Effect of Butyl 3-(1H-Tetrazol-5-Yl) Oxanilate (MTB) on Immunological or Non-Immunological Histamine and SRS(-A) Release from Guinea-Pig, Monkey and Human Lung Tissue

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Accepted April 30, 1987

Abstract—We investigated the influence of butyl 3-(1H-tetrazol-5-yl) oxanilate (MTB) on the release of histamine and slow reacting substance of anaphylaxis (SRS-A) in vitro. MTB dose-dependently inhibited the release of not only histamine but also SRS-A from passively sensitized guinea-pig lung, while disodium cromoglycate (DSCG) hardly affected either release. MTB also resulted in a dose-dependent inhibition of the release of these mediators from the passively sensitized cynomolgus and rhesus monkey and that from human lung at concentrations similar to those inhibiting the release in the guinea-pig. Relatively lower inhibitory activities on the releases of both mediators from the cynomolgus monkey and human lung, but no effects on those from the rhesus monkey were observed with DSCG. MTB dose-dependently inhibited only SRS release from guinea-pig lung induced by phospholipase A₂, although the compound did not show any inhibitory activity on the release of those from the calcium ionophore (A23187)-stimulated one. On the other hand, the release of SRS, but not that of histamine from the lung stimulated with A23187 as well as phospholipase A₂ was inhibited by N-(3,4-dimethoxyccinamoyl)-anthranilic acid (tranilast, N-5'). From these results, MTB was a potent inhibitor of the anaphylactic release of the mediators, particularly SRS-A. It was suggested that the inhibitory mechanism of MTB is different from those of DSCG and N-5'.

A newly synthesized compound, butyl 3-(1H-tetrazol-5-yl) oxanilate (MTB), has been found to potently inhibit 48-hr passive cutaneous anaphylaxis (PCA) in the rat and 8-day PCA in the guinea-pig (1). The inhibitory activity of this compound on PCA was demonstrated by administering it not only intravenously but also perorally and was suggested to be shown through the suppression of mediator release following the antigen-antibody reaction (1).

In this paper, the effect of MTB on the immunological and non-immunological release of histamine and SRS-A from the guinea-pig, monkey and human lung tissue is described.

Materials and Methods

Materials: The materials used and their sources were as follows: butyl 3-(1H-tetrazol-5-yl) oxanilate (MTB), N-(3,4-dimethoxyccinamoyl) anthranilic acid (tranilast, N-5') and disodium cromoglycate (DSCG, supplied from Wakamoto Pharm. Co., Ltd., Ohimachi, Kanagawa, Japan), calcium ionophore A23187 (A23187, Calbiochem-Behring, La Jolla, CA), phospholipase A₂ (from cobra venom), bovine serum albumin (BSA, Cohn Fr. V) and mepyramine maleate (Sigma Chem., St. Louis, MO), atropine sulfate, o-phthalaldehyde (Wako Pure Chem. Ind. Ltd.,
Osaka, Japan), mite extract (from Dermatophagoides farinae, supplied by Dr. H. Nagai of Gifu Pharm. Univ., Gifu, Japan). Other reagents were the highest grade commercially available.

MTB and DSCG were dissolved in methanol and Tyrode’s solution at 2×10⁻² and 4×10⁻³ g/ml, respectively. N-5’ was suspended in distilled water at 4×10⁻³ g/ml and adjusted to pH 7.6 with 1N NaOH. A23187 dissolved in dimethylsulfoxide at 10⁻² g/ml was diluted with Tyrode’s solution before use. Phospholipase A₂ was dissolved in saline.

Animals: Male Hartley guinea-pigs weighing 400–600 g were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). Monkeys weighing 2.1–3.3 kg (cynomolgus monkey) and 3.9 kg (rhesus monkey) and macroscopically normal human lung tissue obtained at the time of resection for carcinoma were used.

Anti BSA serum: The guinea-pig was immunized with an emulsion of an equal volume of 2% BSA and complete Freund’s adjuvant (Difco Lab., Detroit, MI) according to the method of Levine et al. (2). The antiserum (3-hr PCA titer=1:32,000) obtained was kept at -20°C.

Human atopic serum: Human atopic serum against mite (RAST value>30%) was diluted with 4 volumes of Ca²⁺-free Tyrode’s solution and kept at -80°C.

