A guinea-pig model of ultrasonically nebulized distilled water-induced bronchoconstriction

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A guinea-pig model of ultrasonically nebulized distilled water-induced bronchoconstriction. M. Fujimura, M. Amemiya, S. Myou, M. Mizuguchi, T. Matsuda. ©ERS Journals Ltd 1997.

ABSTRACT: Ultrasonically nebulized distilled water-induced bronchoconstriction (UNDW-IB) is specific to asthma. The mechanisms underlying UNDW-IB are not fully understood, and no reproducible animal model has been reported. The purpose of this study was to develop a guinea-pig model of UNDW-IB.

Ultrasonically nebulized distilled water (UNDW) was inhaled 20 min after an aerosolized antigen challenge in passively sensitized and artificially ventilated guinea-pigs. UNDW was also inhaled 5 and 20 min after 0.1 mg·mL^{-1} methacholine inhalation in nonsensitized animals. In addition, 0.1 mg·kg^{-1} S-1452, a thromboxane A$_2$ antagonist, or saline was given intravenously 5 min before UNDW inhalation in sensitized animals.

The inhalation of UNDW caused bronchoconstriction, when inhaled 20 min after an antigen challenge in sensitized guinea-pigs. UNDW inhalation did not produce bronchoconstriction after saline inhalation in nonsensitized or sensitized guinea-pigs, or after antigen inhalation in nonsensitized animals. Methacholine-induced bronchoconstriction did not evoke UNDW-IB. Neither did S-1452 reduce the UNDW-IB. In conclusion, the guinea-pig model of ultrasonically nebulized distilled water-induced bronchoconstriction developed in this study suggests that allergic reaction, but not bronchoconstriction, can induce bronchial hyperresponsiveness to ultrasonically nebulized distilled water, and that thromboxane A$_2$ is not involved in ultrasonically nebulized distilled water-induced bronchoconstriction.

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Ultrasonically nebulized distilled water-induced bronchoconstriction (UNDW-IB) is highly specific for asthma [1–6], especially when compared to methacholine-induced bronchoconstriction [1–4].

Although some researchers have examined the mechanism underlying UNDW-IB, it is not fully understood. The development of an animal model of UNDW-IB may be helpful for understanding the mechanisms of UNDW-IB because procedures that can induce UNDW-IB may clarify the mechanisms that cause it. Furthermore, the involvement of chemical mediators, the autonomic nervous system, tachykinins, and other cytokines in UNDW-IB can be examined using selective inhibitors that cannot be given to asthmatics. However, no reproducible animal model of UNDW-IB has ever been reported.

It has been stated that the inhalation of propranolol or of ultrasonically nebulized distilled water (UNDW) causes bronchoconstriction only in asthmatics [7, 8]. We previously reported a guinea-pig model of propranolol-induced bronchoconstriction [9], in which the inhalation of propranolol 20 min after antigen inhalation caused bronchoconstriction in passively sensitized guinea-pigs. Since specific inhibitors of thromboxane A$_2$ (TxA$_2$) [9] and platelet-activating factor (PAF) [10], but not sensory neuropeptides [11], significantly inhib-

ited the propranolol-induced bronchoconstriction, we postulated that an allergic mediator mechanism was important in propranolol-induced bronchoconstriction.

MORMILE *et al.* [12] have reported that a significant increase in bronchial reactivity to an ultrasonic mist of distilled water is observed after allergen inhalation in humans. UNDW-IB predominantly occurs in asthmatic patients with moderate-to-severe illness [13, 14]. Asthmatic patients who have a positive response to UNDW have increased numbers of mast cells and eosinophils. as assessed by analysis of their bronchial tissue obtained by endobronchial biopsy [14, 15]. These findings suggest that a positive response to UNDW is related to the extent of allergic airway inflammation, and that the sensitization and presence of allergic inflammatory cells and/or mediators in the airways mediate UNDW-IB. These findings prompted us to examine whether UNDW-IB can occur after an allergic reaction.

Materials and methods

Animals

Male albino Hartley strain guinea-pigs weighing 330–380 g were obtained from Sankyou Laboratory

Service (Toyama, Japan). They were kept in conventional animal housing facilities for 1 week prior to use and were allowed to eat and drink *ad libitum*.

