PHARMACOLOGICAL STUDIES ON THE RELEASE OF SLOW REACTING SUBSTANCE OF ANAPHYLAXIS DURING ANTI-IMMUNOGLOBULIN E ANTIBODY MEDIATED PASSIVE PERITONEAL ANAPHYLAXIS IN RATS

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The release of slow reacting substance of anaphylaxis (SRS-A) by anti-immunoglobulin E(IgE; e)-antibody mediated passive peritoneal anaphylaxis (PPA) in rats was investigated immunopharmacologically.

1) A significant amount of SRS-A was released by anti-e-antibody in the peritoneal cavity of rats passively sensitized with IgE. The amount of SRS-A released by anti-e-antibody was about one third less than that released in an anti-γ-antibody and IgG2a system. 2) The release of SRS-A was initiated at 2 min and reached its maximum 5 to 10 min after the injection of anti-e-antibody. 3) Disodium cromoglycate, tranilast and ketotifen inhibited the release of both SRS-A and histamine caused by anti-e-antibody mediated PPA. 4) Glucocorticoids (hydrocortisone, prednisolone and dexamethasone) also inhibited the release of both mediators. 5) p-Bromophenacyl bromide inhibited the release of both mediators. AA-861, a potent 5-lipoxygenase inhibitor, inhibited the release of SRS-A but not histamine. 6) Indomethacin slightly enhanced the release of SRS-A and inhibited the release of histamine. 7) Cytarabine resulted in leucopenia and inhibited the release of histamine but not SRS-A during PPA. 8) Dextran sulfate reduced the number of glass adherent peritoneal cells and inhibited the release of SRS-A but not histamine. These results suggest the suitability of anti-e-antibody mediated rat PPA for investigating the effect of anti-allergic agents on the release of SRS-A.

Keywords — IgE; SRS-A; anti-allergic drug; macrophage; mast cell

INTRODUCTION

Passive peritoneal anaphylaxis (PPA) in the rat is a useful method for the immunopharmacological study of the release of anaphylactic chemical mediators. Previously, Orange and Austen reported the dissociation of immunologic pathways leading to the release of histamine and slow reacting substance of anaphylaxis (SRS-A) in rat PPA with respect to the responsible immunoglobulins, participating target cells and the efficacy of drugs.1-4) They described that SRS-A was released from rat peritoneal polymorphonuclear cells by immunoglobulin G2a (IgG2a) mediated anaphylaxis. They further reported that the release of SRS-A was inhibited by diethylcarbamazine but not by disodium cromoglycate (DSCG). Unlike SRS-A, histamine was released from mast cells by an IgE mediated reaction and the release was inhibited by DSCG. From their results, IgE mediated PPA appears to be unsuitable for investigating the effect of DSCG-like drugs on the release of anaphylactic mediators. The present study, therefore, was conducted to evaluate the suitability of anti-e-antibody mediated rat PPA as a model for the immunopharmacological investigation of anti-allergic drugs.

MATERIALS AND METHODS

Animals — Male Wistar rats weighing 120 to 180 g were used. Animals were purchased from Shizuoka Laboratory Animal Center.

Drugs — DSCG (Fujisawa, Osaka), tranilast (Kissei, Matsumoto), ketotifen (Nippon Sandz, Osaka) and AA-861 (Takeda, Osaka) were kindly supplied from the respective pharmaceutical companies. Hydrocortisone (Nippon Merck-Banyu, Tokyo), prednisolone (Shionogi, Osaka), dexamethasone (Nippon Merck-Banyu), indomethacin (Sumitomo, Osaka), p-bromphenacyl bromide (p-BPB; Wako, Osaka), cytarabine (Sankyo, Tokyo) and dextran sulfate
(Wako) were purchased from the companies indicated. All drugs were dissolved or suspended in saline. Anti-rat-\(\varepsilon\)-antibody and anti-rat-\(\gamma\)-antibody were purchased from Nordic Immunologic Laboratories (Netherlands). Further purification was done by passing the antisera through a Sepharose 4B column coupled with rat IgM.

