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A partial involvement of histamine in ultrasonically nebulized distilled water-induced bronchoconstriction in guinea-pigs

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1 Inhalation of ultrasonically nebulized distilled water (UNDW) can induce bronchoconstriction only in asthmatics, but mechanisms of the response are not well known. We recently reported a guinea-pig model of UNDW-induced bronchoconstriction (UNDW-IB) in which UNDW induces bronchoconstriction when UNDW is inhaled 20 min after a challenge with aerosolized ovalbumin (OA) in passively sensitized, anaesthetized and artificially ventilated guinea-pigs.

2 To elucidate the role of histamine in the UNDW-IB, we examined the effects of antihistamines, diphenhydramine hydrochloride (DH) and chlorpheniramine maleate (CP), and measured histamine content in bronchoalveolar lavage fluid (BALF) in the animal model.

3 DH in doses of 0.1, 1.0 and 10 mg kg^{-1} and CP in doses of 0.01, 0.1 and 1.0 mg kg^{-1} administered intravenously 15 min after the OA challenge partially reduced the UNDW-IB at 1 and 2 min after the UNDW inhalation in a dose-dependent manner. Histamine content in BALF recovered 10 min after the UNDW inhalation following the OA provocation was significantly increased compared with that after saline inhalation and before the UNDW inhalation following the OA challenge.

4 Intravenous atropine in a dose of 0.5 mg kg^{-1} or inhaled disodium cromoglycate in concentrations of 1 and 10 mg ml^{-1} did not alter the UNDW-IB.

5 These results suggest that histamine is involved in part in the UNDW-IB in our animal model.

Keywords: Histamine; antihistamines; ultrasonically nebulized distilled water-induced bronchoconstriction (UNDW-IB); guinea-pigs

Introduction

As it is well known that inhalation of ultrasonically nebulized distilled water (UNDW) causes bronchoconstriction in asthmatics, the UNDW provocation test has been proposed for measuring bronchial responsiveness. It is highly specific for asthma, especially when compared with methacholine provocation (Allegra & Bianco, 1980; Anderson *et al*., 1983; Galdès-Sebaldt *et al*., 1985; Bascom & Bleecker 1986).

Clinically, although some researchers have examined the mechanism of UNDW-induced bronchoconstriction (UNDW-IB), it has not been well understood. It is considered that an animal model of UNDW-IB is helpful for understanding the mechanism of UNDW-IB and we (Fujimura *et al*., 1997a) recently showed that UNDW-IB is induced when UNDW is inhaled 20 min after a challenge with an aerosolized antigen in passively sensitized, anaesthetized and artificially ventilated guinea-pigs. The UNDW inhalation does not produce bronchoconstriction 20 min after a saline inhalation in nonsensitized or sensitized guinea-pigs, or 20 min after the antigen inhalation in nonsensitized animals. Methacholine-induced bronchoconstriction does not evoke UNDW-IB. Accordingly, an allergic reaction or process, excluding bronchoconstriction *per se*, may be one of the mechanisms underlying the UNDW-IB. This proposal is compatible with several clinical studies showing that UNDW-IB is related to allergic reactions and the extent of airway inflammation (Carpi *et al*., 1993; Chetta *et al*., 1996). Furthermore, this animal model is unique because thromboxane A_2 (TXA_2) does not take a part in the UNDW-IB while TXA_2 is involved in many reported animal models of immediate allergic reaction (Fujimura *et al*., 1984), propranolol-induced bronchoconstriction after allergic reaction (Songur *et al*., 1994), and bronchial hyperresponsiveness induced by antigen (Arimura *et al*., 1994), ozone (Aizawa *et al*., 1985), endotoxin (Arimura *et al*., 1993), leukotriene B_4 (O'Byrne, *et al*., 1985), platelet activating factor (Chung *et al*., 1986), and interleukin-8 (Xiou *et al*., 1995).