Passive sensitization of guinea-pigs

Guinea-pig homocytotropic antiserum was obtained by the method elaborated in SANTIVES *et al*. [16]. Briefly, 500 µg of ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally into each guinea-pig at multiple sites. A booster dose was prepared and administered in the same manner 2 weeks later. Serum collected from each animal 2 weeks after the booster dose was pooled, and kept frozen until use. The antibody titre of this serum was 1:12,800, 1:6,400, and 1:512, as estimated by passive cutaneous anaphylaxis at 4 h, 24 h, and 7 days, respectively. Normal guinea-pigs were passively sensitized with 1.0 mL·kg⁻¹ antiserum, *i.p.*

Preparation of animals

Five to 7 days after the passive sensitization, the guinea-pigs were anaesthetized with sodium pentobarbital (75 mg·kg⁻¹ *i.p.*). They were placed in the supine position, and the trachea cannulated with a polyethylene tube (external diameter 2.5 mm; internal diameter 2.1 mm). The left jugular vein was cannulated for the 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, USA). The tidal volume (V_T) was 10 mL·kg⁻¹, and the rate was 60 strokes·min⁻¹. The change in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening; P_{ao}), measured in cmH₂O, was assessed using a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo). As the change in P_{ao} following the inhalation of leukotriene C₄ (LTC₄) represents the average of the changes in pulmonary resistance (R_L) and reciprocal dynamic lung compliance ($1/C_{dyn}$) [17], we used an increase in the P_{ao} as the overall index of bronchial response to bronchoactive agents.

When all procedures were completed, the lungs of the animals were over-inflated by twice V_T for two breaths by clamping the outlet port of the respirator. This resulted in uniformity of relative lung volume [18].

The aerosols used were generated for 30 s with an ultrasonic nebulizer developed for small animals at our institution [18]. The amount of aerosol produced when a physiological saline solution is nebulized is 15.2 µL·min⁻¹ [19], and 46.4% of a nebulized albumin solution is deposited in the lungs when a ⁹⁹ᵐTc-albumin solution is inhaled [18]. The median aerodynamic diameter of the particles of a physiologic saline solution is 3.59±1.96 µm (mean±SD) when measured by a laser particle size analyser [19].

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

Study protocol

Study 1: UNDW inhalation in nonsensitized or sensitized guinea-pigs. Fifteen minutes from the start of artificial ventilation, when P_{ao} had stabilized, sensitized and nonsensitized animals were challenged with either UNDW or saline, without interrupting the constant ventilation (n=6 in each group).

Study 2: UNDW inhalation after antigen challenge. When P_{ao} had stabilized 15 min after the surgical procedure, passively sensitized guinea-pigs were challenged with nebulized ovalbumin (OA) dissolved in physiologic saline (0.1 mg·mL⁻¹). Either UNDW (n=14) or saline (n=9) was inhaled for 30 s, 20 min after the OA provocation. In addition, UNDW (n=8) or saline (n=8) was inhaled 20 min after the OA challenge in nonsensitized animals.

Study 3: UNDW inhalation after methacholine-induced bronchoconstriction. To examine whether bronchoconstriction itself leads to UNDW-IB, UNDW (n=6) or saline (n=6) was inhaled 5 and 20 min after 0.1 mg·mL⁻¹ methacholine was inhaled for 30 s by nonsensitized guinea-pigs.

Study 4: effect of salbutamol. A β₂-agonist, salbutamol (5 mg·kg⁻¹) (n=9), or saline (n=9) was administered intravenously 15 min after the challenge with OA in passively sensitized guinea-pigs. UNDW inhalation was then performed 5 min later, 20 min after the OA challenge.

Study 5: effect of S-1452. To investigate whether TxA₂ is involved in UNDW-IB, a specific TxA₂ receptor antagonist, S-1452 [20], in a single dose of 0.1 mg·kg⁻¹ (n=6), or saline (n=6) was administered intravenously 15 min after the challenge with OA in passively sensitized guinea-pigs. UNDW was then inhaled 5 min later, 20 min after the OA challenge.

