



Genomic and non-genomic effects of glucocorticoids on allergic rhinitis model in mice



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ABSTRACT

Glucocorticoids (GCs) are well known for their anti-inflammatory effects, which are elicited through a transcriptional mechanism via a cytosolic glucocorticoid receptor (cGR)-mediated genomic effect. However, recent *in vitro* studies report that GCs can act as a membrane glucocorticoid receptor (mGR). This study aimed to examine whether mometasone furoate (MF) influences the nasal symptoms induced by histamine, substance P, ATP. Furthermore, the influences of various compounds on MF action were studied *in vivo*. The mice were intranasally administered with nasal symptom-inciting agents, and the occurrences of sneezing and nasal rubbing were counted. MF repressed the nasal symptoms caused when it was administered 10, 30 and 60 min before the induction of nasal symptoms. The repressive effect observed 10 min after the administration of MF was inhibited by RU486, a GR antagonist, but not by actinomycin D, a transcriptional inhibitor. In contrast, the repressive effect observed 60 min after the administration of MF was inhibited by RU486 and actinomycin D. Therefore, the effects observed 10 and 60 min after the MF administration were classified as non-genomic and genomic effects, respectively. The non-genomic effect suppressed the nasal symptoms induced by m-3M3FBS, a phospholipase C (PLC) activator, and was inhibited by U-73122, a PLC inhibitor. The genomic effect was inhibited by N-(p-aminocinnamoyl) anthranilic acid, a phospholipase A₂ (PLA₂) inhibitor. These results indicate that MF has a non-genomic effect through repression of the activation of PLC via the mGR, and MF has also a genomic effect that was influenced by the inhibition of PLA₂ through transcriptional regulation via cGR.

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1. Introduction

Allergic rhinitis is one of the most common immune-mediated diseases, and its main symptoms are sneezing, pruritus, rhinorrhea and nasal obstruction. The pathogenesis of the allergic reaction initially involves the interaction of allergens with a specific immunoglobulin (IgE) antibody bound to the surface of mast cells and basophils on the nasal mucosa. As a result, many symptoms associated with allergic rhinitis are caused by the release of mediators, including histamine, substance P, leukotrienes and cytokines from mast cells and eosinophils [1].

In clinical practice, glucocorticoids (GCs) are used to treat allergic rhinitis [2,3]. GCs are part of the normal feedback mechanism in the immune system that reduces inflammation. Therefore, they are used in medicine to treat diseases that are caused by an overactive immune system, such as allergies, asthma, autoimmune diseases, and sepsis [4]. GCs are a class of steroid hormone that binds to the cytosolic glucocorticoid receptor (cGR), which is a member of the nuclear receptor

superfamily and is present in almost every vertebrate animal cell [5,6]. The activated GR complex in turn upregulates the expression of anti-inflammatory proteins in the nucleus and represses the expression of pro-inflammatory proteins [7–9]. Because of these genomic action mechanisms, a few hours are required after the administration of the GCs for their effects to become apparent [10,11]. In clinical practice, however, the inhibitory effect of topical administration of GCs on nasal symptoms appears in a few minutes after administration. For example, Tillmann et al. [12] reported that nasal itching was markedly reduced 10 min after the administration of dexamethasone. It has thus become increasingly apparent that GRs are also present on the cell membranes (membrane GR [mGR]) and that GCs can exert non-genomic effects via the mGR [13–15].

Genomic effects involve the regulation of the transcription of specific target genes, ultimately resulting in the production of proteins that positively or negatively modulate cell function [9,16,17]. In contrast to genomic effects, which tend to be slow in onset and delayed in recovery, non-genomic effects occur within minutes, are reversible immediately after the removal of the GCs, and are insensitive to substances that block RNA transcription [18,19]. Therefore, we propose that GCs have some effects in addition to those mediated by transcriptional regulation. Non-genomic effects of GCs have been demonstrated *in vitro*, but are uncertain *in vivo*. The present study was

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therefore undertaken to clarify the genomic and non-genomic effects of GCs on nasal symptoms using a mouse allergic rhinitis model.

2. Materials and methods

2.1. Animals

Five week-old female ICR mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in an air-conditioned room with controlled temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 15\%$). Food and water were given *ad libitum*. Ten animals were used for the investigation of the nasal symptoms. All the procedures involving the animals were conducted in accordance with the guidelines for animal experiments at Okayama University Advanced Science Research Center, and all the procedures were licensed by the Animal Research Control Committee of Okayama University.

2.2. Materials

The following reagents were obtained from the sources shown in the parentheses: histamine dihydrochloride (Sigma, St. Louis, MO, USA) and substance P (Peptide Institute, Inc., Osaka, Japan). These reagents were dissolved in saline. Adenosine 5'-triphosphate disodium salt hydrate (ATP, Sigma) was dissolved in phosphate-buffered saline (PBS). RU486 (Cayman Chemicals, Ann Arbor, MI, USA) and U-73122 (Tocris, Bristol, UK) were suspended in 10% dimethyl sulfoxide (DMSO)-saline. Actinomycin D (Wako Pure Chemicals, Osaka, Japan) was suspended in 10% ethanol-saline. m-3M3FBS (Sigma) was suspended in 10% DMSO 10% Tween80-saline. N-(p-aminocinnamoyl) anthranilic acid (ACA, Sigma) was suspended in 20% DMSO-PBS. A mometasone furoate (MF, MSD, Tokyo, Japan) nasal spray (500 $\mu\text{g}/\text{mL}$) was used as the model GCs. The mice were given a nasal instillation of the all drugs in volume of 2 μL into the bilateral cavities using a micropipette.

2.3. Evaluation of the nasal symptoms in mice

Before the experiment, the animals were placed in an observation cage ($31 \times 25 \times 18\text{ cm}$) for approximately 10 min for acclimatization. After the nasal instillation of 2 μL of the drugs into the bilateral cavities using a micropipette, the animals were placed back into the observation cage (1 animal per cage), and the frequencies of sneezing and nasal rubbing in 15 min were counted.

