Anaphylactic Release of Histamine and Slow Reacting Substance of Anaphylaxis (SRS-A) from Guinea-Pig Lung and its Modulation by Prostaglandins

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The regulatory mechanism of anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung tissue was studied. The release of histamine and SRS-A from sensitized lung tissue was markedly decreased by preincubating with isoproterenol, aminophylline, cyclic AMP-Na and PG E2. While the release of both chemical mediators was increased by preincubating with cyclic GMP-Na and PG F2 alpha, and by preincubating with the mixture of PG E2 and PG F2 alpha (1:9) the release of both chemical mediators was markedly increased comparing with that by preincubating with PG F2 alpha alone.

Key Words : Anaphylaxis, SRS-A, Prostaglandins, Ovalbumen, Guinea-pig lung.

Histamine and slow reacting substance of anaphylaxis (SRS-A) are released during anaphylactic reaction. It has been postulated that a variety of sympathomimetic amines were capable of inhibiting the immunologic release of histamine from passively sensitized human lung. It has also been described that the antigen-induced release of histamine from peripheral leukocytes was inhibited by both a beta-adrenergic agent and a methylxanthine. They attributed this effect to increases in cellular levels of cyclic AMP.

The present investigation was done to clarify the regulatory mechanism of anaphylactic release of histamine and SRS-A from guinea-pig lung tissue by preincubating with various drugs.

MATERIALS AND METHODS

Mepyramine maleate (Merck, Darmstadt), scopolamine hydrobromide (Tokyo Kasei, Tokyo), histamine dihydrochloride (Takara Kohsan, Tokyo), isoproterenol hydrochloride (Kaken Kagaku, Tokyo), aminophylline (neophyllin, Eisai, Tokyo), cyclic AMP-Na, cyclic GMP-Na (Daichi Kagaku, Tokyo) and prostaglandin E2, F2 alpha (Ono Pharmaceutical Co, Osaka) were all supplied as noted.

Male white rabbits weighing 2.7-3.0 kg were sensitized with ovalbumen and gamma globulin were prepared from their peripheral sera by salting out method using ammonium sulfate.

Male Hartley strain guinea-pig weighing 250-300 g were used. The lung tissue was dissected free of pleura, cartilage and large vessels with fine scissors and minced into fragments (30-50 mg) which were washed repeatedly with Krebs-Henseleit solution until the washing were grossly free of blood. The lung fragments were divided into ap-
proximately 500 mg replicates. The replicates were suspended in 24 ml of gamma globulin solution diluted 1:4 in Krebs-Henseleit solution, incubated at 37°C for 3-4 hours bubbling with oxygen and carbon dioxide (95:5, v/v).

All drugs used for preincubation of lung tissue before antigen challenge were prepared in Krebs-Henseleit solution just before use, and at the concentration studied did not induce a non-specific release of these mediators in the absence of antigen challenge.

Histamine and SRS-A released from the lung tissue were quantitated by the bioassay method, the former on the guinea-pig ileum (GPI) and the latter also on the GPI with continuous infusion of mixture of mepyramine maleate (100 ng/ml) and scopolamine hydrobromide (100 ng/ml).

Fig. 1 shows the schema of the tissue bioassay system. The lung fragments were put on a mesh stretched over a glass funnel. Guinea-pig ileum (GPI) and chick rectum (CR) were removed and suspended in a bioassay glass jackets. All of these were superfused with Krebs-Henseleit solution at 37°C saturated with oxygen and carbon dioxide (95:5, v/v). The rate of superfusion was constant at 20 ml/min.

Fig. 1. The schema of tissue bioassay system.

Histamine, ovalbumen and mixture of blockers were infused by an infusion pump (Harvard Apparatus, USA) at the rate of 0.97 ml/min. Contraction of these bioassay tissues were detected by an isotonic transducer (ME Commercial, Tokyo, Japan) and displayed on a polyrecorder. The size of contraction was expressed as the contraction index which consist of the area (cm²) encircled by the both baseline and the response curve. The amount of SRS-A was expressed as the international units; one unit of SRS-A is the dose which causes the same size of contraction on GPI with 5 ng of histamine.