Chemicals. The following chemicals were used: distilled water and physiological saline (Otsuka Pharmaceutical Co., Ltd., Osaka, Japan); OA (Sigma, St. Louis, MO, USA); Freund's complete adjuvant (Sigma); sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA); salbutamol sulphate (Sigma); methacholine (Wako Pure Chemical Ind., Osaka, Japan); and S-1452 (Calcium 5(z)-1R, 2S, 3S, 4S-7- (3-phenylsulphonylaminobicyclo (2.2.1) hept-2-yl)-5-heptenoate hydrate) (Shionogi Pharmaceutical Ind., Osaka, Japan) [20].

Statistical analysis

Data are shown as the mean±SEM. Statistical differences were determined by unpaired t-test. Differences in the time course of the percentage increase in P_{ao} from the baseline value after provocation with OA or saline, or after the inhalation of UNDW or saline were analysed using a two-factor repeated analysis of variance (ANOVA). The time course curve after OA challenge or the inhalation of UNDW was compared between animals treated with salbutamol or saline, or with S-1452

or saline by a two-factor repeated ANOVA. A p-value of 0.05 or less was considered statistically significant.

Results

Study 1: UNDW inhalation in nonsensitized or sensitized guinea-pigs

The mean values (±SEM) of the P_{ao} before the UNDW and saline challenge were 9.2±0.3 and 9.0±0.0 cmH_2O in nonsensitized guinea-pigs and 9.0±0.3 and 9.2±0.4 cmH_2O in sensitized guinea-pigs, respectively. There were no significant differences between these values. The percentage increase in P_{ao} after the inhalation of UNDW was not different from that after the inhalation of saline in both sensitized and nonsensitized guinea-pigs.

Study 2: UNDW inhalation after antigen challenge

The baseline P_{ao} values before the OA challenge were 9.4±0.2 and 9.3±0.3 cmH_2O in passively sensitized animals given UNDW or saline, respectively. These values did not differ significantly. The peak P_{ao} values after the OA challenge were 223.5±22.7% with UNDW and 218.1±32.9% with saline (fig. 1). These values were not significantly different. The percentage increases in P_{ao} immediately before UNDW or saline inhalation (20 min after the OA challenge) were 210.8±19.5% and 218.1±32.9%, respectively, and these values were not significantly different. Percentage increases in the P_{ao} at 1, 2, 3, and 5 min after the inhalation of UNDW were significantly greater than those after the inhalation of saline (p<0.05) (fig. 1). UNDW inhalation did not cause a significant increase in the P_{ao} compared with saline inhalation, in nonsensitized guinea-pigs.
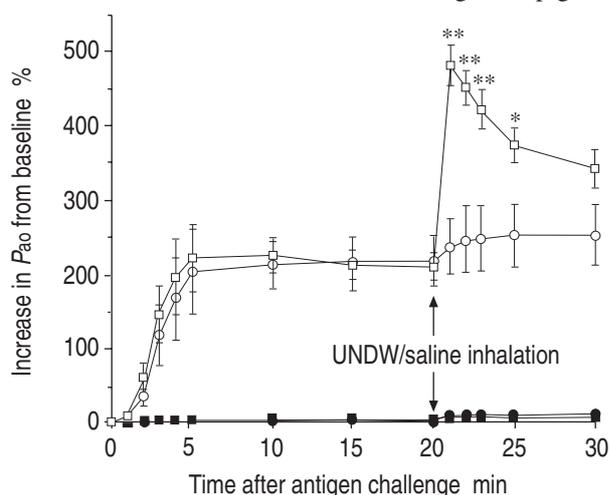

Fig. 1. – Time course of the percentage increase in pressure at the airway opening (P_{ao}) after ovalbumin (OA) challenge followed by inhalation of ultrasonically nebulized distilled water (UNDW) or saline 20 min after OA provocation in passively sensitized or nonsensitized guinea-pigs. Values are presented as mean±SEM. ❑ : UNDW inhalation in passively sensitized animals (n=14); ○ : saline inhalation in passively sensitized animals (n=9); ■ : UNDW inhalation in nonsensitized animals (n=8); ● : saline inhalation in nonsensitized animals (n=8). *,**: p<0.05, p<0.01, compared with the passive sensitization and saline inhalation group.