**PPA** — Rats were passively sensitized by intraperitoneal injection of antiserum containing homocytotropic antibody against dinitrophenylated ascaris (DNP-As) or bovine serum albumin (BSA). Antiserum against DNP-As was prepared according to the method of Tada and Okumura.\(^{5}\) Anti-DNP-As IgE was detected by a 48 h homologous passive cutaneous anaphylaxis (PCA). After passing through a Sepharose 4B column coupled with anti-\(\varepsilon\)-antibody, the PCA causing capacity of anti-DNP-As serum was diminished. However, similar abrogation was not seen when anti-\(\gamma\)-antibody was used as an absorbant. This evidence suggests that rat antiserum against DNP-As contains IgE homocytotropic antibody. Anti-BSA antibody was prepared according to the method of Orange et al.\(^{3}\) IgG\(_2\)a antibody against BSA was detected in a similar manner as described above for anti-DNP-As IgE antibody. A three hour homologous PCA was used for detection of IgG\(_2\)a homocytotropic antibody. Thirty six hours after the injection of each of the above antisera, anti-immunoglobulin antibody dissolved in 10 ml Tyrode solution was injected into the peritoneal cavity. Ten min later, the animals were killed by exsanguination and the peritoneal fluid was collected. Following gentle centrifugation at 400 \(\times\) \(g\) for 5 min at 4 °C, the amount of mediators in the supernatant fluid was measured. The partially purified SRS-A fraction was assayed on a stripped longitudinal smooth muscle of guinea pig ileum, which had been pretreated with atropine (5 \(\times\) 10\(^{-7}\) M) and pyrilamine (10\(^{-7}\) M). By definition, one unit (U) of SRS-A produces a contraction equivalent to 5 \(\times\) 10\(^{-8}\) g/ml histamine. Partial purification of SRS-A was done by 80% ethanol extraction and stepwise elution by different concentrations of ethanol from SEPPAK column (Waters Associates, Mass., USA). The SRS-A fraction was eluted by 70% ethanol after washing the column with water and 35% ethanol (pH 6.8) solution. In preliminary experiments, histamine, acetylcholine and serotonin were removed and more than 90% leukotriene C\(_4\) (LTC\(_4\)) and LTD\(_4\) were recovered by this procedure. Histamine was measured according to the method of May et al.\(^{8}\)

**Statistics** — Statistical analysis was performed by using the Student's \(t\) test.

**RESULTS**

**Effect of Anti-\(\varepsilon\) and Anti-\(\gamma\)-antibodies on the Release of SRS-A**

The results of experiments designed to measure the release of SRS-A from sensitized rat peritoneal cells by anti-\(\varepsilon\) and anti-\(\gamma\)-antibodies

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**FIG. 1. SRS-A and Histamine Release in Rat Passive Peritoneal Anaphylaxis**

Each column represents the mean ± S.E. of 5 to 6 experiments. Anti-DNP As and anti-BSA antibody was injected intraperitoneally 48 and 3 h prior to challenge, respectively.

**FIG. 2. Time Course for the Release of SRS-A and Histamine in Anti-\(\varepsilon\)-antibody Mediated Passive Peritoneal Anaphylaxis in Rat**

Each point represents the mean ± S.E. of 5 to 6 animals.
are shown in Fig. 1. From the peritoneal cells sensitized with anti-DNP-As IgE antibody, seven to twelve units/ml SRS-A were released by 50 or 200 times diluted anti-ε-antibody. Conversely, no SRS-A release was caused by anti-γ-antibody from the cells which were sensitized with anti-DNP As antibody. Similar results were obtained in the case of histamine release. Contrary to IgE mediated PPA, a three-fold greater release of SRS-A was observed by IgG_{2a} (against BSA) and anti-γ-antibody mediated PPA. Histamine was released to a much less extent in this system compared to that released by IgE antibody mediated system. The time course for release of SRS-A and histamine during IgE antibody mediated PPA is indicated in Fig. 2. While histamine release was initiated within 30 s by the injection of anti-ε-antibody, SRS-A release began 2 min after the injection of antiserum. Maximum release of SRS-A was observed 5 to 10 min after challenge.

Effect of Drugs on the Release of SRS-A by IgE Antibody Mediated PPA

Figure 3 shows the effect of DSCG, tranilast and ketotifen on the release of SRS-A and histamine by PPA. All drugs examined inhibited the release of both SRS-A and histamine when administered intravenously 1 min prior to challenge. A 48 h homologous PCA, caused by anti-DNP-As IgE and antigen, was clearly inhibited by each of the aforementioned drugs when administered under the same conditions as mentioned above (data not shown). Figure 4 shows the effect of glucocorticoids on the release of mediators in PPA. Hydrocortisone, prednisolone and dexamethasone clearly inhibited the release...
FIG. 6. Effect of Cytarabine and Dextran Sulfate on the Release of SRS-A and Histamine

Each column represents the mean ± S.E. of 4 to 6 experiments. Cytarabine was administered intraperitoneally for 3 d. Polymorphonuclear cells in blood were decreased 36% at a dose of 10 mg/kg and 76% at a dose of 37.5 mg/kg respectively. Dextran sulfate was administered intraperitoneally 24 h prior to challenge. Percentage of glass adherent cell in peritoneal exudate cells was decreased 10% at a dose of 50 mg/kg and 52% at a dose of 100 mg/kg, respectively.

of both mediators when administered subcutaneously 6 h prior to challenge. Dose and the timing of administration were determined in accordance with the results of PCA reaction reported previously. Effects of p-BPB, AA-861 and indomethacin are indicated in Fig. 5. While p-BPB, at a dose of 5 mg/kg, inhibited the release of histamine but not SRS-A, at a dose of 25 mg/kg, the drug inhibited the release of both mediators. AA-861, a potent 5-lipoxygenase inhibitor, inhibited the release of SRS-A but not histamine. Indomethacin slightly enhanced the release of SRS-A but inhibited the release of histamine. Figure 6 shows the effect of cytarabine and dextran sulfate on the release of SRS-A and histamine. Cytarabine caused leukopenia and decreased the population of polymorphonuclear cells and mast cells in peritoneal exudate cells. When cytarabine, at a dose of 37.5 mg/kg, was administered intraperitoneally for 3 d, the release of histamine was clearly inhibited but the release of SRS-A was not significantly inhibited. Dextran sulfate, when administered intraperitoneally at a dose of 100 mg/kg 24 h prior to challenge, reduced the number of glass adherent cells in peritoneal exudate cells while inhibiting the release of SRS-A but not histamine.