2.4. Statistical analysis

All the values are expressed as the mean $\pm$ SEM. The statistical evaluation of the results was performed by one-way ANOVA, followed by the Dunnett's test or, when only two means were to be compared, the unpaired Student's *t*-test. A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of MF on the nasal symptoms induced by histamine in mice

Fig. 1A shows the incidence of sneezing and nasal rubbing over the 15 min period after the intranasal administration of histamine. Histamine at a dose of 5 $\mu\text{mol}/\text{site}$ significantly elicited nasal symptoms compared with the saline treatment. MF at a dose of 1 $\mu\text{g}/\text{site}$ was administered 5, 10, 30, 60 or 120 min before the induction of nasal symptoms by histamine (5 $\mu\text{mol}/\text{site}$). As shown in Fig. 1B, MF inhibited the sneezing and the nasal rubbing induced by histamine. Significant effects of MF were observed when it was administered 10, 30 and 60 min before the induction of nasal symptoms by histamine compared with the saline treatment.

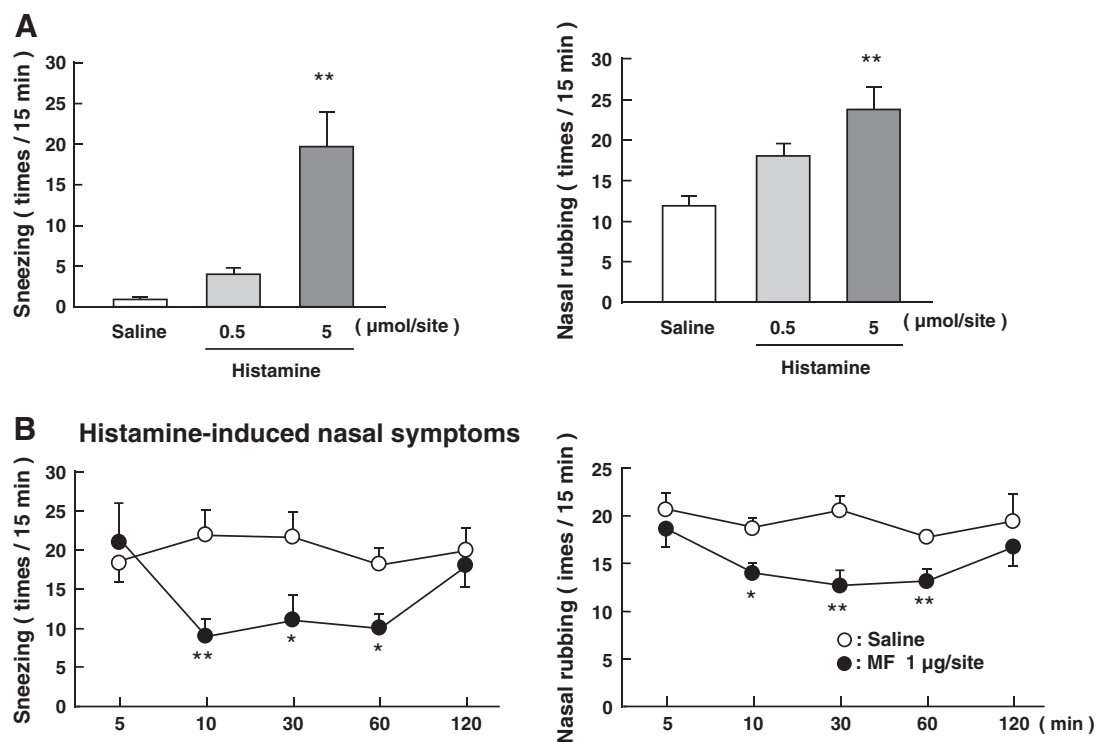


Fig. 1. (A) Sneezing and nasal rubbing induced by an intranasally administration of histamine in mice. The mice were given an intranasal administration of saline or histamine (0.5 and 5 $\mu\text{g}/\text{site}$) and the sneezing and nasal rubbing were counted for 15 min. Each column and vertical bar represents the mean $\pm$ SEM ($n = 10$). **: Significantly different from saline-treated group at $p < 0.01$. (B) Effects of MF on sneezing and nasal rubbing induced by histamine. The mice were given an intranasal administration of saline or MF (1 $\mu\text{g}/\text{site}$) 5, 10, 30, 60 or 120 min before the induction of nasal symptoms. Each value represents the mean $\pm$ SEM ($n = 10$). *, **: Significantly different from saline-treated group at $p < 0.05$ and $p < 0.01$, respectively.

3.2. Influence of a GR blocker and a transcriptional inhibitor on the effect of MF on the nasal symptoms induced by histamine in mice

Pretreatment with a GR blocker (RU486) 10 min before the administration of MF (1 $\mu\text{g}/\text{site}$) dose-dependently antagonized the effects of MF administered 10 and 60 min before the induction of nasal symptoms induced by histamine compared with the vehicle treatment (Fig. 2A, B). In both cases, significant effects of RU486 on sneezing and nasal rubbing were observed at a dose of 30 $\mu\text{g}/\text{site}$. By contrast, the pretreatment with a transcriptional inhibitor (actinomycin D) led to a significant decrease in the effect of MF at a dose of 10 $\mu\text{g}/\text{site}$ compared with the vehicle treatment, but only when MF was administered 60 min before the induction of nasal symptoms (Fig. 3A, B). Therefore, we defined the effect observed 10 min after MF administration as a non-genomic effect and the effect observed 60 min after MF administration as a genomic effect.

3.3. Induction of sneezing and nasal rubbing by substance P and ATP in mice

The administration of substance P and ATP caused a dose-related increase in the occurrence of sneezing and nasal rubbing (Fig. 4A, B). A significant effect of substance P on sneezing was observed at a dose of 30 nmol/site, and on nasal rubbing, at doses higher than 10 nmol/site compared with the saline treatment. ATP at a dose of 300 nmol/site significantly induced sneezing and nasal rubbing compared with the PBS treatment.