Study 3: UNDW inhalation after methacholine-induced bronchoconstriction

Increases in P_{ao} from baseline values after the inhalation of either UNDW or saline, 5 and 20 min after aerosolized provocation with 0.1 $mg \cdot mL^{-1}$ methacholine did not differ (fig. 2).

Study 4: Effect of salbutamol

The P_{ao} values before the OA challenge were 9.1±0.1 and 9.0±0.3 cmH_2O in animals treated with saline and salbutamol, respectively. These values were not significantly different. The percentage increase in the P_{ao} 15 min after the OA provocation (just before the salbutamol treatment) was 200.3±27.5 and 201.0±13.2% with saline and salbutamol, respectively. There was no significant difference between them. Compared with saline treatment, salbutamol reduced the percentage increase in P_{ao} and completely abolished the UNDW-induced increase in the P_{ao} (fig. 3).

Study 5: Effect of S-1452

The P_{ao} values before the OA provocation were 8.7±0.3 and 8.8±0.2 cmH_2O in animals pretreated with 0.1 $mg \cdot kg^{-1}$ S-1452 or saline, respectively. These values were not significantly different. The peak values after the OA challenge were 205.3±24.3 and 207.9±10.7% with saline and S-1452, respectively. These values did not differ significantly. The percentage increases in P_{ao} immediately before UNDW (20 min after the OA challenge) were 190.7±26.4 and 162.7±11.6% in the groups given saline and S-1452, respectively. These values were not significantly different. The percentage increase in P_{ao} after the UNDW inhalation was not significantly different between treatment with S-1452 and treatment with saline (fig. 4). Similarly, when the percentage increase in the P_{ao} after UNDW inhalation and the value immediately before UNDW inhalation were compared, the time course curve did not differ significantly between treatment with S-1452 and treatment with saline.
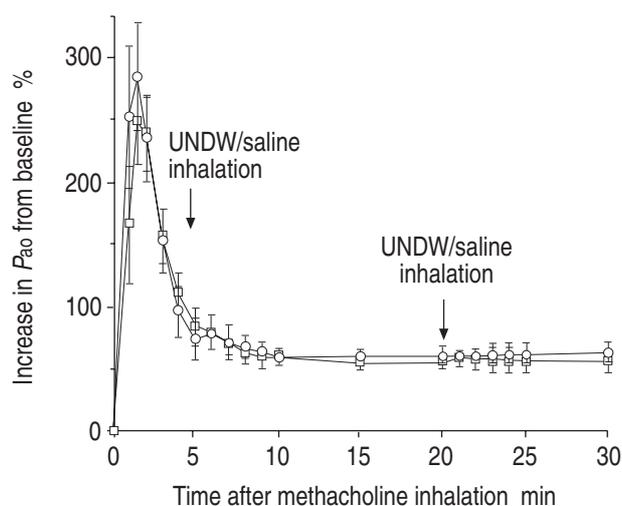