Some investigators (Allegra & Bianco, 1980; Allegra *et al*., 1993; Anderson *et al*., 1983; Kaliner *et al*., 1974; Azevedo *et al*., 1990; Goose & Blair, 1969; Wells *et al*., 1986; Robuschi *et al*., 1987; del Bufalo *et al*., 1989) have reported that histamine, one of the mediators in allergic response, may play a role in UNDW-IB of asthmatics. The purpose of the present study was to examine whether histamine is involved in the UNDW-IB of our animal model. We assessed effects of antihistamine on the UNDW-IB and measured histamine content in bronchoalveolar lavage fluid (BALF) recovered 10 min after the response.

Methods

Animals

Male, albino, Hartley strain guinea-pigs weighing 330–380 g were obtained from Sankyou Laboratory Service (Toyama, Japan). After arriving at the Institute of Animal Experiments in our university, they were kept in conventional animal housing facilities for 1 week before use. They were allowed to drink and eat *ad libitum*.

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Passive sensitization of guinea-pigs

Guinea-pig homocytotropic antiserum was obtained according to the method of Santives *et al*. (1976). Briefly, 500 μg of ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally to each guinea-pig at multiple sites. A booster dose was prepared and administered in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster dose, pooled, and kept frozen until use. The antibody titre of this serum was 1 : 12800, 1 : 6400, and 1 : 512, as estimated by passive cutaneous anaphylaxis (PCA) at 4 h, 24 h and 7 days, respectively. Normal guinea-pigs were passively sensitized with 1.0 ml kg^{-1} antiserum intraperitoneally.

Preparation of animals

Five to 7 days after the passive sensitization, guinea-pigs were anaesthetized with sodium pentobarbitone (75 mg kg^{-1} intraperitoneally). They were placed in the supine position, and the trachea was cannulated with a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm). The left jugular vein was cannulated for administration of drugs.

After surgery, each guinea-pig was artificially ventilated by a small animal respiratory pump (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA, U.S.A.). The tidal volume was 10 ml kg^{-1} and the rate was 60 strokes min^{-1}. The changes in lung resistance to inflation, defined as the lateral pressure of the tracheal tube or pressure at the airway opening [Pao (cmH_2O)], was measured using a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo, Japan). Since the change in the Pao following antigen challenge in passively sensitized guinea-pigs with or without pretreatment with antihistamines (Minami, 1983) or inhalation of leukotriene C_4 in naive animals represented the average of the changes in pulmonary resistance and reciprocal dynamic lung compliance (Fujimura, 1983), we used the increase in the Pao as an overall index of bronchial response to bronchoactive agents.

When all procedures were completed, the animals were overinflated by two times tidal volume for two breaths by clamping the outlet port of the respirator to unify the volume history of the lung (Fujimura, 1983). All kinds of aerosols were generated for 30 s with an ultrasonic nebulizer developed for small animals at our institution (Minami *et al*., 1983). The amount of aerosol produced is 15.2 μl min^{-1} when a physiologic saline solution is nebulized (Kurashima *et al*, 1997), and 46.4% of a nebulized albumin is deposited in the lungs when a ^{99m}Tc-albumin solution is inhaled (Minami *et al*., 1983). The median aerodynamic diameter of the particles of a physiologic saline is 3.59 ± 1.96 μm (means $\pm$ s.d.), when measured by a laser particle size analyser (Kurashima *et al*., 1997).

All the animal procedures in this study complied with the standards set out in the guidelines of the care and use of laboratory animals on the Takara-machi campus of Kanazawa University.

Study protocol

Studies one to four were carried out to elucidate the role of histamine in the UNDW-IB. Study five was conducted to examine whether bronchoconstriction mediated in part by secondarily released mediators can induce UNDW-IB or not. Studies six and seven were performed to assess the effects of atropine and disodium cromoglycate on the UNDW-IB. Each study was performed separately.