3.4. Effects of MF on the nasal symptoms induced by substance P and ATP in mice

Similar to the observations for histamine, the nasal symptoms induced by substance P was also inhibited by MF (1 $\mu\text{g}/\text{site}$), and a significant effect was observed in the cases in which MF was administered 10, 30, and 60 min before the induction of nasal symptoms compared with the saline treatment (Fig. 5A). On the other hand, MF has an inhibitory effect on the induction of nasal symptoms by ATP (300 nmol/site), compared with the saline treatment, when it was administered 60 min before symptom induction (Fig. 5B).

3.5. Effects of MF on the nasal symptoms induced by phospholipase C (PLC) activator in mice

After the administration of a PLC activator (m-3M3FBS) at a dose of 30 nmol/site, there was no significant effect on the nasal symptoms; however, a significant induction of sneezing and nasal rubbing was observed at doses of 100 and 300 nmol/site compared with the vehicle treatment (Fig. 6A). To confirm that the m-3M3FBS-induced symptoms are mediated via PLC activation, mice were pre-treated with the PLC inhibitor U-73122 (30 $\mu\text{g}/\text{site}$). U-73122 significantly inhibited the nasal symptoms induced by m-3M3FBS (data not shown). On the other hand, the pretreatment with ACA (100 nmol/site) had no influence on the m-3M3FBS-induced nasal symptoms (data not shown). The administration of MF (1 $\mu\text{g}/\text{site}$) 10 min before the induction of nasal symptoms by m-3M3FBS (300 nmol/site) resulted in a significant inhibition

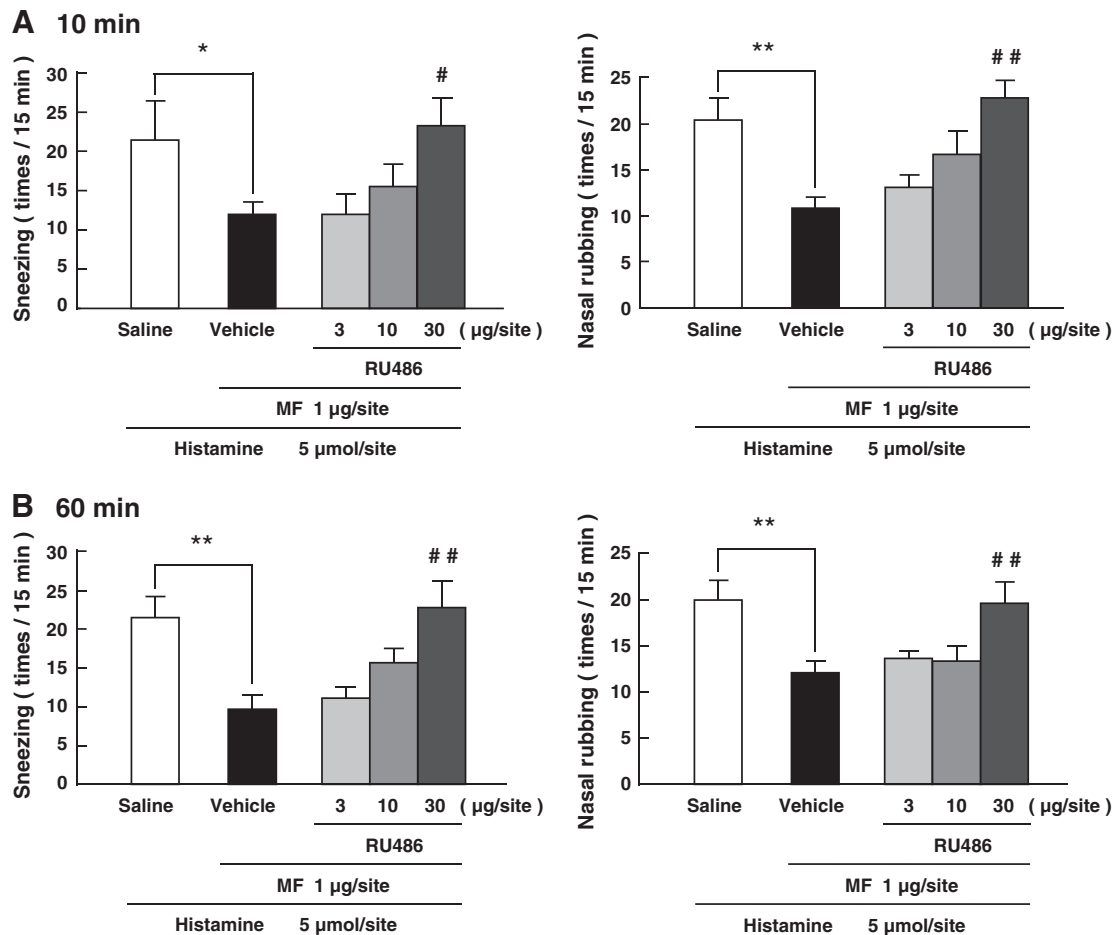


Fig. 2. Influence of RU486 on the effect of MF observed 10 min (A) or 60 min (B) after the administration. The mice were given an intranasal administration of RU486 (3, 10 and 30 $\mu\text{g}/\text{site}$) 10 min before the administration of MF. Each column and vertical bar represents the mean $\pm$ SEM ($n = 10$). *, **: Significantly different from saline-treated group at $p < 0.05$ and $p < 0.01$, respectively. #, ##: Significantly different from vehicle-treated group at $p < 0.05$ and $p < 0.01$, respectively.

of the nasal symptoms compared with the saline treatment (Fig. 6B). On the other hand, when MF was administered 60 min before the induction of nasal symptoms, it had no effect on sneezing and nasal rubbing caused by m-3M3FBS (Fig. 6C).