DISCUSSION

The study reported here indicates that SRS-A was released by anti-ε-antibody mediated PPA in rats and that this model is suitable for investigating the anti-allergic action of certain drugs such as DSCG and glucocorticoids. Previously, Orange et al. reported that SRS-A is released by antigen from polymorphonuclear cells sensitized with IgG₂a homocytotropic antibody but not from mast cells sensitized with IgE antibody.\(^2\)\(^3\)\(^0\) Notwithstanding their detailed study, there are few reports demonstrating SRS-A release by PPA using antiserum containing IgE homocytotropic antibody and antigen in rats.\(^8\)\(^1\)\(^0\) However, there have been no trials to clarify the role of IgE antibody in the release of SRS-A during PPA. In the present study, we demonstrated the release of SRS-A by anti-ε-antibody from rat peritoneal cells which were sensitized with IgE. Since this reaction occurred between IgE and anti-ε-antibody, SRS-A release was caused only by IgE antibody and released from IgE Fc receptor bearing cells. Lewies et al. reported the possible role of secondary cells that are affected by certain mediators released from primary cells (mast cells) in IgE mediated SRS-A release in lung tissue.\(^1\)\(^1\) According to their findings, many kinds of cells may be involved in IgE mediated SRS-A release. The cells concerned in this scheme will be discussed later. However, it is evident that this reaction was initiated only by IgE antibody. In addition, the present results indicated that the release of SRS-A by anti-ε-antibody was clearly suppressed by anti-allergic drugs such as DSCG, tranilast and glucocorticoids. Therefore, this model is suitable for investigation of the potency and mechanism of anti-allergic drugs.

Concerning the mechanism of SRS-A release in anti-ε-antibody mediated PPA, the present data show the importance of the 5-lipoxygenase pathway as demonstrated in many other systems.\(^1\)\(^2\)\(^1\)\(^3\)\(^1\)\(^4\) In our study, glucocorticoids and p-BPB clearly inhibited the reaction. Hirata et al. reported the existence of a phospholipase inhibitory mechanism in the suppression of histamine release by glucocorticoids in rats.\(^1\)\(^0\) In addition, when p-BPB at a dose of 25 mg/kg was administered intraperitoneally in rats, clear suppression of phospholipase A₂ activity was shown.\(^1\)\(^6\) These data suggest that phospholipase A₂ activation is involved in the release of SRS-A during rat PPA. However, since the above two agents could not be considered as true phospho-
lipase $\Lambda_2$ inhibitors, clarification of the precise role of phospholipase $\Lambda_2$ in the release of SRS-A must be investigate by using a specific enzyme inhibitor. In order to investigate the role of 5-lipoxygenase, AA-861, which is reported as a specific 5-lipoxygenase inhibitor, was used.\(^{10}\) In the present study, AA-861 clearly inhibited the release of SRS-A in a dose related fashion. These data suggested the involvement of the 5-lipoxygenase pathway in rat anti-\(\epsilon\)-antibody induced PPA. The role of 5-lipoxygenase was supported by the results of indomethacin. Indomethacin slightly enhanced SRS-A release, probably shifting the arachidonic metabolism to lipoxygenase pathway. But, whether this enhancement is related to the inhibition of cyclooxygenase activity or not is unclear. Nevertheless, the above data strongly suggest the participation of the 5-lipoxygenase pathway in the release of SRS-A during rat PPA.

Regarding the cells involved in PPA, it is reported that polymorphonuclear cells play an important role in the release of SRS-A.\(^{2,3}\) However, the present data also suggest the influence of mast cells and macrophages rather than polymorphonuclear cells in this reaction. Cytarabine, which decreased a number of polymorphonuclear cells, resulted in the inhibition of the release of histamine but not SRS-A. Dextran sulfate decreased the number of glass adherent cells, mainly macrophages and apparently inhibited the release of SRS-A without affecting the release of histamine. These data suggest the participation of macrophages in the release of SRS-A. While, the specific role of macrophages is still uncertain, there are two possible explanations. One is the direct participation of IgE on the macrophage surface because of the existence of an IgE Fc receptor on macrophage, as some investigators have previously reported.\(^{17,18}\) The other is the stimulation of macrophage by a humoral factor released from primary reacting cells (mast cells). This possibility is suggested by Lewis and Austen.\(^{11}\) Experiments using purified mast cells and macrophages are needed, however, to determine the precise role of cells.

In addition, since pharmacological investigation of anti-\(\gamma\)-antibody mediated release of SRS-A has not yet been conducted more immunopharmacological studies on this reaction are needed.

In conclusion, we suggest that the anti-\(\epsilon\)-antibody mediated rat PPA provides a useful model to study the effect of anti-allergic drugs on the release of SRS-A and to investigate its mechanisms.

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