Study 1: Effect of diphenhydramine hydrochloride (DH) on the UNDW-IB

An antihistamine drug, diphenhydramine hydrochloride (DH) in a dose of 0.1 ($n=9$), 1.0 ($n=8$) or 10 mg kg^{-1} ($n=9$), or saline ($n=10$), was administered intravenously 15 min after the challenge with OA in passively sensitized guinea-pigs, and then UNDW was inhaled 5 min later; 20 min after the OA challenge.

Study 2: Effect of chlorpheniramine maleate (CP) on the UNDW-IB

Another antihistamine drug, chlorpheniramine maleate (CP) in a dose of 0.01 ($n=7$), 0.1 ($n=10$) or 1.0 mg kg^{-1} ($n=10$), or saline ($n=10$), was administered intravenously 15 min after the challenge with OA in passively sensitized guinea-pigs, and then UNDW was inhaled 5 min later; 20 min after the OA challenge.

Study 3: Effect of DH administered 15 min after antigen challenge on the antigen-induced bronchoconstriction

DH ($n=6$) or saline ($n=6$) was administered intravenously 15 min after the OA challenge in passively sensitized guinea-pigs and the Pao was measured without an inhalation of UNDW or saline during a 30 min period after the OA provocation.

Study 4: Histamine content in bronchoalveolar lavage fluid (BALF) after the UNDW-IB

To investigate whether UNDW inhalation in this animal model induces histamine release or not, bronchoalveolar lavage (BAL) was carried out 20 min after the OA challenge (immediately before the UNDW inhalation) ($n=8$) or 10 min after UNDW ($n=10$) or saline ($n=8$) inhalation following the OA challenge (30 min after the OA challenge) in passively sensitized guinea-pigs. Normal saline 10 ml aliquot was injected into the lungs and the lavage effluent was allowed to drain into a 50 ml conical tube. This procedure was repeated twice. Histamine content in the BALF was determined by a commercially available radio immunoassay (SRL, Inc., Tokyo, Japan).

Study 5: UNDW inhalation after leukotriene D_4-induced bronchoconstriction

To assess whether prior bronchoconstriction induces UNDW-IB, UNDW was inhaled 10 min after an aerosolized leukotriene D_4 in a concentration of 1.0 μg ml^{-1} ($n=6$) or vehicle (saline, $n=6$) was administered for 30 s.

Study 6: Effect of atropine on the UNDW-IB

Atropine sulphate monohydrochloride at a dose of 0.5 mg kg^{-1} ($n=7$) or saline ($n=7$) was administered intravenously 15 min after the challenge with OA, and then UNDW was inhaled 5 min later; 20 min after the OA challenge.

Study 7: Effect of disodium cromoglycate on the UNDW-IB

Disodium cromoglycate at a concentration of 1.0 ($n=6$) or 10 mg ml^{-1} ($n=6$) or saline ($n=6$) was inhaled for 30 s 15 min after the challenge with OA, and then UNDW was inhaled 5 min later.

Chemicals

The following chemicals were used: atropine sulphate monohydrochloride (Sigma, St. Louis, MO, U.S.A.), chlorpheniramine maleate (Sigma), diphenhydramine hydrochloride (Sigma), distilled water (Otsuka Pharmaceutical Co., Ltd., Osaka, Japan), Freund's complete adjuvant (Sigma), ovalbumin (Sigma), physiologic saline (Otsuka), leukotriene D_4 (Sigma), sodium pentobarbital (Abbott Laboratories, North Chicago, IL., U.S.A.), and disodium cromoglycate (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan).

Statistical analysis

Data are shown as means ± s.e.mean. Statistical differences were determined by analysis of variance (ANOVA). A P value of 0.05 or less was considered statistically significant.

Results

Baseline value of the Pao before the OA provocation was not significantly different between groups in each of the following studies.