3.6. Effects of a PLC inhibitor on nasal symptoms induced by histamine and ATP in mice

When a PLC inhibitor (U-73122) was administered 10 min before the induction of histamine-induced nasal symptoms, sneezing and nasal rubbing were dose-dependently inhibited. Significant effects of U-73122 were observed at doses of 30 $\mu\text{g}/\text{site}$ on sneezing, a dose higher than that used to elicit an effect on nasal rubbing (3 $\mu\text{g}/\text{site}$) compared with the vehicle treatment (Fig. 7A). In contrast, the pretreatment with U-73122 had no influence on the ATP-induced nasal symptoms, even when at a dose of 30 $\mu\text{g}/\text{site}$ (Fig. 7B).

3.7. Effects of a PLA_2 inhibitor (ACA) on nasal symptoms induced by histamine and ATP in mice

Fig. 8 shows the effect of the ACA pretreatment 30 min before the induction of nasal symptoms by histamine and ATP. After the administration of ACA at a dose of 100 nmol/site, significant inhibitory actions on sneezing and nasal rubbing elicited by histamine (5 $\mu\text{mol}/\text{site}$) were observed compared with the vehicle treatment. In addition, the ATP-

induced nasal symptoms were significantly inhibited by ACA administered at a dose of 100 nmol/site.

4. Discussion

Many previous studies have demonstrated that the topical application of GCs affects the symptoms of allergic rhinitis and that this effect takes up a few hours to appear after administration. We confirmed in this study that MF inhibited the nasal symptoms induced by histamine in the healthy ICR mice only a short time after administration. Significant effects of MF were observed after administration at 10, 30, and 60 min before the induction of nasal symptoms by histamine. It is well known that GCs influence the incidence of nasal symptoms through a transcriptional anti-inflammatory effect several hours after administration [7–10]. However, in clinical practice, the inhibitory action of intranasal administration of GCs on nasal symptoms appeared much more rapidly. Thus, the inhibitory effect of GCs on the nasal symptoms was mediated not only by anti-inflammatory effects. Tillmann et al. [12] reported that nasal itching was markedly reduced within 10 min of administration of betamethasone and nasal obstruction was beneficially affected 150 or 240 min after administration. Therefore, it seems likely that GCs inhibited nasal symptoms by a known anti-inflammatory effect and a more rapid action that did not involve a transcriptional effect.

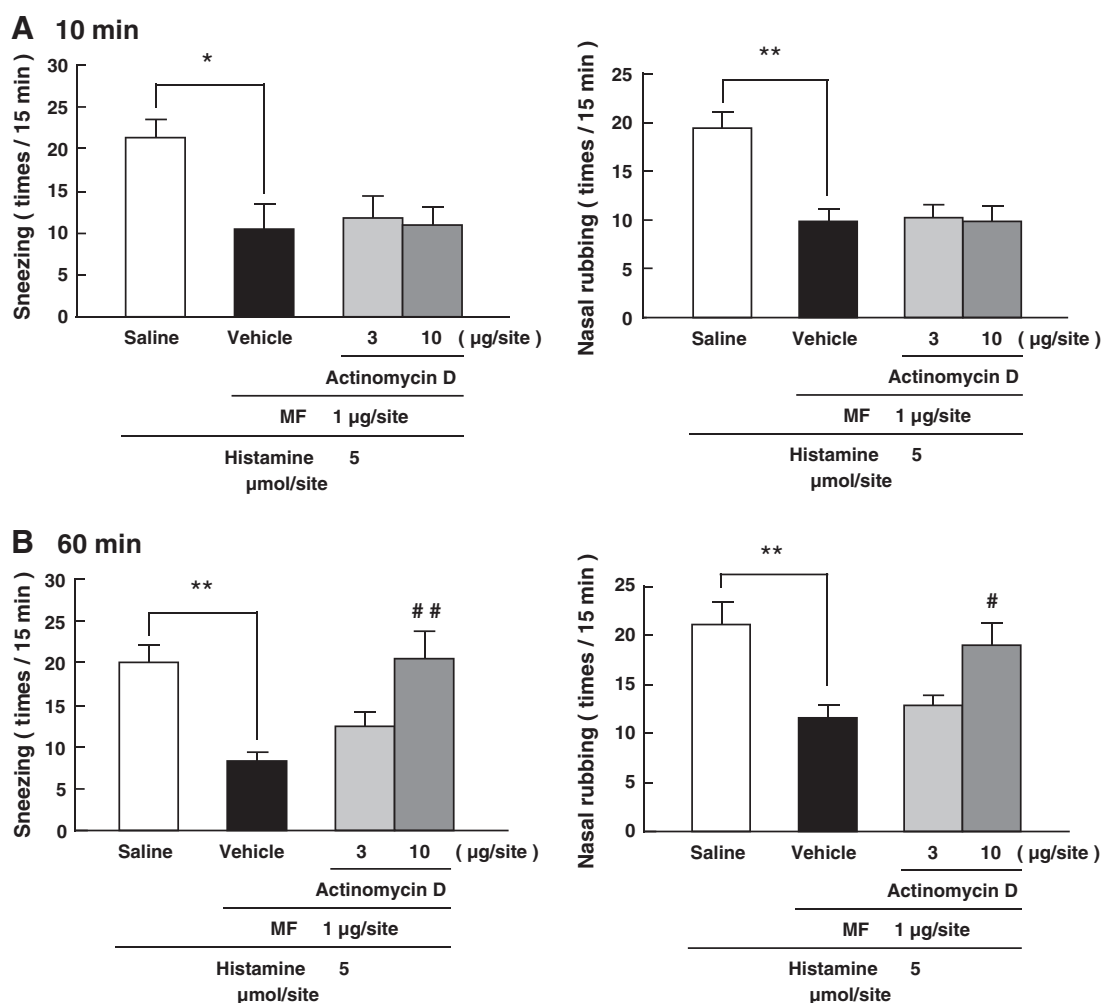


Fig. 3. Influence of actinomycin D on the effect of MF observed 10 min (A) or 60 min (B) after the administration. The mice were given an intranasal administration of actinomycin D (3 and 10 $\mu\text{g}/\text{site}$) 10 min before the administration of MF. Each column and vertical bar represents the mean $\pm$ SEM ($n = 10$). *, **: Significantly different from saline-treated group at $p < 0.05$ and $p < 0.01$, respectively. #, ##: Significantly different from vehicle-treated group at $p < 0.05$ and $p < 0.01$, respectively.