RESULTS

Fig. 2 is the actual record of the release of histamine and SRS-A from sensitized guinea-pig lung fragments infusing 1 and 10 mg of ovalbumen. The upper GPI shows the amount of histamine, while the lower GPI shows the amount of SRS-A. The right upper column shows the standard response to histamine. It is obvious from this figure that the release of histamine occurs promptly. While the release of SRS-A occurs very slowly. And it is also obvious that the amount of the release of these chemical mediators became smaller by repeating the infusion of ovalbumen.

Fig. 3 summarized the data from 32 experiments. Hatched bars and clear bars mean the amount of SRS-A and histamine released from guinea-pig lung fragments, respectively. By the 1st time infusion of ovalbumen (1 mg), the mean values of released histamine and SRS-A were 1.85 ± 0.52 µg and 174.6 ± 115.0 units, respectively. On the other hand by the 2nd time infusion
of ovalbumen (1 mg), the mean values of released histamine and SRS-A were 0.20±0.20 μg and 11.8±29.2 units, respectively, and they were significantly lower than those by the 1st time ovalbumen infusion (P<0.001, P<0.001, n=32, paired t-test). By the 3rd time infusion of ovalbumen (10 mg), the mean values of released histamine and SRS-A were 0.84±0.44 μg and 18.8±28.2 units, respectively.

**Fig. 3.** The amount of histamine and SRS-A released from passively sensitized guinea-pig lung fragments by repeating the infusion of ovalbumen.

Fig. 4 shows the anaphylactic release of histamine and SRS-A from sensitized guinea-pig lung fragments and its inhibition by preincubating with isoproterenol and aminophylline (wet weight, 500 mg).

**Fig. 4.** The anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments by preincubating with isoproterenol (1×10⁻⁵ M) and aminophylline (1×10⁻² M). Hatched bars and clear bars mean the amount of SRS-A and histamine released from guinea-pig lung fragments, respectively. The mean values of released histamine and SRS-A in the control were 1.84±0.17 μg and 172.0±19.6 units, respectively. While the mean values of released histamine and SRS-A preincubated with isoproterenol were 0.78±0.19 μg and 106.8±35.6 units, respectively, and they were significantly lower than control values (P<0.001, P<0.001, n=14, paired t-test). The mean values of released histamine and SRS-A preincubated with cyclic AMP·Na and cyclic GMP·Na. The mean values of released histamine and SRS-A in the control were 1.64±0.17 μg and 172.0±19.6 units, respectively. While the mean values of released histamine and SRS-A preincubated with cyclic AMP·Na were 0.78±0.19 μg and 106.8±35.6 units, respectively, and they were significantly lower than control values (P<0.001, P<0.001, n=14, paired t-test). The mean values of released histamine and SRS-A preincubated with cyclic GMP·Na were 2.16±0.30 μg and 337.8±70.8 units, respectively, and they were significantly higher than the control values (P<0.001, P<0.001, n=14, paired t-test).

**Fig. 5.** Shows the anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments prein-
Fig. 5. The anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments (wet weight, 500 mg) and its modulation by preincubating with cyclic AMP-Na and cyclic GMP-Na. It is obvious from this figure that the release of histamine and SRS-A was decreased by preincubating the lung fragments with PG E2 in dose dependently. And there were highly significant differences between control values and those of PG E2 (0.1 μg/ml) and PG E2 (1 μg/ml), PG E2 (1 μg/ml) and PG E2 (10 μg/ml) (P < 0.001, P < 0.001, P < 0.001, n=19, paired t-test).

Fig. 6. The anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments (wet weight, 500 mg) and its inhibition by preincubating with various doses of PG E2.

Fig. 7 shows the anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments preincubated with various doses of PG F2alpha. The release of histamine and SRS-A was increased by preincubating the lung fragments with 0.1 μg/ml of PG F2alpha comparing with the control values (P < 0.01, P < 0.001, n=6, paired t-test). Then the amount of these chemical mediators decreased significantly by preincubating with the increasing dose of PG F2alpha.