Study 1: Effect of diphenhydramine hydrochloride (DH) on the UNDW-IB

Percentage increases in the Pao from the baseline value after an inhalation of UNDW following the OA challenge in four groups are shown in Figure 1. The peak value after the OA challenge or the value immediately before the UNDW inhalation (20 min after the OA challenge) were not significantly different from each other. Percentage increases in the Pao at 1 min after the UNDW inhalation from the pre-OA challenge values were 429.8 ± 42.0, 267.7 ± 29.6, 303.9 ± 28.4 and 354.1 ± 36.6% with saline and 10, 1.0 and 0.1 mg kg^{-1} of DH. The value was significantly lower with 10 and 1.0 mg kg^{-1} of DH than with saline treatment ($P<0.01$ and $P<0.05$, respectively). Similarly, percentage increase in the Pao at 2 min after the UNDW inhalation was 348.5 ± 18.5, 203.4 ± 18.9, 244.9 ± 18.7 and 289.7 ± 28.7% with saline and 10, 1.0 and 0.1 mg kg^{-1} of DH, respectively, and the values with 10 and 1.0 mg kg^{-1} of DH were significantly different from that with saline treatment ($P<0.01$).

Study 2: Effect of chlorpheniramine maleate (CP) on the UNDW-IB

Percentage increases in the Pao from the baseline value after the inhalation of UNDW following the OA challenge are shown in Figure 2. The peak values after the OA challenge or the value just before the UNDW inhalation were not significantly different among the groups. The percentage increases in the Pao at 1 min after the UNDW inhalation from the pre-OA challenge values were 408.2 ± 37.1, 277.5 ± 19.6, 316.2 ± 36.4 and 354.6 ± 36.2% with saline and 1.0, 0.1 and 0.01 mg kg^{-1} of CP. The value with 1.0 mg kg^{-1} of CP was significantly different from that with saline treatment ($P<0.01$). Similarly, the percentage increases in the Pao at 2 min after the UNDW inhalation were 357.1 ± 35.2%, 277.5 ± 19.6%, 251.5 ± 23.0% and 291.5 ± 18.7% with saline, 1.0, 0.1 and 0.01 mg kg^{-1} of CP, and the value was significantly lower with 1.0 and 0.1 mg kg^{-1} of CP than with saline treatment ($P<0.01$ and $P<0.05$, respectively).

Study 3: Effect of DH administered 15 min after antigen challenge on the antigen-induced bronchoconstriction

Time course of percentage increases in the Pao caused by the OA challenge in animals given DH or saline 15 min after the OA provocation is shown in Figure 3. There was no significant difference in the time course curve after the OA challenge or the administration of DH or saline between the two groups.

Study 4: Histamine content in bronchoalveolar lavage fluid (BALF) after the UNDW-IB

The total recovered bronchoalveolar lavage fluid (BALF) volume was 14.6 ± 0.6, 13.5 ± 0.8 and 14.2 ± 1.1 ml immediately before the UNDW inhalation, 10 min after the UNDW and the saline inhalation following the OA provocation

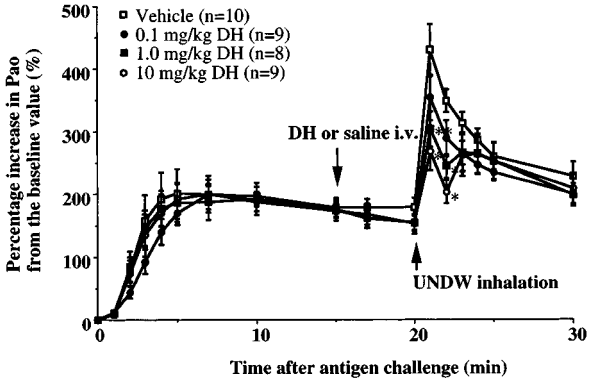

Figure 1 Effect of diphenhydramine hydrochloride (DH) on ultrasonically nebulized distilled water-induced bronchoconstriction (UNDW-IB). Ultrasonically nebulized distilled water (UNDW) was inhaled 20 min after ovalbumin (OA, 0.1 mg ml^{-1}) challenge in passively sensitized guinea-pigs. DH or vehicle (saline) was intravenously administered 5 min before the UNDW inhalation (15 min after the OA challenge). Each point represents the mean and vertical lines show s.e.mean. *$P<0.01$, **$P<0.05$ compared with vehicle treatment group.