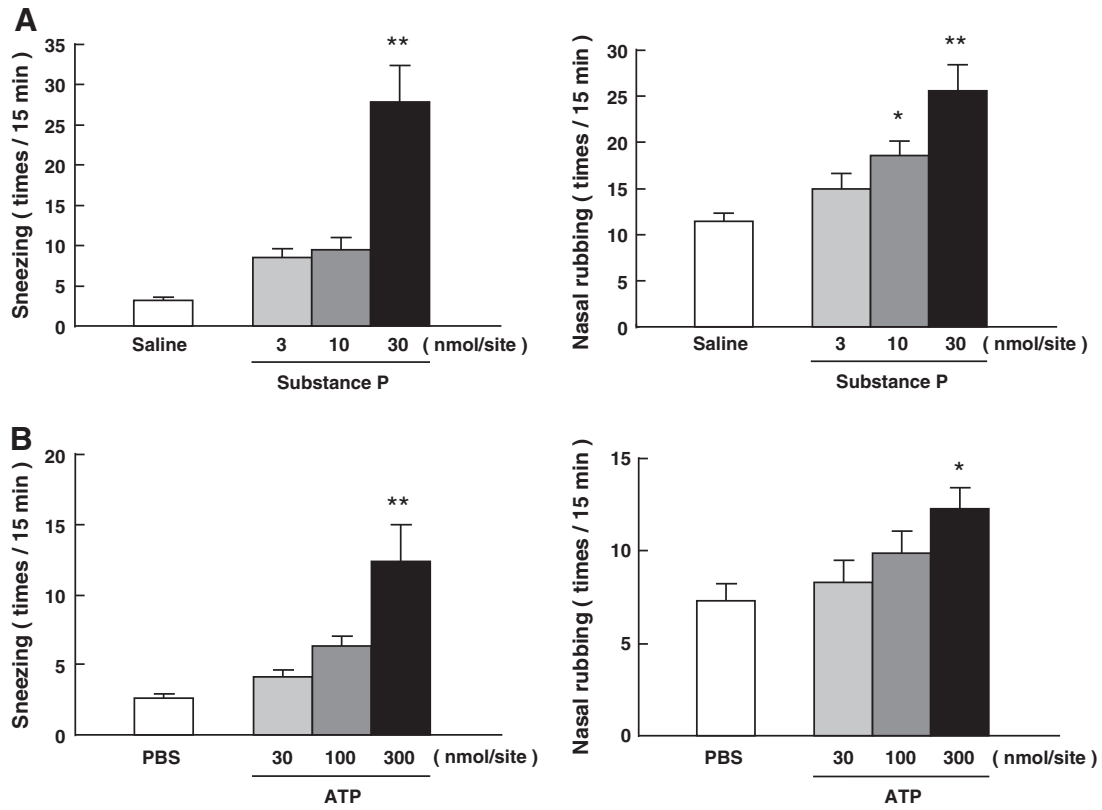


Fig. 4. Sneezing and nasal rubbing induced by an intranasal administration of substance P (A) and ATP (B) in mice. The mice were given an intranasal administration of saline, substance P (3, 10 and 30 nmol/site), PBS or ATP (30, 100 or 300 nmol/site), and sneezing and nasal rubbing were counted for 15 min. Each column and vertical bar represents the mean $\pm$ SEM ($n = 10$). *, **: Significantly different from saline- or PBS-treated group at $p < 0.05$ and $p < 0.01$, respectively.