Fig. 2. – Time course of the percentage increase in P_{ao} from the premethacholine value after the inhalation of UNDW (n=9) or saline (n=9), 5 and 20 min after aerosolized provocation with 0.1 $mg \cdot mL^{-1}$ methacholine in nonsensitized guinea-pigs. ○ : saline inhalation; ❑ : UNDW inhalation. For definitions, see legend to figure 1.
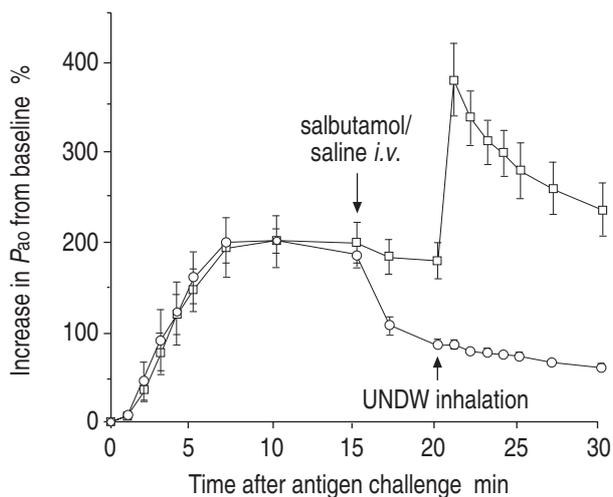
Fig. 3. – Time course of the percentage increase in Pao after the inhalation of UNDW following OA challenge in passively sensitized guinea-pigs treated intravenously with saline (n=9) or salbutamol (n=9). ○ : salbutamol treatment; ❏ : saline treatment. For definitions, see legend to figure 1.
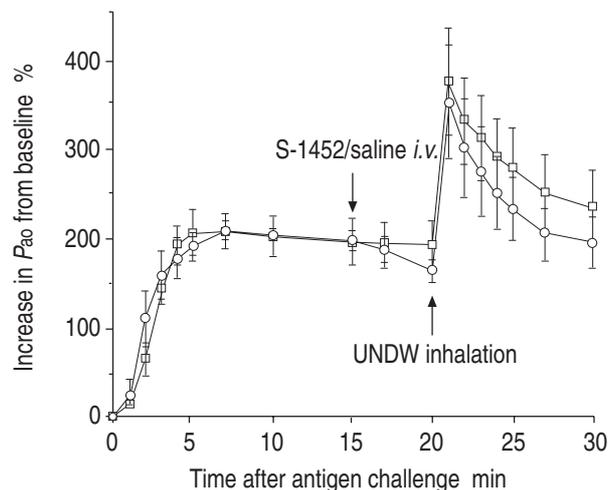

Fig. 4. – Time course of the percentage increase in Pao after the inhalation of UNDW following OA challenge in passively sensitized guinea-pigs treated intravenously with S-1452 or saline 5 min before the UNDW inhalation (15 min after OA challenge). ○ : treated with 0.1 mg·kg^{-1} S-1452 (n=6); ❏ : treated with saline (n=6). For definitions see legend to figure 1.

Discussion

We examined whether the inhalation of UNDW can cause bronchoconstriction after antigen-induced bronchoconstriction in passively sensitized guinea-pigs. The study clearly demonstrated that bronchoconstriction occurred when UNDW was inhaled 20 min after challenge with an antigen in passively sensitized guinea-pigs. The inhalation of UNDW without an antigen prechallenge did not produce bronchoconstriction in either nonsensitized or sensitized guinea-pigs. In addition, methacholine-induced bronchoconstriction was not accompanied by UNDW-IB when UNDW was inhaled 5 or 20 min after the methacholine inhalation. These results suggest that an allergic reaction or process, excluding bronchoconstriction, may be one of the mechanisms underlying 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 [14, 15].

The Pao measurement used in this study does not clearly distinguish between airway oedema and airway smooth muscle contraction. As the change in the Pao, but not the absolute value of the Pao, represents the average of changes in the RL and $1/C$dyn when leukotriene C_4 is inhaled [17], we have used ch.anges in the Pao as an overall index of changes in airway calibre when oedema or inflammation of the lung parenchyma is negligible [9–11, 17–19]. UNDW inhalation caused an acute and sharp increase in the Pao in the present study, suggesting the presence of bronchoconstriction but not airway oedema. In addition, we examined the effect of a bronchodilator, salbutamol, on the increase in the Pao caused by UNDW inhalation. Salbutamol completely inhibited the UNDW-induced increase in the Pao. It has been shown that intravenously administered salbutamol does not inhibit airway microvascular leakage induced by vagal stimulation [21], substance P [21], or platelet-activating factor [22], and that it has little or no inhibitory effect on histamine-induced microvascular permeability in proximal and distal intrapulmonary airways [23]. Taken together, it is likely that the increase in Pao is indicative of bronchoconstriction induced by the inhalation of UNDW in our guinea-pig model.