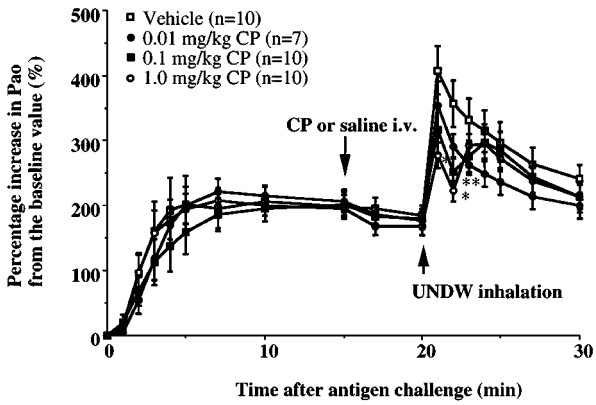

Figure 2 Effect of chlorpheniramine maleate (CP) on ultrasonically nebulized distilled water-induced bronchoconstriction (UNDW-IB). Ultrasonically nebulized distilled water (UNDW) was inhaled 20 min after ovalbumin (OA, 0.1 mg ml^{-1}) challenge in passively sensitized guinea-pigs. CP or vehicle (saline) was intravenously administered 5 min before the UNDW inhalation (15 min after the OA challenge). Each point represents the mean and vertical lines show s.e.mean. *$P<0.01$, **$P<0.05$ compared with vehicle treatment group.

(30 min after the OA challenge), respectively, in passively sensitized guinea-pigs. These values were not significantly different. Histamine contents in the BALF were 29.2 ± 8.4, 58.7 ± 11.7, and 28.5 ± 6.2 nmol l^{-1} 20 min after the OA challenge, 10 min after the UNDW inhalation and 10 min after the saline inhalation following the OA provocation, respectively (Figure 4). The value after the UNDW inhalation following the OA provocation was significantly greater than those after the OA challenge alone and after the saline inhalation following the OA challenge ($P<0.05$ each).

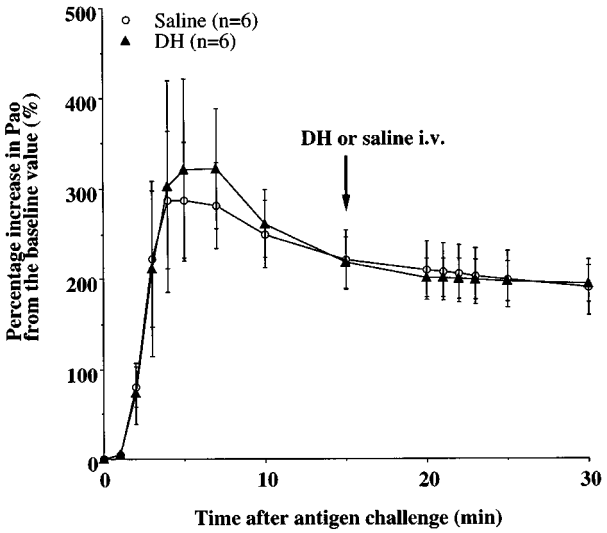

Figure 3 Effect of diphenhydramine hydrochloride (DH) on ovalbumin (OA, 0.1 mg ml^{-1})-induced bronchoconstriction without inhalation of ultrasonically nebulized distilled water (UNDW). DH or vehicle (saline) was intravenously administered 15 min after the OA challenge. Each point represents the mean and vertical lines show s.e.mean.