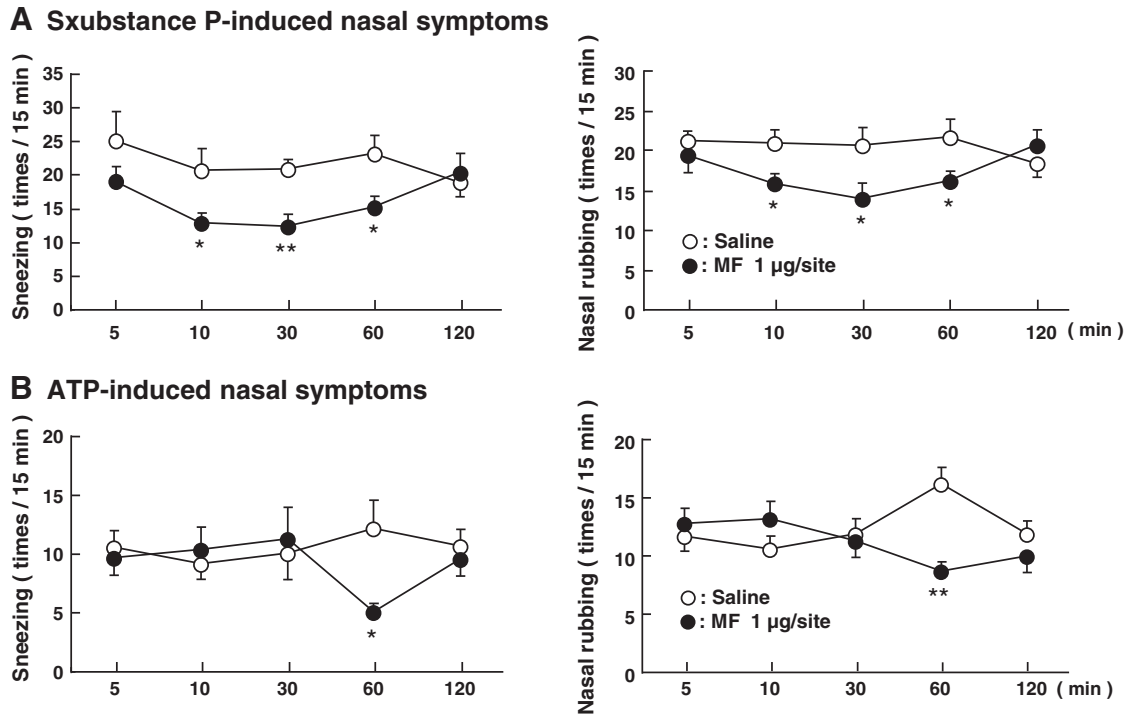


Fig. 5. Effect of MF on sneezing and nasal rubbing induced by substance P (A) and ATP (B). The mice were given an intranasal administration of saline or MF (1 μ g/site) 5, 10, 30, 60 or 120 min before the induction of nasal symptoms by substance P and ATP. Each value represents the mean $\pm$ SEM ($n = 10$). *, **: Significantly different from saline-treated group at $p < 0.05$ and $p < 0.01$, respectively.

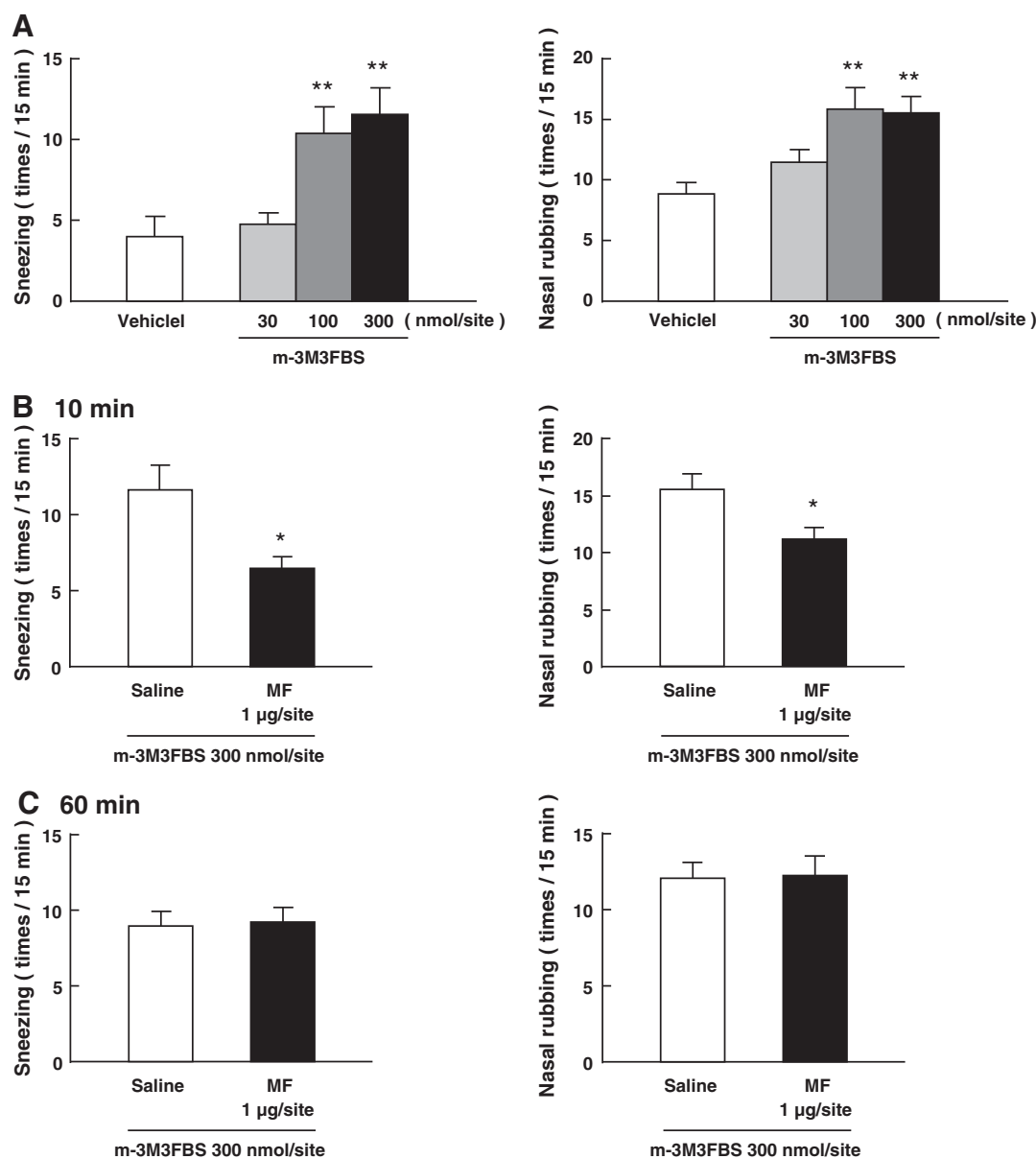


Fig. 6. (A) Sneezing and nasal rubbing induced by an intranasal administration of m-3M3FBS in mice. The mice were given an intranasal administration of vehicle or m-3M3FBS (30, 100 and 300 nmol/site) and the sneezing and nasal rubbing were counted for 15 min. Each column and vertical bar represents the mean $\pm$ SEM ($n = 10$). **: Significantly different from vehicle-treated group at $p < 0.01$. Effects of MF in a case of administration 10 min (B) or 60 min (C) before the induction of nasal symptoms by m-3M3FBS. Each column and vertical bar represents the mean $\pm$ SEM ($n = 10$). *: Significantly different from vehicle-treated group at $p < 0.05$.