Fig. 8 shows the anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments preincubated with 1 μg/ml of PG E2, PG F2alpha and mixture of PG E2 and PG F2alpha (1:9). It is obvious from this figure that the release of histamine and SRS-A was decreased by preincubating the lung fragments with 1 μg/ml of PG E2 (P < 0.001, P < 0.001, n=10, paired t-test) and was markedly increased by preincubating with 1 μg/ml of PG F2alpha (P < 0.001, P < 0.001, n=10, paired t-test). While the release of histamine and SRS-A was increased by preincubating the lung fragments with mixture of PG E2 and PG F2alpha (1:9) comparing with that by preincubating with PG F2alpha (1 μg/ml) alone (P < 0.001, P < 0.001, n =10, paired t-test).
Anaphylactic Release of Histamine and SRS-A

Fig. 8. The anaphylactic release of histamine and SRS-A from passively sensitized guinea-pig lung fragments (wet weight, 500 mg) and its modulation by preincubating with prostaglandins.

DISCUSSION

Present investigation was done in an effort to clarify the regulatory mechanism of release of histamine and SRS-A from passively sensitized guinea-pig lung tissue by preincubating with various drugs. The anaphylactic reaction in ovalbumine sensitized guinea-pig is a result of the interaction between non-reaginic antibodies of the IgG class and challenging antigen3). Guinea-pig lung tissue can be passively sensitized with IgG antibody by incubating in sera from ovalbumine sensitized rabbit. Histamine and SRS-A are known to be released when lung tissue sensitized in this way is subsequently challenged with the corresponding antigen. In addition to these chemical mediators, Piper and Vane demonstrated the release of prostaglandins45. But in this experiment it was impossible to detect the release of prostaglandins (PGs) by the chick rectum which was very sensitive to PGs.

Beta-adrenergic agents appear capable of interacting with specific beta-receptors and thus increase cellular levels of cyclic AMP. In the present experiments, isoproterenol, the predominantly beta-adrenergic agents, inhibited the antigen-induced release of both histamine and SRS-A. Methylxanthines such as aminophylline act as competitive inhibitors of the cytoplasmic phosphodiesterase that catabolizes cyclic AMP to 5'-AMP. In the present experiments, aminophylline effectively inhibited the anaphylactic release of both histamine and SRS-A.

Thus the increase of cyclic GMP produces an enhancement of the anaphylactic release of chemical mediators while cyclic AMP is inhibitory5). In the present experiments, cyclic AMP·Na inhibited, while cyclic GMP·Na enhanced the anaphylactic release of chemical mediators from passively sensitized guinea-pig lung fragments.

PG E2 inhibited the anaphylactic release of histamine and SRS-A from sensitized guinea-pig lung fragments in dose dependently. While PG F2 alpha enhanced the anaphylactic release of histamine and SRS-A from sensitized guinea-pig lung fragments, but such an enhancement became less dominant by increasing its dose.

Previously we reported that the mixture of PG E2 and PG F2 alpha (50 ng/ml) showed the maximal contraction of guinea-pig tracheal strips at the mixture ratios of PG E2:PG F2 alpha=1:96). White Nemoto et al7) demonstrated that both of the serum prostaglandin E2 and F2 alpha were significantly higher in asthmatic patients than in normal subjects, and that the ratios of PG E2 and PGF2 alpha were 1:9-3:7. In the present experiments, the release of histamine and SRS-A from passively sensitized guinea-pig lung fragments was markedly increased by preincubating these lung fragments with mixture of PG E2 and PG F2 alpha (1:9) comparing with that by preincubating with PG F2 alpha alone. These results may suggest that the patients in the status asthmaticus shows the ratios of serum PG E2:PG F2 alpha=1:9 and that at this ratio bronchoconstriction and the release of chemical mediators occur most predominantly.

REFERENCES

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