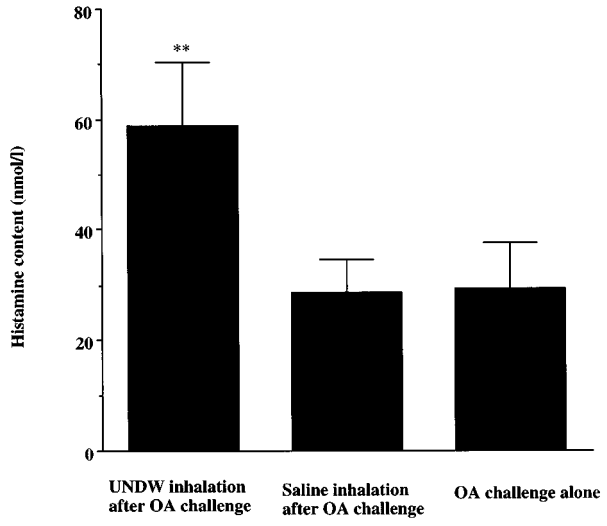

Figure 4 Histamine content in bronchoalveolar lavage fluid (BALF) recovered 20 min after ovalbumin (OA) challenge (control, $n=8$), or 10 min after ultrasonically nebulized distilled water (UNDW) (UNDW inhalation, $n=10$) or saline (saline inhalation, $n=8$) inhalation following the OA provocation, 30 min after the OA challenge. Each bar represents the mean and vertical lines show s.e.mean. $^{**}P<0.05$ compared with saline inhalation following OA provocation and OA challenge alone.

Study 5: UNDW inhalation after leukotriene D_4-induced bronchoconstriction

Percentage increase in the Pao reached to the peak value at 4 min after an inhalation of LTD_4 as shown in Figure 5. The percentage increases in the Pao after the inhalation of UNDW and saline were not different when inhaled 10 min after the LTD_4 inhalation.

Study 6: Effect of atropine on the UNDW-IB

The peak values of percentage increase in the Pao after the OA challenge were 239.5 ± 15.1 and 229.5 ± 7.7% and the values just before the UNDW inhalation were 171.5 ± 6.0 and 175.2 ± 5.5% in guinea-pigs treated with atropine and saline 15 min after the OA challenge, respectively. These values were not significantly different between treatments with atropine and saline. The maximum percentage increases in the Pao after UNDW inhalation were 367.3 ± 35.1% and 405.2 ± 34.2% with atropine and saline, respectively, and these values did not differ significantly.

Study 7: Effect of disodium cromoglycate on the UNDW-IB

The peak percentage increases in the Pao after the OA challenge were 369.7 ± 40.0, 388.3 ± 45.6 and 346.8 ± 59.8% in guinea-pigs given saline and 1.0 and 10 mg ml^{-1} disodium cromoglycate, respectively, and these values were not significantly different. The percentage increases in the Pao immediately before the UNDW challenge were 345.5 ± 44.7, 343.0 ± 44.5 and 349.9 ± 51.8% with saline and 1 and 10 mg ml^{-1} cromoglycate, respectively, which did not significantly differ from each other. The maximum percentage increases in the Pao after the UNDW inhalation were 634.5 ± 86.7, 716.9 ± 106.2 and 669.9 ± 104.5% with saline and 1.0 and 10 mg ml^{-1} of cromoglycate, respectively. These values were not significantly different.

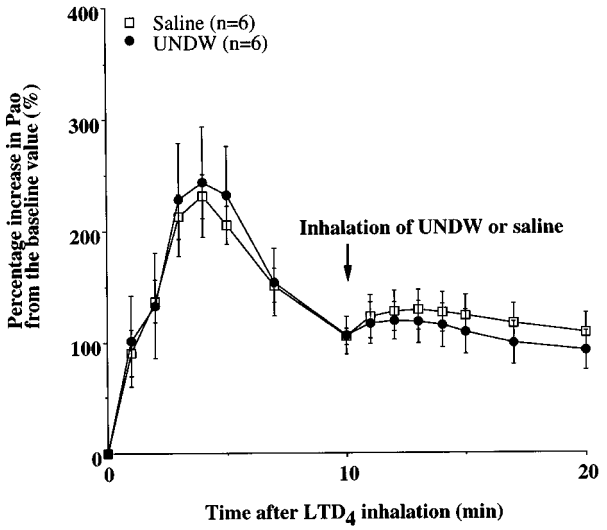

Figure 5 Time course of the percentage increase in Pao from the pre-leukotriene D_4 (LTD_4) inhalation value after the inhalation of ultrasonically nebulized distilled water (UNDW) or saline, 10 min after aerosolized provocation with 1.0 μg ml^{-1} LTD_4 in nonsensitized guinea-pigs. Each point represents the mean and vertical lines show s.e.mean.