Recently, some studies reported that GRs exist not only in the cytoplasm but also on the cell membrane [13,14]. In addition, it was proposed that GCs act via the mGR without transcriptional regulation and the expression of these effects occurs more rapidly than the genomic effect elicited through the cGR [13–15]. As a result of deliberation concerning whether the GR and transcriptional regulation were associated with the effects of MF, we showed that the effect observed 60 min after the administration of MF was inhibited by a GR antagonist and an mRNA transcriptional inhibitor. In contrast, the effect observed 10 min after the administration of MF was inhibited by a GR antagonist but not by an mRNA transcriptional inhibitor. RU486 is a high-affinity antagonist of the GC receptor that has been demonstrated to be effective in blocking the classical genomic effects of GC. On the other hand, Powell et al. [20] reported that RU486 exhibited the highest affinity for mGR on mouse T lymphoma cells. So far, RU486 also have successfully antagonized some rapid GC actions in

the brain [21]. However, the membrane-binding place for GC was identified that did not have any affinity for the RU486 in a pituitary-derived cell line [22]. In contrast, a rapid and non-genomic translocation of annexin-I by GC was prevented by RU486 treatment in a pituitary-derived cell line [23]. Thus, the consequence of the mGR for ligand binding is not well understood. We, therefore, consider that the effects observed 10 and 60 min after MF administration were GR-mediated non-genomic and genomic effects, respectively. Shipp et al. [24] have shown that the transcription of DUSP1 is induced rapidly, increasing after 30 min of dexamethasone treatment in human A549 cells. Chinenov et al. [25] also have evaluated the effect of GCs on cytokine gene expression, they treated bone marrow-derived macrophages from mice with ligands for TLR in the presence of dexamethasone. Dexamethasone markedly decreased the expression of TLR-induced genes. Although the kinetics and magnitude of inhibition was gene-specific, it was evident as early as 30 min at dexamethasone. In this study, we

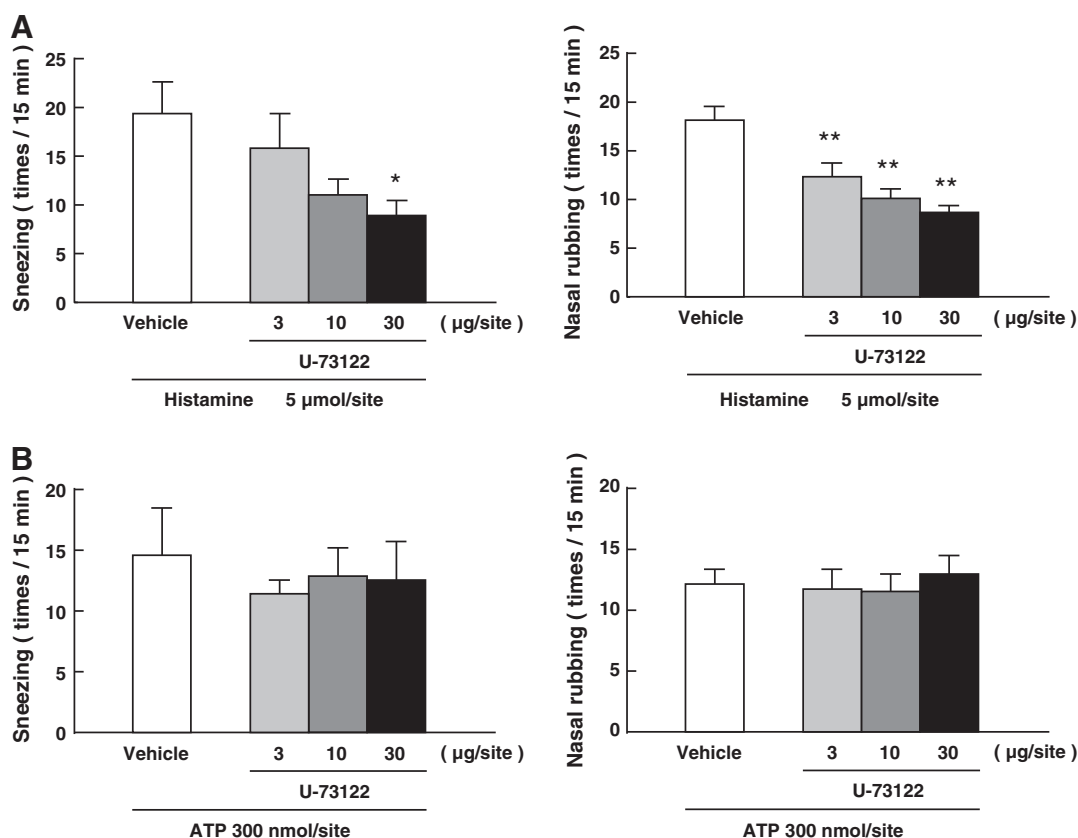


Fig. 7. Effect of U-73122 on sneezing and nasal rubbing induced by histamine (A) and ATP (B). The mice were given an intranasal administration of U-73122 (3, 10 and 30 µg/site) 10 min before the induction of nasal symptoms by histamine (5 µmol/site) and ATP (300 nmol/site). Each column and vertical bar represents the mean ± SEM. (n = 10). *, **: Significantly different from vehicle-treated group at $p < 0.05$ and $p < 0.01$, respectively.

examined that MF inhibited the histamine-induced nasal symptom (sneezing and nasal rubbing), which is not inflammatory reaction. It is well known that GCs inhibit other various nasal symptoms (for example nasal obstruction) by anti-inflammatory effect via the cGR. Therefore, we think that the genomic effects of MF remain over 60 min.

Next, we studied the effect of MF on the nasal symptoms induced by substance P and ATP. MF inhibited the nasal symptoms induced by substance P in the healthy mice 10, 30 and 60 min after the MF administration, similar to the results seen for histamine. In contrast, the inhibitory effect of MF on the nasal symptoms induced by ATP took effect 60 min after its administration. Based on these differences in effect, we investigated the mechanisms of action of MF on the nasal symptoms.