Passive sensitization of the guinea-pig: The guinea-pig was passively sensitized by an i.v. injection of 0.5 ml/animal of anti BSA guinea-pig serum. Two days after the injection, the guinea-pig was sacrificed by bleeding from the femoral arteries. The lung was perfused with 20 ml/animal of Ca²⁺-free Tyrode’s solution through the pulmonary artery and removed from the body.

Preparation of lung fragments: The isolated guinea-pig, monkey or human lung was freed from large bronchi and vessels, and it was fragmented into pieces of about 0.5×0.5×0.5 mm (in guinea-pigs) or 0.7×1×2 mm (in monkeys and humans) with a Mcllwain tissue chopper. After rinsing with 50 ml/g wet tissue of Ca²⁺-free Tyrode’s solution, the lung fragments of guinea-pig were used in the release experiments of histamine and SRS-A, and those of monkeys and humans were passively sensitized in vitro and then used for the release experiments.

Passive sensitization of monkey and human lung fragments: Monkey or human lung fragments were passively sensitized with 5 or 10 ml/g wet tissue of human atopic serum at 37°C for 2–4 hr. After the completion of sensitization, the lung fragments, supported on gauze, were washed with 50 ml/g wet tissue of Ca²⁺-free Tyrode’s solution.

Anaphylactic histamine and SRS-A release: Two hundred to 500 mg of passively sensitized guinea-pig, monkey or human lung fragments were distributed into individual tubes, suspended in 0.92 ml/100 mg wet tissue of Tyrode’s solution and preincubated at 37°C for 5 min. Following the addition of 0.03 ml/100 mg wet tissue of drug solutions or vehicles and incubation for 5 min, the suspended lung fragments were challenged with 0.05 ml/100 mg wet tissue of antigen (final concentrations: 10⁻⁶ g/ml BSA in the guinea-pig, 5×10⁻⁵ g/ml mite extract in the monkey and humans) at 37°C for 15 min. After removal of fragments on gauze, the resultant anaphylactic filtrate was centrifuged at 1,700×g for 30 min at 4°C, and the supernatant was stored at -20°C until assay.

Non-immunological histamine and SRS release: Three hundred to 500 mg of normal guinea-pig lung fragments were distributed into individual tubes and suspended in 0.92 or 0.965 ml/100 mg wet tissue of Tyrode’s solution. After preincubation at 37°C for 5 min, addition of 0.03 ml/100 mg wet tissue of drug solutions or vehicles, and incubation for 5 min, the suspended lung fragments were stimulated with 0.05 ml/100 mg wet tissue of phospholipase A₂ (final concentrations: 1 U/ml for histamine release, 0.5 U/ml for SRS release) at 37°C for 20 min or with 0.005 ml/100 mg wet tissue of A23187 (final concentration: 10⁻⁸ g/ml) at 37°C for 120 min. The conditions of filtration, centrifugation and storage were the same as those of the anaphylactic histamine and SRS-A release experiments.

Assay of histamine and SRS(-A): Hista-
Results

1. Effect on the histamine and SRS-A release from the passively sensitized guinea-pig lung: As shown in Fig. 1, the release of histamine and SRS-A from passively sensitized guinea-pig lung fragments was dose-dependently inhibited by $10^{-7}$ to $10^{-4}$ g/ml of MTB. At $10^{-4}$ g/ml, the inhibition of histamine release was by 36% and that of SRS-A release was by 61%. On the other hand, $10^{-5}$ and $10^{-4}$ g/ml of DSCG did not inhibit the release of both mediators. The same concentration of N-5’ scarcely affected histamine release, but SRS-A release was reduced to 50% of the control at $10^{-4}$ g/ml.

2. Effect on the histamine and SRS-A release from the passively sensitized monkey lung: In Fig. 2 is shown the effect of MTB on histamine and SRS-A release from passively sensitized cynomolgus monkey lung fragments. MTB dose-dependently inhibited the release of anaphylactic mediators. At $10^{-5}$ g/ml, the inhibition was 47% for histamine and 53% for SRS-A release. N-5’ at $10^{-4}$ g/ml reduced histamine and SRS-A release to 40 and 32% of the control value, respectively. Although DSCG seemed to inhibit the release of both mediators at $10^{-4}$ g/ml, it was much less potent than MTB. The release of histamine and SRS-A from passively sensitized rhesus monkey lung fragments was also inhibited by $10^{-6}$ to $10^{-4}$ g/ml of MTB (Fig. 3).