Discussion

Both antihistamines, diphenhydramine hydrochloride (DH) and chlorpheniramine maleate (CP), given 15 min after an antigen challenge significantly inhibited the early phase (1–2 min after UNDW inhalation) of the UNDW-IB in our guinea-pig model (Fujimura *et al*., 1997a), suggesting involvement of histamine in the UNDW-IB. When UNDW was not inhaled, DH administered 15 min after the antigen challenging did not influence the time course curve of the antigen-induced bronchoconstriction. The histamine content after the inhalation of UNDW following the antigen challenge was significantly increased compared with those after the saline inhalation following the antigen challenge and the antigen challenge without the UNDW inhalation in sensitized guinea-pigs. UNDW did not cause a significant bronchoconstriction when inhaled 10 min after an inhalation of LTD_4.

We previously showed that UNDW did not elicit bronchoconstriction when inhaled at 5 or 20 min after an inhalation of methacholine, a direct bronchonconstrictor (Fujimura *et al*., 1997a). Although it has been shown that an inhaled noncontractile concentration of LTD_4-induced airway hyperresponsiveness to acetylcholine reaches the peak 8 min after the inhalation and persists for 30 min in guinea-pigs (Sato *et al*., 1996), UNDW did not produce bronchoconstriction when inhaled 10 min after an inhalation of a bronchoconstrictive concentration of LTD_4 in this study. LTD_4 is an indirect bronchoconstrictor because it causes bronchoconstriction in part *via* secondary release of TXA_2 (Fujimura *et al*, 1988). These findings suggest that allergic reaction may be necessary for UNDW to induce bronchonconstriction rather than bronchoconstriction *per se*.

In *in vitro*, it has been demonstrated that mast cells can release histamine in hypotonic solutions (Kaliner *et al*, 1974). Clinically, antihistamine drugs (H_1-blockers) have been used to assess whether or not histamine is involved in this response. Azevedo *et al*. (1990) have reported that the decrease in FEV_1 induced by UNDW inhalation is significantly reduced with terfenadine. Allegra *et al*. (1993) have also shown that the magnitude and duration of both $PtcO_2$ and $PtcCO_2$ changes due to UNDW inhalation are progressively normalized by ketotifen. Sodium cromoglycate is a well-established prophylactic drug for asthma and this substance has been shown to inhibit the mediator release from mast cells (Goose *et al*., 1969). Nedocromil sodium also inhibits the mediator release from mast cells (Wells *et al*., 1986). Both of them have been shown to be effective in preventing UNDW-IB (Allegra & Bianco, 1980; Anderson *et al*., 1983; Robuschi, 1987; de Bufalo *et al*., 1989). From these findings UNDW-IB may result in part from bronchoconstrictor mediators released from mast cells, such as histamine. In our animal model, treatment with antihistamine drugs, DH and CP, dose-dependently inhibited the UNDW-IB. Both DH and CP have anti-cholinergic effect. Sheppard *et al*. (1983) reported previously that an inhaled atropine aerosol significantly reduced the magnitude of UNDW-IB and ipratropium bromide reduced the bronchial response to UNDW. But Allegra & Bianco, (1980) and Anderson *et al*. (1983) reported that inhaled atropine or ipratropium bromide did not reduce UNDW-IB. These results suggest that it is not clear whether parasympathetic efferent activity is involved in UNDW-IB or not. In the present study atropine in a dose of 0.5 mg kg^{-1} given 15 min after the antigen provocation did not influence the UNDW-IB, it is thought that the preventing effects of DH and CP in our study is due to the anti-histamine activity. On the other hand, as high doses of barbiturates suppress neural reflexes our anaesthetized guinea-pig model is considered to be inappropriate for studying the role of autonomic nerve activity in the UNDW-IB.