We studied the effect of a TxA_2 receptor antagonist, S-1452 [20], on UNDW-IB in the present animal model to determine whether TxA_2 is involved in UNDW-IB. TxA_2, a cyclo-oxygenase product of arachidonic acid metabolism, has been implicated in acute bronchoconstriction after antigen inhalation in asthmatic patients [24, 25], and this effect has been shown to be mediated by the stimulation of thromboxane receptors [26]. We have shown that S-1452 reduces nonspecific bronchial hyperresponsiveness in stable asthmatic patients [27]. S-1452 has been shown to antagonize the binding of ^{3}H-U46619 in washed rat platelets with stereo specificity and high potency (dissociation constant of the antagonist (K_i), 25 nM), but to have no inhibitory activity on the binding of ^{3}H-prostaglandin PGE_1, ^{3}H-PGD_2, or ^{3}H-$PGF_{2\alpha}$ to rat platelet membranes [20]. It has also been shown that S-1452 (100 nM) inhibits collagen-induced shape changes and aggregation of rat platelets [20]. The inhalation of propranolol causes bronchoconstriction only in asthmatics. We previously reported a guinea-pig model of propranolol-induced bronchoconstriction in which the inhalation of propranolol 20 min after antigen inhalation caused bronchoconstriction in passively sensitized guinea-pigs [9]. When S-1452, in doses of 0.01 and 0.1 mg·kg^{-1}, was given intravenously, 15 min after an antigen challenge, it completely inhibited the bronchoconstriction induced by propranolol inhaled 20 min after the antigen challenge in passively sensitized guinea-pigs [9], suggesting that an allergic mediator mechanism regulated propranolol-induced bronchoconstriction. In contrast, 0.1 mg·kg^{-1} S-1452 did not inhibit UNDW-IB in this study. These findings suggest that both UNDW and propranolol cause bronchoconstriction when they are inhaled after an allergic reaction, but that the mechanisms of action are different.

The inhalation of furosemide has been shown to inhibit the bronchoconstriction induced by UNDW [28, 29]. SHIMIZU et al. [30] have shown that it is possible

that osmotic changes in periciliary fluid induced by UNDW cause rapid changes in ion transport or epithelial cell swelling. It has been postulated that water transport into the luminal side of the airway epithelium, or rapid cell swelling in small airways, may induce a transient fall in the forced expiratory volume in one second (FEV_1) [30]. Sodium cromoglycate (SCG) is a well-established prophylactic drug for asthma, and this substance has been shown to inhibit mediator release from mast cells [31]. Nedocromil sodium also inhibits mediator release from mast cells [32]. Both drugs are effective in preventing UNDW-IB in asthmatics [1, 2, 33, 34]. These findings suggest that UNDW-IB may result in part from bronchoconstrictor mediators released from mast cells, such as histamine. We did not examine the effects of SCG and furosemide in the present study, as we considered SCG and furosemide to be inactive or less active in guinea-pig airways because of the following findings: it has been shown that SCG is inactive on tissue mast cells of the guinea-pig [35] and SCG does not inhibit respiratory distress caused by antigen inhalation in sensitized guinea-pigs [36]. Our previous study confirmed the lack of effect on antigen-induced bronchoconstriction in passively sensitized guinea-pigs (unpublished data), although nebulized furosemide has been shown to inhibit antigen-induced bronchoconstriction [37], but not histamine- or acetylcholine-induced bronchoconstriction [38], our previous study did not show an inhibitory effect of inhaled furosemide on antigen-induced bronchoconstriction in passively sensitized guinea-pigs (unpublished data). It has been postulated that furosemide inhibits cholinergic and excitatory non-adrenergic, noncholinergic neurotransmission [39], but our previous study showed that tachykinin is not involved in antigen-induced bronchoconstriction [40] or propranolol-induced bronchoconstriction after antigen challenge in passively sensitized guinea-pigs [11]. Future studies will be needed to assess the effects of these agents on our guinea-pig model of UNDW-IB.

In conclusion, we developed a guinea-pig model of ultrasonically nebulized distilled water-induced bronchoconstriction. This animal model is different from our animal model of propranolol-induced bronchoconstriction [9] regarding the role of thromboxane A_2. We believe that this is the first report of an animal model of ultrasonically nebulized distilled water-induced bronchoconstriction. Our model may be helpful for investigating the mechanism of one of the specific types of bronchial hyperresponsiveness in asthma.

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