3. Effect on the histamine and SRS-A release from the passively sensitized human lung: In the case of human lung fragments, the release of histamine and SRS-A was dose-dependently inhibited by MTB at $10^{-8}$ to $10^{-4}$ g/ml. Inhibition of histamine release was 21% and that of SRS-A release was 46% at $10^{-6}$ g/ml of MTB. The inhibition by MTB was more potent than those by DSCG and N-5’ (Fig. 4).

4. Effect on the histamine and SRS release from the guinea-pig lung stimulated by A23187: As shown in Fig. 5, $10^{-8}$ to $10^{-4}$ g/ml of MTB had little effect on the release of histamine and SRS from the guinea-pig lung fragments stimulated by A23187, while N-5’ at $10^{-4}$ g/ml markedly inhibited the SRS release.

5. Effect on the histamine and SRS release from the guinea-pig lung stimulated by phospholipase A2: Figure 6 illustrates the effect of MTB on the release of histamine and
Fig. 2. Effect of MTB, DSCG and N-5' on histamine and SRS-A release from passively sensitized cynomolgus monkey lung fragments. ■: Histamine, ●●●: SRS-A. Each column represents the mean±S.E. of 3 experiments, with the exception of 2 experiments (mean) for N-5'.

Fig. 3. Effect of MTB and DSCG on histamine and SRS-A release from passively sensitized rhesus monkey lung fragments. ■: Histamine, ●●●: SRS-A.

SRS from the guinea-pig lung fragments stimulated by phospholipase A₂. MTB dose-dependently inhibited the SRS release, while the histamine release was hardly affected. N-5' also considerably inhibited the SRS release exclusively.

Discussion

In the present study, the effect of MTB on histamine and SRS-A release was investigated and compared with those of DSCG and N-5'.
MTB dose-dependently inhibited the release of anaphylactic histamine and SRS-A from the lung fragments of all the species examined. DSCG also inhibited the release of both anaphylactic mediators from cynomolgus monkey and human lung, but the inhibitory potency was 1/10-1/100 as small as that of MTB. Furthermore, DSCG did not show any inhibition of the release of histamine or SRS-A from the guinea-pig and rhesus monkey lung. The precise site of action of DSCG for the inhibition of the mediator
release is still not known; however, the following possible mechanisms have been proposed from the results of the experiments using mainly sensitized isolated rat mast cells: 1) inhibition of phosphodiesterase (5), 2) inhibition of calcium influx (6), and 3) regulation of phosphorylation of protein (7). On the other hand, the mechanisms responsible for the inhibition of MTB on the mediator release have not been examined yet. However, from the present results showing different effects of MTB and DSCG on the release from lungs of the guinea-pig and rhesus monkey, it was suggested that the inhibitory mechanisms of MTB are different from those of DSCG and, conversely, that some different mechanisms or factors influencing the release of histamine and SRS-A among those species exist. It is likely that MTB inhibits not only the common step for the release of histamine and SRS-A but also the production of SRS-A from the finding that both histamine and SRS-A release was inhibited by the compound, particularly the latter.

On the contrary, MTB hardly affected the release of histamine and SRS from the guinea-pig lung fragments induced by A23187, but N-5’ inhibited it. Accordingly, it is suggested that the mechanism of action of MTB may also be different from that of N-5’. It was reported that the antiallergic effect of N-5’ was not mediated through an adrenergic mechanism (8), but it inhibited some calcium dependent process (9). Considering these reports and the present results, it was suggested that MTB may inhibit a calcium independent process.

MTB dose-dependently inhibited the SRS release from the guinea-pig lung fragments induced by phospholipase A₂. This is in contrast to that by A23187. It was reported that not only mast cells but also other cell types may be involved in the SRS release from the guinea-pig lung fragments induced by non-immunological stimuli (10). In rat mast cells, it was reported by Imai et al. (11) that stimulation by A23187 and compound 48/80 produced the activation of different membrane phospholipid turnover. Krell and Kusner (12) indicated that the respective formation of SRS(–A) by antigen and A23187 was induced by different mechanisms as well as different cell sources in the guinea-pig lung. From the reports mentioned above, it is considered to be better to utilize mast cell or tissue and an antigen-antibody reaction system for further elucidation of the mechanisms of action of MTB.

Summarizing the results, it is concluded that MTB is a potent inhibitor of the release of anaphylactic histamine and SRS-A, and suggested that the mechanisms through which MTB inhibits these mediator releases may be different from those of DSCG or N-5’.

References


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