, A., Carnuccio, R., Ilentl, A. and Sautebin, L.: Multiple control of inflammation by glucocorticoids. Agents Actions 17, 285–289 (1985)