Interestingly, DH and CP significantly inhibited bronchoconstriction only at 2 and 1 min after the inhalation of UNDW. Histamine may partially contribute to the UNDW-IB. As it is considerable that histamine released by the UNDW inhalation presents its maximal effect at 2 min after the inhalation of UNDW, it may be claimed that measurement of histamine content in BALF must be performed at 1 or 2 min after the inhalation of UNDW. However, as BALF is poorly recovered from animals with severe bronchoconstriction, we measured the histamine content at 10 min after the inhalation of UNDW when bronchonconstriction decreased as shown in Figures 1 and 2. The result showed the significant increment of histamine content after the inhalation of UNDW compared with inhalation of saline. It is likely that the histamine content may be higher at 1 or 2 min than at 10 min after the inhalation of UNDW because of histamine metabolism. In addition, DH given 15 min after the antigen challenge did not influence the time course of antigen-induced bronchoconstriction without a following UNDW inhalation. This result is in agreement with our previous data that in passively sensitized guinea-pigs histamine is involved in the antigen-induced bronchoconstriction in the early phase (1–3 min after the antigen challenge) and leukotrienes are involved in the late phase (5–10 min after the antigen challenge) (Minami, 1983; Fujimura, 1983), indicating that antigen-induced histamine-mediated bronchoconstriction terminates within 15 min after the antigen challenge. On the other hand, in our series of animal experiments using the same experimental system (Songur *et al*., 1994; Fujimura *et al*., 1996, 1997b), thromboxane antagonists, leukotriene antagonists and platelet activating factor antagonists decreased the antigen-induced bronchoconstriction by a small degree when given intravenously 15 min after the antigen challenge. Taken together, it may be concluded that UNDW inhalation causes bronchoconstriction *via* newly released histamine in our animal model.

Inhibitory effect of anti-histamines on UNDW-IB in our animal model is compatible with the previous studies in asthmatic subjects (Azevedo *et al*., 1990; Allegra *et al*., 1993). However, the partial inhibition of anti-histamines shown both in asthmatics and in our animal model suggests that the mechanisms of UNDW-IB may involve not only histamine release but also release of other mediators such as sensory neuropeptides, lipid mediators excluding TXA_2 (Fujimura *et al*., 1997a) and so on. Indeed, we (Fujimura *et al*, 1998; Mizuguchi *et al*, 1996) recently reported that sensory neuropeptides were involved in the UNDW-IB but not in the antigen-induced bronchoconstriction.

Sodium cromoglycate and nedocromil sodium, inhibitors of mediator release from mast cells (Goose & Blair, 1969; Wells *et al*., 1986), have been shown to be effective in preventing UNDW-IB (Allegra & Bianco, 1989; Anderson *et al*., 1983; Robuschi, 1987; del Bufalo *et al*., 1989). We examined the effect of inhaled sodium cromoglycate in our guinea-pig model. This agent had no effect on the UNDW-IB. It has been clearly shown that sodium cromoglycate does not inhibit anaphylactic release of histamine or slow-reacting substance of anaphylaxis from sensitized guinea-pig lung strip *in vitro* while it inhibits anaphylactic mediator release from lung tissues of rats and monkeys (Kohda, *et al*., 1970). *In vivo* experiments (Armour & Temple, 1982; Bottomley *et al*., 1984) have also shown a small

or no effect of sodium cromoglycate on anaphylactic bronchial response in guinea-pigs. Namely, guinea-pigs are unsuitable in studying the effect of this compound.

In conclusion, it is suggested that histamine is partially involved in the UNDW-IB which is caused after an antigen challenge. Our animal model may be useful for understanding the mechanisms of the UNDW-IB. Further studies are needed to clarify the involvement of other mediators in the UNDW-IB both in animals and asthmatics.

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