First, we tried to confirm the mechanisms behind the genomic effect of MF. The genomic effect of MF inhibited the sneezing and the nasal rubbing induced by histamine, substance P, and ATP. It is common knowledge that GCs stimulate the production of annexin A1, a phospholipase A₂ (PLA₂) inhibitor [19,26]. PLA₂ is an enzyme that plays an important role in the arachidonic acid cascade [27]. In general, the arachidonic acid cascade is triggered by variant inflammatory action. Activated PLA₂ generates leukotriene (LT) and prostaglandin (PG) and complicates inflammation. It has been reported that PLA₂ is also activated by an increase in the intracellular calcium level [28]. Therefore, we considered if MF inhibited the activation of PLA₂ in association with an elevation of the intracellular calcium level by chemical mediators through the production of annexin A1. Therefore, we studied whether the induction of nasal symptoms by histamine and ATP required the activation of PLA₂. We determined that histamine and ATP need the activation of PLA₂ for the induction of nasal symptoms. From these results, it seems likely that the genomic effect of MF is expressed through the production of annexin A1.

Second, we studied the non-genomic effect of MF. The non-genomic effect of MF inhibited the sneezing and the nasal rubbing elicited by histamine and substance P, but did not affect the nasal symptoms induced by ATP. Histamine and substance P act via the histamine H1 receptor and neurokinin 1 (NK1) receptor, respectively. These receptors are Gq-protein-coupled-receptors and activate phospholipase C (PLC) [29,30]. Activated PLC produces inositol triphosphate (IP₃), and IP₃ leads to the release of Ca²⁺ release from the endoplasmic reticulum (ER) [31–33]. In contrast, ATP acts via the P2X receptor, a calcium ionotropic receptor, and directly leads to a calcium ion influx [34]. As a result, intracellular calcium levels graduate, nerve impulses are transmitted, and nasal symptoms appear [35]. It has been suggested that the non-genomic effect of GCs is expressed through the inhibition of PLC activation. We initially sought to study the effect of MF on nasal symptoms induced by a PLC activator. MF inhibited the nasal sneezing and the nasal rubbing induced by the PLC activator after administration 10 min before the induction of nasal symptoms. Moreover, we examined whether the nasal symptoms induced by histamine and ATP were elicited through the activation of PLC. It was revealed that only the nasal symptoms induced by histamine were accompanied with the activation of PLC. Thus, we concluded that the non-genomic effect of MF was expressed through the activation of PLC. The mGR has been found on lymphoma cells and peripheral blood leukocytes [36–38]. Therefore, it is thought that mGR plays an important role in the pathogenesis of inflammatory reaction in allergic diseases.

In conclusion, we reveal that MF has two types of effect; a non-genomic effect via the mGR and a genomic effect via the cGR. Moreover, our findings elucidated that the non-genomic effect occurred as a result of the inhibition of activated PLC and the genomic effect was caused by the production of annexin A1.

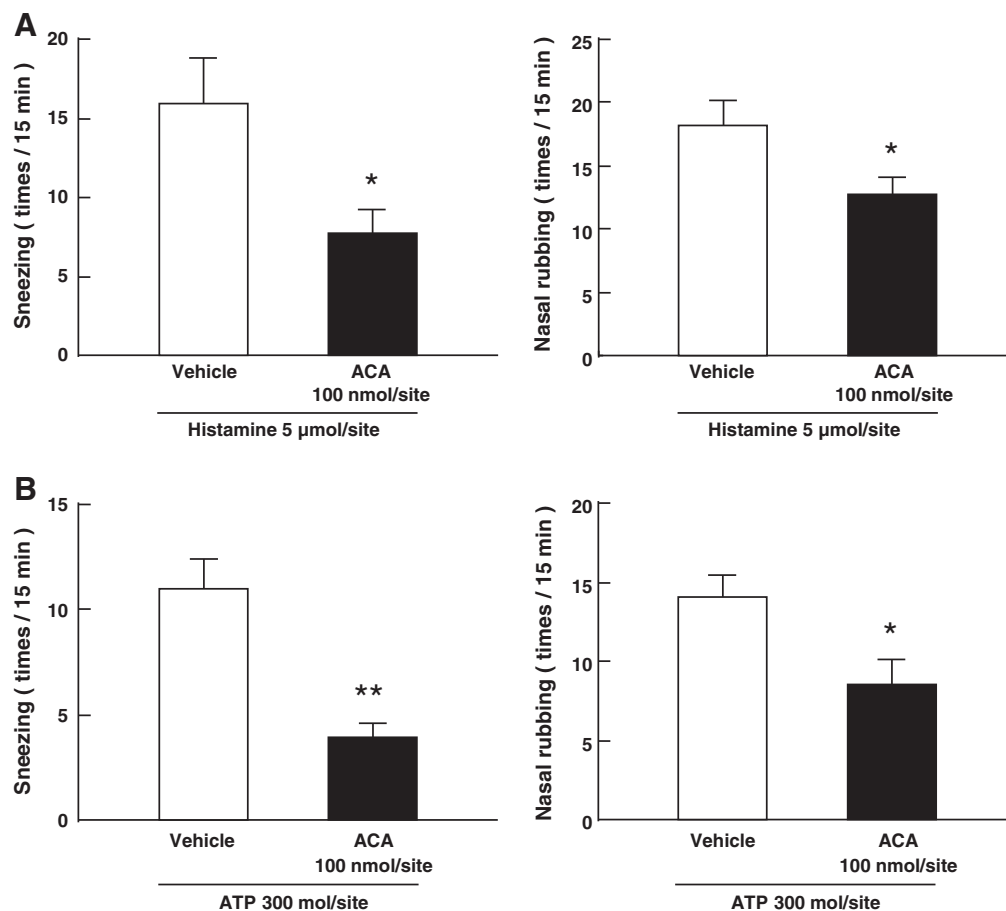


Fig. 8. Effect of ACA on sneezing and nasal rubbing induced by histamine (A) and ATP (B). The mice were given an intranasal administration of ACA (100 nmol/site) 30 min before the induction of nasal symptoms by histamine (5 μmol/site) and ATP (300 nmol/site). Each column and vertical bar represents the mean ± SEM. (n = 10). *, **: Significantly different from vehicle-treated group at p < 0.05 and p < 0.01, respectively.

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